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Movers and shakers: nutrient subsidies and benthic disturbance predict biofilm biomass and stable isotope signatures in coastal streams

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SUMMARY

1. Nutrient subsidies and physical disturbance from migrating species can have strong impacts on primary producers. In the north Pacific, adult salmon (*Oncorhynchus* spp.) transport marine-derived nutrients back to freshwater streams and can also significantly disrupt the substratum during spawning events. We tested for effects of spawning pink (*O. gorbuscha*) and chum (*O. keta*) salmon on stream biofilm. Biofilm is a mix of algae, fungi and bacteria that provides food and habitat and forms the base of these aquatic food webs.
2. We collected rock biofilm samples to compare stable isotopes and biomass prior to and following peak salmon spawning in 16 catchments on the central coast of British Columbia, Canada.
3. We conducted two separate analyses. The first was a within-stream comparison, which focused on 5 catchments that had a barrier to pink and chum salmon migration. The second was an among-stream analysis that included all 16 catchments and explicitly considered biotic and abiotic factors, in addition to salmon density, known to influence biofilm growth and isotope ratios.
4. Salmon density proved to be the best predictor of biofilm $\delta^{15}N$. Biofilm $\delta^{13}C$ was best predicted by salmon density and catchment size. While spring chlorophyll *a* increased with mean salmon density, it was on average lower during spawning in the autumn, probably due to physical disturbance from spawning salmon.
5. These results show that of the several variables considered to affect biofilm isotopes and biomass, salmon density and catchment size are among the most influential in coastal streams where salmon spawn.

Keywords: aufwuchs, ecosystem-based management, fisheries, nutrient pulse, periphyton

Introduction

Streams are dynamic bidirectional nutrient highways. They are the primary conduits for the export of terrestrial and freshwater resources to coastal environments but nutrients can also move upstream with species migration (Flecker *et al.*, 2010). Subsidies through species movements can increase production in nutrient-limited recipient ecosystems (Vanni, 2002; Vanni *et al.*, 2004; Payne & Moore, 2006) and have been shown to have the greatest effects in such hydrologically linked systems (Polis & Hurd, 1996; Bain & Stevenson, 1999; Marczak, Thompson & Richardson, 2007; Leroux & Loreau, 2008). While these habitats are inherently variable and disturbed frequently through natural events such as floods and droughts, species migrations can be an additional mechanism that alters the trophic interactions and physical landscape in these communities (Lake, 2000; Moore, Schindler & Scheuerell, 2004; Vanni *et al.*, 2004; Verspoor, Braun & Reynolds, 2010; Winemiller, Flecker & Hoeinghaus, 2010).

Spawning Pacific salmon (*Oncorhynchus* spp.) can play dual and opposing roles in nutrient pathways in freshwater habitats. They are both a source of high-quality nutrients, accumulating over 99% of their body mass at

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sea, and a cause of disturbance during spawning, which can export nutrients downstream (Wolman, 1954; Quinn, 2005). Spawning females can disturb large areas of the streambed when digging their nests (redds; Moore *et al.*, 2004; Moore & Schindler, 2008), but through spawning and subsequent death, all adults deposit the majority of their accumulated biomass in the form of excretory products, eggs, milt and carcasses.

Here, we examine the net impact of nutrient subsidies and disturbance on stream biofilm from Pacific Salmon. Biofilm is a matrix of algae, fungi, bacteria, microzoans and detritus held together by a polysaccharide matrix, living on rocks and logs in streams, rivers and lakes (Lock *et al.*, 1984). Biofilm provides energy and habitat structure to higher trophic levels; changes in its abundance can have far-reaching effects on species composition and food-web linkages (Biggs, 1996; Allan & Castillo, 2007). Many factors influence biofilm biomass, including species composition (Robson *et al.*, 2008), light (Lamberti & Steinman, 1997; Merritt, Cummins & Berg, 2008), nutrients (Rosemond, Mulholland & Elwood, 1993; Peterson *et al.*, 2001; Steinman, Lamberti & Leavitt, 2007; Verspoor *et al.*, 2010), temperature (Schuldt & Hershey, 1995; Polis & Hurd, 1996; Lamberti & Steinman, 1997; Bain & Stevenson, 1999; Marczak *et al.*, 2007; Leroux & Loreau, 2008; Verspoor *et al.*, 2010), flow (e.g. flooding, droughts, scour; Biggs, 1996; Lake, 2000; Vanni *et al.*, 2004; Moore *et al.*, 2004; Verspoor *et al.*, 2010; Winemiller *et al.*, 2010), substratum complexity (Wolman, 1954; Robson, 1996; Biggs, Smith & Duncan, 1999; Quinn, 2005; Holtgrieve *et al.*, 2010), grazer size and density (Rosemond *et al.*, 1993; Robson, 1996; Moore *et al.*, 2004; Moore & Schindler, 2008), catchment size (Lock *et al.*, 1984; Lamberti & Steinman, 1997) and the leaf litter of a common riparian nitrogen-fixing tree species, red alder (*Alnus rubra*; Biggs, 1996; Helfield & Naiman, 2002; Compton *et al.*, 2003; Allan & Castillo, 2007; Rüegg *et al.*, 2011). These influences can be broadly categorised into resources, which regulate growth and predation and physical disturbance, which cause biomass loss (Grime, 1977; Biggs, 1996; Robson *et al.*, 2008). In this study, we focus on how resources and disturbance interact to affect epilithic biofilm biomass, while accounting for the presence of grazers.

Some experimental studies with live salmon, salmon carcasses or analogues of carcasses have shown a positive effect on biofilm chlorophyll *a* (chl *a*; a measure of algal abundance) and ash-free dry mass (AFDM; a measure of total organic matter) in natural streambeds and experimental channels (Lamberti & Steinman, 1997; Wipfli *et al.*, 1999; Chaloner *et al.*, 2004; Johnston *et al.*, 2004; Merritt *et al.*, 2008; Rüegg *et al.*, 2012). However, other studies have found no clear relationship or a negative response when measuring biofilm in streams during and after spawning (Minakawa & Gara, 1999; Moore *et al.*, 2004; Mitchell & Lamberti, 2005; Verspoor *et al.*, 2010). The lack of consensus in the literature on the net effects of salmon is understandable, considering the methodological differences, contrasts between species, differences in catchment structure, differences between natural streams and experimental channels and differences in the effects of carcasses, carcass analogues and live salmon (Janetski *et al.*, 2009). Few studies to date have worked in 10 or more streams with live salmon during spawning (except Moore & Schindler, 2008; Verspoor *et al.*, 2010), nor taken into consideration the many habitat variables known to affect biofilm.

Here, we attempt to resolve conflicting views of impacts of salmon on epilithic stream biofilm by providing the first study on naturally occurring biofilm that combines a variety of spatial and temporal controls across a large number of coastal streams that varied in spawning salmon density. These sites had low dissolved nutrient concentration in the absence of spawning salmon, typical of coastal streams (Gende *et al.*, 2004). In 2009, we sampled rock biofilm before and during salmon spawning in 16 small- to medium-sized coastal streams in British Columbia, Canada. These sites naturally spanned a range in pink (*O. gorbuscha*), chum (*O. keta*) and coho (*O. kisutch*) salmon density, and in chemical, physical and biological landscape-level characteristics. Five of these sites were chosen because they had waterfalls or logjams, considerably limiting salmon migration and provided an additional within-stream comparison where samples were taken upstream and downstream of the barriers. Such barriers provided the opportunity for a 'natural' experiment in this, and other studies (Hocking & Reimchen, 2002; Mathewson, Hocking & Reimchen, 2003; Christie & Reimchen, 2008; Moulton *et al.*, 2010) because they excluded salmon from upstream reaches, creating natural controls. While these comparisons controlled for variation in physical and chemical aspects of the catchments, they did not account for any effects that the waterfalls and logjams may have had on algal biomass, independent of other factors. Longitudinal continuity in streams is an important factor controlling algal dynamics (Growns & Growns, 2001; Robson *et al.*, 2008) but there are no known studies to separate barrier effects from water regime effects on algae. It is possible that these natural barriers may have had some effect on algal dynamics although there were no differences in stream flow rates upstream and downstream of the barriers in

the present study. We used stable isotopes to determine uptake of salmon-derived nutrients in biofilm, which can be used as a powerful tool to identify nutrient sources (Peterson & Fry, 1987). We then examined chlorophyll *a* and ash-free dry mass to test for the effects of salmon density on biofilm biomass.

The objective of our study was to test hypotheses for the net effects of salmon as both a resource subsidy and mechanism of disturbance on stream biofilm biomass, while considering key habitat variables known to affect biofilm accrual. We sampled biofilm before and after peak spawning and predicted that uptake of salmon-derived nutrients would be reflected by enriched biofilm $\delta^{15}N$ and $\delta^{13}C$, especially in the autumn. We also predicted that biofilm biomass might take 'one step back' in autumn, by showing a decrease due to disturbance by spawning salmon, but potentially 'two steps forward' in spring, whereby streams with higher salmon densities might have more biofilm biomass due to carry-over effects of nutrients from salmon the previous autumn or in previous years. We also predicted that light, high temperature, large substratum size, high dissolved nitrogen and phosphorus, and large catchment size would have positive effects on biofilm biomass, while invertebrate grazers and high flow would have the opposite effect. The rationale for these predictions for isotopes and biomass can be found in Tables 1 and 2, respectively. These predictions were tested using both among-stream comparisons, and comparisons within streams, upstream and downstream of waterfalls and logjams.

Methods

Study sites

We sampled rock biofilm from 16 streams within 45 km of Bella Bella (52°9′N, 128°8′ W), on British Columbia's central coast during the spring (June) and autumn (September to October) of 2009 (Fig. 1). This area is in the Coastal Western Hemlock Biogeoclimatic zone and is characterised by heavy annual rainfall (>2200 mm), a mean temperature of 7.9 °C and nutrient-poor soils (Klinka, Pojar & Meidinger, 1991). Our study streams are dominated by chum (*O. keta*) and pink (*O. gorbuscha*) salmon but also include limited numbers of coho (*O. kisutch*) and sockeye (*O. nerka*). Spawning occurs from late August to early November in most streams, in densities ranging from 0 to 6 kg of chum and pink salmon m^{-2} over a median spawning channel length of 0.8 km (range = 0.3–5.8) and median bankfull width of 12.8 m (range = 2.7–23.5). Sampling occurred in stream reaches directly above the estuary, which was demarcated by the highest extent of saline water intrusion. Site-specific data are provided in Table 3.

Salmon enumeration

Live and dead salmon were counted from stream and bank walks in the autumn by Fisheries and Oceans Canada (DFO), the Heiltsuk First Nation and by field crews from Simon Fraser University. Details are given in Hocking & Reynolds (2011). Briefly, each site was counted at least three times, weather permitting. We calculated the abundance of each salmon species using the area under the curve method when three or more estimates were available (Irvine, Morris & Cobb, 1993) or using peak abundance plus carcasses where two or fewer counts were recorded in a given year. Both methods yielded similar results (Hocking & Reynolds, 2011). We calculated mean body mass from 10 fish (five males and five females) of each species measured at each site and used this value to calculate region-specific mean biomass estimates.

Environmental data collection

We collected data for three biotic and eight abiotic variables in addition to salmon density. Hypotheses for each variable considered in our analyses are detailed in Table 1 and 2. The length of each study reach was determined by multiplying the mean bankfull width by 30 (median = 228 m, range = 60–520 m; Bain & Stevenson, 1999). Each reach was divided into four equal sections, and three transects per section were assigned using a random number generator. Light availability was calculated as percentage canopy open at each transect from mean spherical densiometer readings taken at each bank and in the deepest part of the stream (median = 60%, range = 11–71%). We did not have long-term flow data for our sites so we used percentage gradient as a proxy (Verspoor *et al.*, 2010; median = 1.7°, range = 0.7 to 3.8°). Mean sediment size at each transect was calculated using the Wolman pebble count method where the intermediate axis (β) of 10 randomly selected rocks was measured at 10 transects for a total of 100 measurements per site (Wolman, 1954; median = 10.8 cm, range = 0.5–400 cm). Dissolved nutrients were assayed from three water samples per stream that were taken 3 months prior to and again following peak salmon spawning. Personnel at the Fisheries and Oceans Canada Cultus Lake Research Facility quantified soluble reactive

Table 1 Hypotheses for salmon and habitat variables for $\delta^{13}C$ and $\delta^{15}N$ analyses

Isotope	Variable	Mechanism	Metric	Level	Predicted response	Reference
$\delta^{15}N$ and $\delta^{13}C$	Salmon	Salmon are naturally high in $\delta^{15}N$ and $\delta^{13}C$ based on their largely marine diet. Via diffusion, biofilm can incorporate both heavy nitrogen and carbon in the water column from the excretory products of salmon or from decomposing tissue, eggs and milt	2006–2009 mean salmon density (kg m^{-2})	Catchment	Enriched	Quinn (2005) and Janetski *et al.* (2009)
$\delta^{15}N$ and $\delta^{13}C$	Invertebrate grazers	Grazers can decrease biofilm biomass, increasing light exposure to cells lower in the algal mat, which has been shown to decrease heavy isotope discrimination during photosynthesis. A reduction in algal mat thickness can also increase exposure to dissolved inorganic carbon, which is lower in $\delta^{13}C$ concentration	Grazer density (number m^{-2})	Catchment	Depleted	MacLeod & Barton (1998) and Hill & Middleton (2006)
$\delta^{15}N$ and $\delta^{13}C$	Light	Increases metabolic activity and photosynthetic rates, which can decrease the discrimination against heavy isotope uptake during photosynthesis	% Open canopy	Transect	Enriched	MacLeod & Barton (1998) and Ishikawa, Doi & Finlay (2012)
$\delta^{15}N$ and $\delta^{13}C$	Flow	Higher flow leads to a smaller boundary layer which reduces both carbon and nitrogen stable isotope signatures	Gradient degrees	Catchment	Depleted	Trudeau & Rasmussen (2003) and Ishikawa *et al.* (2012)
$\delta^{15}N$ and $\delta^{13}C$	Temperature	Increases metabolic activity and photosynthetic rates, which can decrease the discrimination against heavy isotope uptake during photosynthesis	Maximum weekly average temperature (°C)	Catchment	Enriched	Mook, Bommerson & Staverma (1974), MacLeod & Barton (1998) and Hill & Middleton (2006)
$\delta^{15}N$	Alder	Alder fix atmospheric nitrogen and consequently have a $\delta^{15}N$ closer to 0	Alder basal area (m^{-2})	Catchment	Depleted	Helfield & Naiman (2002) and Hicks *et al.* (2005)
$\delta^{13}C$	Biofilm Biomass	An increase in biofilm mat thickness increases carbon cycling within the algal community and reduces access to dissolved inorganic carbon, leading to $\delta^{13}C$ enrichment in the biofilm	AFDM (mg cm^{-2}) (total organic matter)	Catchment	Enriched	Hill & Middleton (2006), Staal *et al.* (2007) and Ishikawa *et al.* (2012)
$\delta^{13}C$	pH	Affects carbon availability for photosynthesis	pH	Catchment	Enriched	Hill & Middleton (2006) and Staal *et al.* (2007)
$\delta^{13}C$	Catchment Size	A composite measure for stream size and affects carbon availability for photosynthesis	Catchment Size PC 1	Catchment	Enriched	Vannote *et al.* (1980) and Finlay (2001)

phosphorus (SRP; $median_{spring}$ = 0.4 μg L^{-1}, $range_{spring}$ = 0–2.1 μg L^{-1}, $median_{autumn}$ = 6.4 μg L^{-1}, $range_{autumn}$ = 0.5–244.6 μg L^{-1}) and total dissolved inorganic nitrogen (DIN; $median_{spring}$ = 17.5 μg L^{-1}, $range_{spring}$ = 4.3–113.4 μg L^{-1}, $median_{autumn}$ = 90.5 μg L^{-1}, $range_{autumn}$ = 10.5–3,665.8 μg L^{-1}), measured separately as ammonium (NH_3^+) and nitrate (NO_3) following the American Public Health Association methods (APHA, 1989). Temperature was measured continuously using waterproofed temperature loggers (iButtons DS1922L)

Table 2 Hypotheses for salmon and habitat variables for chlorophyll *a* and ash-free dry mass analyses

Variable	Mechanism	Metric	Level	Predicted response	Reference
Salmon	Nutrients can fertilise biofilm but benthic disturbance during spawning can decrease biofilm biomass	2006–2009 mean salmon density (kg m^{-2})	Catchment	Positive spring, negative autumn	Wipfli *et al.* (1999), Chaloner *et al.* (2004) and Moore *et al.* (2004)
Invertebrate grazers	Reduce overall biofilm abundance.	Grazer density (number m^{-2})	Catchment	Negative	Rosemond *et al.* (1993)
Alder	Can increase dissolved and particulate nitrogen availability, mitigating nutrient limitation.	Alder basal area (m^{-2})	Catchment	Positive for low salmon density sites, neutral for sites beyond a nutrient threshold	Compton *et al.* (2003) and Rüegg *et al.* (2011)
Light	Higher light benefits algal growth.	% Open canopy	Transect	Positive	Lamberti & Steinman (1997)
Flow	Steeper gradient increases flow, which can increase scour and decrease biofilm	Gradient degrees	Catchment	Negative	Lamberti & Steinman (1997)
Substratum	Larger size can increase community stability.	Mean pebble size (cm)	Transect	Positive	Biggs *et al.* (1999), Janetski *et al.* (2009) and Holtgrieve *et al.* (2010)
Nitrogen	Biofilm can be nitrogen limited.	Dissolved inorganic nitrogen (μg L^{-1})	Catchment	Positive	Rosemond *et al.* (1993) and Peterson *et al.* (2001)
Phosphorus	Biofilm can be phosphorus limited.	Soluble reactive phosphorus (μg L^{-1})	Catchment	Positive	Rosemond *et al.* (1993) and Verspoor *et al.* (2010)
Temperature	Can affect metabolic activity and thus biofilm growth.	Maximum weekly average temperature (°C)	Catchment	Positive	Schuldt & Hershey (1995) and Lamberti & Steinman, 1997)
Catchment Size	Correlated with nutrient cycling and catchment size, which can influence primary productivity.	Catchment PC 1	Catchment	Positive	Lamberti & Steinman (1997)

anchored to boulders in the stream and set to record every 2 h (median = 8.8 °C, range = 7.0–10.2 °C). To quantify catchment size (median = 0.2, range = −2.8 to 3.3), we used the first axis from a principal components analysis of bankfull width (mean width of the stream at its highest point before breaching its banks), bankfull height (the mean maximum depth of the stream before breaching its banks), mean depth (mean actual stream depth, measured on sampling dates) and watershed area (calculated from the Government of British Columbia's *iMap*BC website (Government of British Columbia, DataBC, 2006; Hocking & Reynolds, 2011; Field & Reynolds, 2011). The first principal component accounted for 81% of the variation in bankfull width and height, mean depth and watershed area, which all loaded positively and were correlated with each other (correlation coefficient ≥ 0.63). Alder basal area was calculated from the diameter at breast height for each tree >5 cm in diameter in six 35-m-long by 10-m-wide belt transects that extended perpendicular from each stream into the riparian zone (median = 6,413 m^2, range = 0–54 326 m^2; Hocking & Reynolds, 2011).

To calculate invertebrate consumer density, we first collected benthic invertebrates from each stream using a surber sampler (500-μm mesh, metal frame area = 0.09 m^2) and pooled samples from three riffle habitats per transect, at three transects per site. We disturbed the substratum to a depth of 7 cm for 2 min, excluding the time it took to scrub larger rocks. We stored the samples in 95% ethanol until further processing. Using a similar method to Verspoor *et al.* (2010), samples were split using a Folsom Plankton Splitter. Invertebrates were separated from the stream organic matter and identified to Order (Ephemeroptera, Plecoptera, Trichoptera, Diptera, Other) to a total count of 300 or greater. Ephemeropterans, plecopterans and trichopterans were identified to family using Merritt *et al.* (2008). Chironomidae (Order Diptera) were distinguished from the other Dipteran families. All families were categorised into the dominant functional feeding groups as identified by Merritt *et al.* (2008). Although a single invertebrate family may represent several functional feeding groups, for the purpose of this study, we used the dominant feeding group to represent the entire

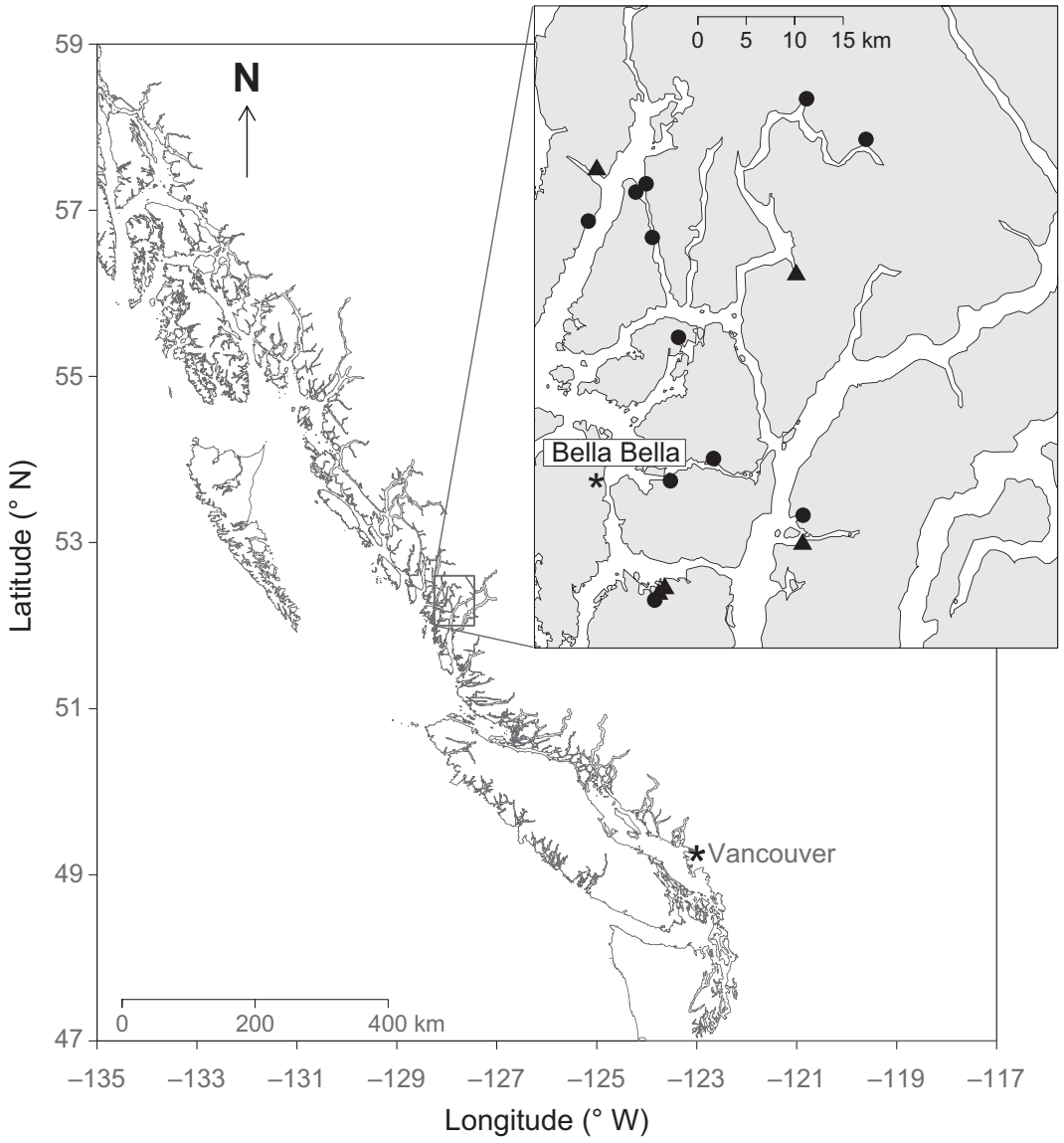

Fig. 1 Field sites on the central coast of British Columbia. The asterisks indicate the location of Bella Bella and Vancouver, British Columbia, Canada. Circles indicate streams along the salmon density gradient (0–3.2 kg of salmon m^{-2}). Triangles indicate streams with waterfalls or log jams blocking spawning pink and chum migration used for the within-stream analysis. The 'downstream' sites were subsequently used in a separate among-stream comparison.

family. Considering this, grazers were summed to estimate invertebrate consumer density per stream (number m^{-2}). The dominant grazers included baetids and heptageniids from the order Ephemeroptera, which comprised 45% and 44% of the total grazer abundance, respectively.

Biofilm isotopes and biomass

Epilithic biofilm isotope samples were scrubbed from four cobble-sized rocks (secondary axis <256 mm) using brushes. The rocks were haphazardly picked across the wetted width of the stream channel from six randomly selected transects within each study reach (n = 24 samples per stream). Invertebrates, if present, were removed prior to scrubbing. Dominant taxonomic composition was not assessed for this study. These samples were left unfiltered and stored in the dark at −20 °C until further processing. Each sample was defrosted in the dark at 4 °C, dried at 60 °C, then ground into a fine powder using a heavy duty Wig-L-Bug©. Dried samples (2.0–3.0 mg) were analysed for nitrogen and carbon natural abundance by the University of California Davis Stable Isotope facility using a PDZ Europa ANCA-GSL

Table 3 Site-specific stream data. Abbreviations correspond to Fig. 3 and stream location refers to upstream or downstream of waterfalls or logjams, which block adult pink and chum salmon migration. Stream magnitude is the sum of stream orders for the tributaries and the mainstem for a given site. Salmon densities were calculated over the entire spawning channel

Site	Abbreviation	Stream location	Stream magnitude	Catchment area (km^{-2})	Spawning channel length (m)	Mean Bankfull width (m)	Study reach (m)	2006–2009 mean salmon density ($kg\ m^{-2}$)
Ada	AD	Downstream	24	9.8	435	11.1	228	0.62
Ada	AD	Upstream	24	9.7	0	10.7	224	0.00
Beales Left	BL	Downstream	9	6.5	300	10.9	147	1.15
Bullock Main	BM	Downstream	2	3.3	622	10.9	250	1.32
Clatse	CL	Downstream	3	24.3	900	22.8	520	1.13
Clatse	CL	Upstream	3	23.9	0	17.8	321	0.00
Fannie Left	FL	Downstream	16	16.4	1500	12.8	400	0.46
Fell Creek	FE	Upstream	10	7.0	0	10.9	183	0.00
Hooknose	HN	Downstream	9	14.8	1800	16.9	373	0.32
Jane	JA	Downstream	5	1.3	500	4.6	124	0.01
Jane	JA	Upstream	5	1.3	0	2.7	124	0.00
Kill Creek	KI	Downstream	2	0.5	453	3.5	60	0.92
Kunsoot Main	KM	Downstream	3	4.9	1280	13.1	246	0.66
Mosquito Bay Left	MR	Downstream	4	2.1	250	5.7	130	1.45
Neekas	NK	Downstream	23	16.0	2100	17.7	486	3.21
Neekas	NK	Upstream	23	10.8	0	12.8	263	0.00
Quartcha	QU	Downstream	6	29.4	5500	21.7	229	0.11
Roscoe Main	RM	Downstream	10	33.6	5800	23.5	439	0.31
Sagar	SA	Downstream	8	36.6	800	15.5	151	0.15
Sagar	SA	Upstream	8	36.6	0	13.6	114	0.00
Troupe North	TN	Downstream	2	1.6	332	4.4	131	0.01

elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Stable isotopes are expressed as the difference between the sample and a standard, δ. Air and Vienna PeeDee Belemnite are the standards used for nitrogen and carbon, respectively. The difference is expressed as parts per thousand according to

$$\delta^{15}\mathrm{N} \text{ or } \delta^{13}\mathrm{C} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000 \quad (1)$$

where R is the ratio of the heavy isotope to the light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$).

Chlorophyll *a* (chl *a*) and ash-free dry mass (AFDM) were used as measures of algal and total biofilm biomass, respectively. Rocks were selected using the same method as for isotopes and any invertebrates were removed. We scrubbed two 1-cm^2 sections (one for chl *a* and one for AFDM) from each rock with a brush for 1 min. Each section was rinsed thoroughly through a 500-μm mesh with distilled water and stored in separate opaque containers. Chl *a* samples were filtered onto a glass fibre filter (Whatman, 47 mm, 0.7 μm), and AFDM samples were filtered onto pre-weighed, ashed glass fibre filters. Samples were stored in the dark at −20 °C until further processing. Biomass samples were processed following Steinman *et al.*, (2007).

Statistical analyses

Within streams: upstream versus downstream of salmon barriers. Five of the 16 streams had a waterfall or logjam that blocked pink and chum migration, providing an upstream versus downstream comparison of spring and autumn biofilm isotopes and biomass, while controlling for catchment-specific characteristics. We assessed the difference between the downstream mean minus the upstream mean by season for each response variable ($\delta^{15}N$, $\delta^{13}C$, chlorophyll *a* and AFDM) using a two-way ANOVA. We then ran post hoc Tukey HSD tests to compare between the upstream and downstream reaches. We visually inspected our models to ensure they met the assumptions of linear regressions. We log-transformed $\delta^{15}N$ and $\delta^{13}C$ and square-root-transformed chlorophyll *a* and AFDM to satisfy assumptions of normality.

Comparisons among streams. We compared the effect of salmon and several habitat variables on biofilm isotopes and biomass across 16 streams that spanned a natural gradient in salmon density and habitat characteristics. Because there was no clear consensus in the literature on what metric to use for salmon, we first calculated different salmon indices from the salmon enumeration data that were deemed biologically relevant to assess both

salmon as a subsidy and as a source of disturbance. Single- and multiple-year density means were calculated using the following formula,

$$D = \frac{\sum \frac{B_i}{L \times W}}{n} \quad (2)$$

where D is the mass of salmon by unit area, B is the total mass of pink and chum in year i, L is the spawning channel length, W is the mean bankfull width based on 12 or more measurements per site and n is the number of years used to calculate D (Verspoor *et al.*, 2010). We also summed the contribution of salmon over a 4-year period by negatively weighting salmon biomass from previous years as follows,

$$D' = \sum D_i \times e^{-\lambda t} \quad (3)$$

where D' is the 4-year sum of salmon biomass per unit area, D as calculated in equation 2, for a given year i, λ is the rate of biomass loss and t is time in months from autumn 2009 (t = 2, 6, 12 and 24; Verspoor *et al.*, 2010). Using Akaike Information Criterion corrected for small sample sizes (AICc), we first competed each index for each response variable in a linear model. We then assessed the individual contribution each salmon index contributed to the variation in each of our response variables using hierarchical partitioning (MacNally, 2006). Both methods concluded that the 2006–2009 mean mass of salmon per square metre (kg m^{-2}) explained the most variation in our response variables and was therefore the best index to use as a proxy for salmon's impact on biofilm.

We then checked for multicollinearity among all variables included in each analysis using variance inflation factors (VIF) and correlation coefficients (Zuur, Ieno & Elphick, 2010). A VIF score >3.5 and a correlation coefficient >0.6 were used to eliminate habitat variables considered to have a high degree of collinearity (Zuur *et al.*, 2009). There was a higher VIF score and degree of collinearity (0.7) between DIN and SRP, and DIN and salmon density in the autumn. We therefore excluded DIN from the final analyses because it was highly correlated with the other two variables. All remaining environmental variables did not significantly correlate with salmon density. Finally, we visually inspected our models to ensure they met the assumptions of linear regressions. We also square-root-transformed AFDM and log-transformed

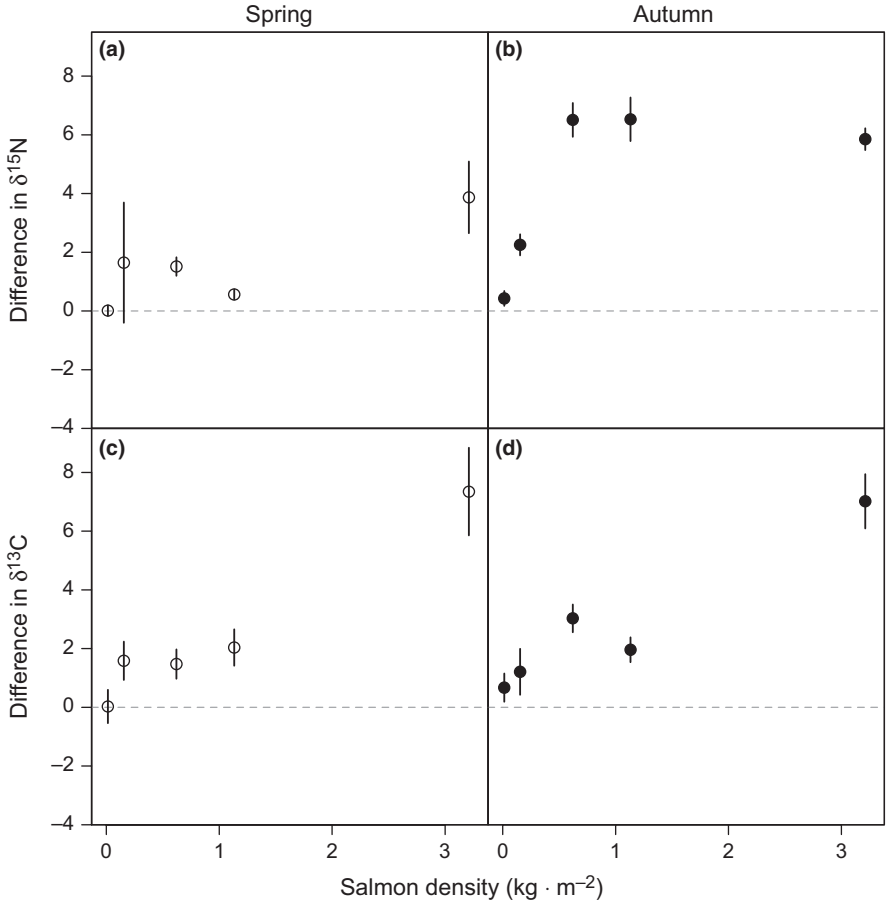

Fig. 2 Biofilm isotopes – The mean difference (downstream minus upstream of natural spawning barriers) with 95% confidence intervals for site pairs used in the within-stream analysis. Positive means indicate a higher value downstream of the barrier where salmon spawn in the autumn.

chlorophyll *a* and salmon density to satisfy assumptions of normality.

For the among-stream analyses, we identified three biotic and eight abiotic variables, from the literature, that influenced biofilm biomass. We generated a suite of linear mixed effects models, limiting the number of predictor variables to one per 10 data points (Harrell, 2001) to avoid the chance of spurious results. This generated 128 models for each season for $\delta^{15}N$ and $\delta^{13}C$ (w_i 6.05×10^{-8} to 0.16) and 511 models for each season for chl *a* and AFDM (model weights, w_i, 5.63×10^{-6} to 0.07). Due to low model weights, we accounted for model uncertainty using multimodel averaging (Burnham & Anderson, 2002). We standardised the independent data to a mean of 0 and standard deviation of 2 so that comparisons could be made among independent variables (Grueber *et al*., 2011). Models with delta AICc <2 were retained to form candidate model sets and were averaged using the natural method (Burnham & Anderson, 2002; Grueber *et al*., 2011) in the MuMIn package in R (Barton, 2012; see Table S1 in Supporting Information). We used three lines of evidence to evaluate the effect of salmon and habitat variables on biofilm isotopes and biomass among streams: (i) the magnitude and direction of the averaged coefficient, (ii) whether the 95% confidence intervals spanned zero and (iii) the relative variable importance (RVI) of each variable, which is the sum of the model weights of all the models in the final confidence set in which the variable appears (Burnham & Anderson, 2002). All analyses were performed in R (R Development Core Team, 2011).

Results

Within streams: upstream versus downstream of salmon migration barriers

Biofilm $\delta^{15}N$ was higher downstream of the barriers regardless of season, across all sites, as shown by positive mean differences in Fig. 2a,b, and generally increased with salmon density. As predicted, this difference was greatest in the autumn (ANOVA, $F_{1,49} = 40.81$, $P < 0.001$); however, these differences varied by site and season (ANOVA, $F_{4,49} = 3.68$, $P = 0.01$). Biofilm $\delta^{13}C$ was consistently greater downstream of salmon migration barriers than upstream, (Fig. 2c & d) and the difference between downstream versus upstream increased with salmon density. However, the magnitude of the differences between biofilm $\delta^{13}C$ downstream of the barriers versus upstream did not differ by season (ANOVA, $F_{1,49} = 0.72$, $P = 0.40$). The trends for $\delta^{15}N$ and $\delta^{13}C$ are also apparent in Fig. 3 where upstream sites (grey) were less enriched in both the spring and autumn than the downstream counterparts (black). The upstream sites had isotope signatures much closer to the terrestrial vegetation signature (T) than the salmon signature (S). There was also a notable shift in

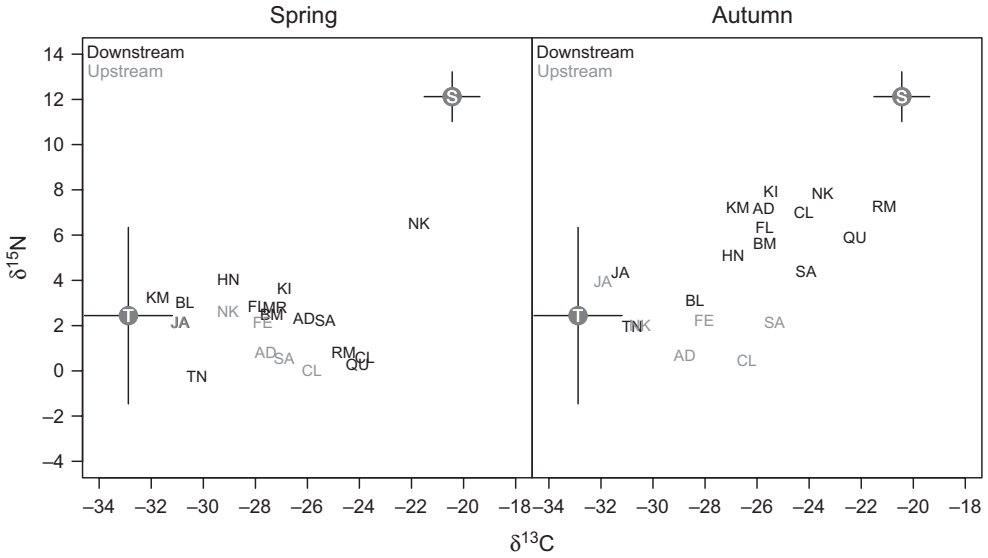

Fig. 3 Biofilm nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes for all study sites. Site codes correspond to Table 3. T is the mean terrestrial $\delta^{15}N$ and $\delta^{13}C$ of herbaceous plant species from our sampling sites (Hocking & Reynolds, 2011) and S is the mean chum and pink salmon $\delta^{15}N$ and $\delta^{13}C$ signatures from our sites ± SD. Sites downstream of salmon migration barriers are black, and sites upstream of the barriers are grey. Note AD, CL, JA, NE and SA were the five paired sites included in the within-stream comparison. FE is a site upstream of a salmon migration barrier but it does not have a corresponding downstream site and therefore was not included in the within-stream comparison.

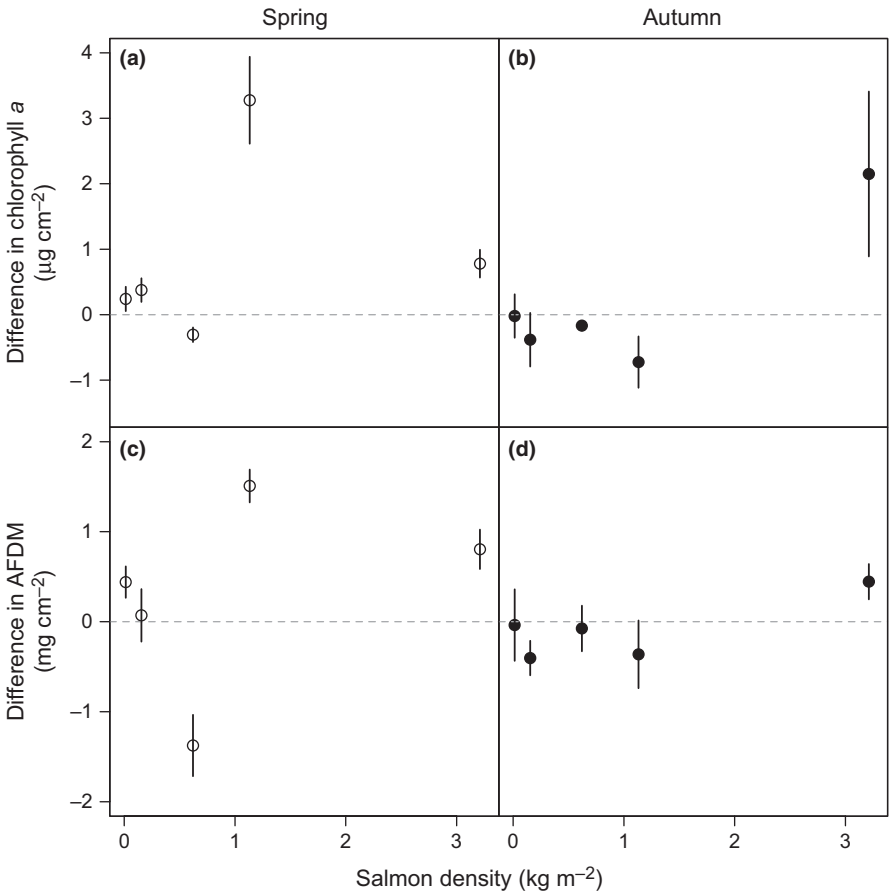

Fig. 4 Biofilm biomass – The mean difference (downstream minus upstream of natural spawning barriers) with 95% confidence intervals for site pairs used in the within-stream analysis. Positive means indicate a higher value downstream of the barrier where salmon spawn in the autumn.

the autumn towards the salmon signature in all sites downstream of salmon migration barriers. Interestingly, the greatest shifts for $\delta^{15}N$ occurred in sites with the largest catchments despite having among the lowest mean salmon densities [e.g. Roscoe Main (RM), Quartcha (QU), Clatse (CL) and Sagar (SA)].

Algal biomass (chl *a*) was typically higher downstream of waterfalls and logjams in the spring and lower in the autumn during salmon spawning (Fig 4a & b; ANOVA, $F_{1,44} = 8.24$, $P = 0.006$) but this difference varied by site and season (ANOVA, $F_{4,44} = 7.65$, $P < 0.001$). The two exceptions were Ada (salmon density = 0.62 kg m^{-2}) and Neekas (salmon density = 3.21 kg m^{-2}). Downstream of the waterfalls, chlorophyll a was lower in the spring at Ada and was higher in the autumn at Neekas (Fig. 4a & b). Total biofilm biomass (AFDM) followed a similar pattern to algal biomass (Fig. 4c & d).

Comparisons among streams

As predicted, streams with higher salmon densities had higher biofilm $\delta^{15}N$ in both spring and autumn (Figs 5 & 6a), with a positive salmon effect almost twice that of any other variable included in the averaged model (spring and autumn effect sizes = 2.56 and 2.52; Fig. 5). In contrast, spring biofilm $\delta^{15}N$ declined in streams with more alder (effect size = −1.57, Figs 5 & 6b).

Biofilm $\delta^{13}C$ also increased with salmon density in both seasons (Figs 5 & 6c). For every unit increase in salmon density (kg m^{-2}), spring and autumn biofilm $\delta^{13}C$ were enriched by a factor of 3.19 and 2.57, respectively (Fig. 5). Biofilm $\delta^{13}C$ also increased with catchment size (Figs 5 & 6d), with an equally strong effect as salmon, in both the spring (effect size = 2.93) and autumn (effect size = 3.79; Fig. 5). Among the other habitat variables included in the analysis, spring biofilm $\delta^{13}C$ declined with grazer density (effect size = −2.19), but increased with stream pH (effect size = 1.67). The confidence intervals for the remaining parameter estimates included zero, so they were considered to have uncertain effects on biofilm isotopes.

Chl *a* was best described by a suite of biotic and abiotic variables that varied by season. Salmon was a good predictor of chl *a* but its effect was strongest in the autumn (Figs 5 & 7a). Though the confidence intervals marginally spanned zero in the spring, chl *a* generally increased with salmon density (effect size = 0.62; Fig. 5) and declined in the autumn with increasing salmon density (effect size = −1.87). This effect was dampened

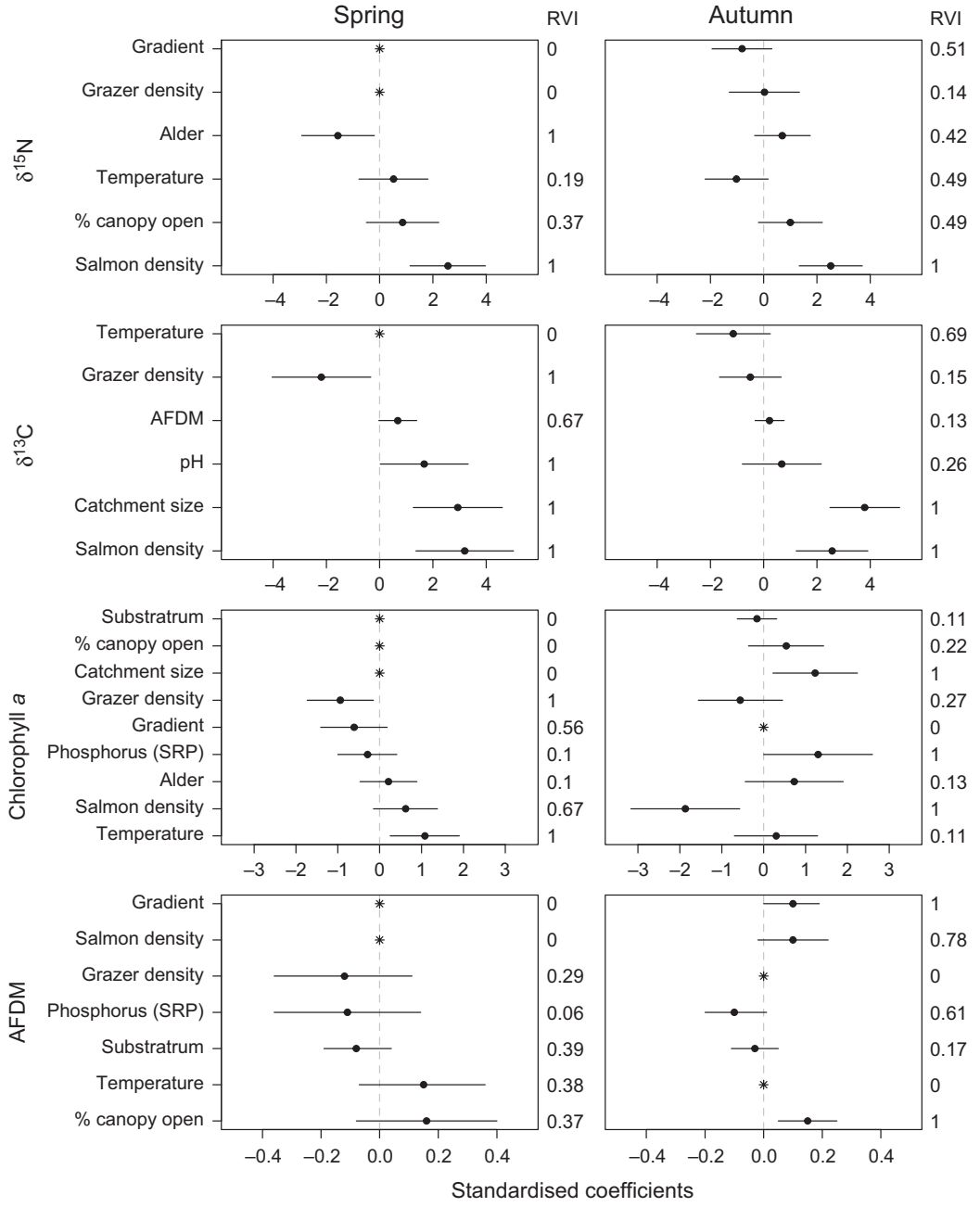

Fig. 5 Standardised coefficients (mean = 0, SD = 2) and 95% confidence intervals for spring and autumn from averaged models with a ΔAICc <2. Note the different *x*-axis scale for each response. Variables with asterisks were included in the original analysis but were not retained in the final averaged model set. Subsequently, standardised coefficients and confidence intervals were not calculated.

by Neekas (salmon density = 3.21 kg m^{-2}), which had a high leverage effect (Fig. 7a). In warmer streams, spring chl *a* was higher (effect size = 1.08), but this trend was not as strong in the autumn (effect size = 0.30; Figs 5 & 7b). Generally, streams with higher grazer densities had lower chl *a;* however, this relationship was also not as strong in the autumn when salmon were present (spring effect size = −0.94; autumn effect size = −0.56; Fig. 5). Conversely, streams in larger catchments and with higher SRP had more chl *a*, but this was limited to the autumn sampling period (Fig. 5). There was higher uncertainty of the effects of alder, light, and substratum size on chl *a* abundance, which corresponded to a low relative variable importance and large confidence intervals that spanned zero (Fig. 5).

AFDM was highly variable but increased with light and stream gradient, with the strongest effect observed in the autumn (autumn effect size for light = 0.15 and

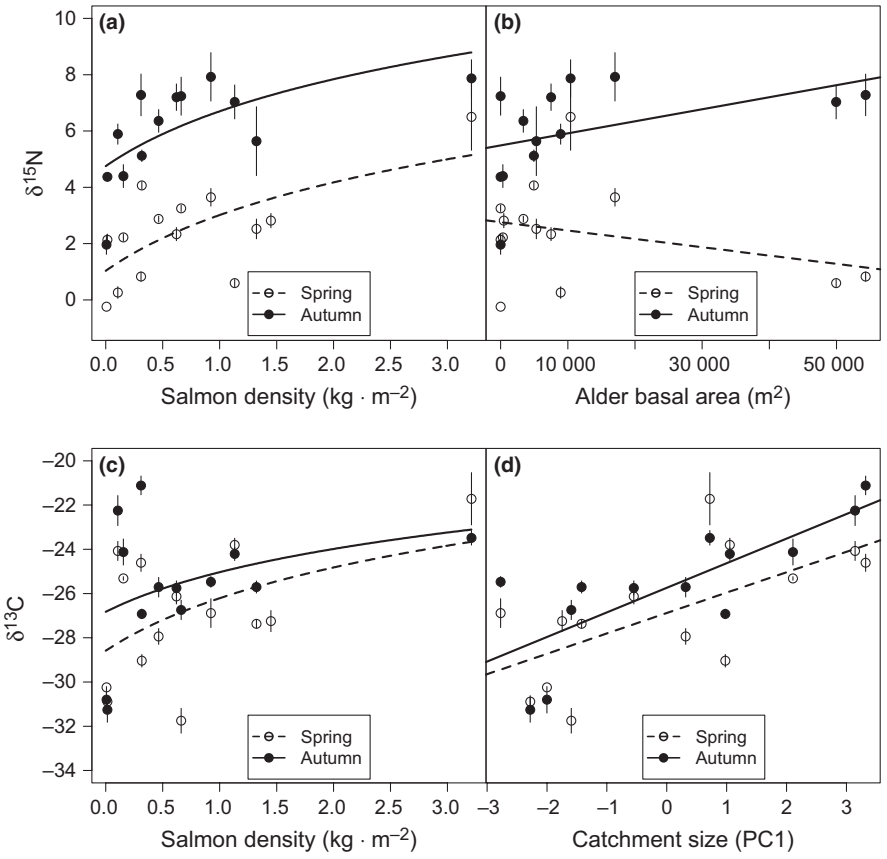

Fig. 6 Bivariate plots of $\delta^{15}N$ versus (a) salmon density, and (b) alder, and $\delta^{13}C$ versus (c) salmon density and (d) catchment size. Salmon density was $\log_{10}$-transformed for both analyses, whereas alder and catchment size were not. Model lines reflect the $\log_{10}$-transformed (a and c) and linear (b and d) relationships. Each data point represents a different stream.

gradient = 0.10; Figs 5 & 7d). The relatively weak parameter estimates combined with confidence intervals that spanned zero (Fig. 5) suggest that the remaining predictor variables included in the analyses could not explain the variation in AFDM in either season.

Discussion

This analysis of 16 streams showed that biofilm isotopes and biomass were correlated with salmon densities. Spawning salmon were an important source of short- and long-term nutrients as well as a mechanism of disturbance. However, we also found that habitat features, both on land and in streams, played an important role in mediating these effects. These results support the idea that nutrient subsidies and disturbance are important determinants in stream communities, with effects that depend on catchment-scale processes and habitat features.

Effect of salmon density on biofilm isotopes and biomass

The five streams with barriers to pink and chum salmon migration provided a novel opportunity to control for catchment-specific characteristics while testing for the effect of spawning salmon on biofilm isotopes and biomass. As seen in other studies, and consistent with our predictions, biofilm from salmon spawning areas had consistently higher $\delta^{15}N$ and $\delta^{13}C$ than sites with no spawning adults, regardless of season (Kline *et al.*, 1990; Bilby, Fransen & Bisson, 1996; Verspoor *et al.*, 2010; Reisinger *et al.*, 2013). This was particularly true in the autumn for $\delta^{15}N$. Also, of interest was the difference between downstream and upstream sites, which generally increased with salmon density. Biofilm biomass (chlorophyll *a* and AFDM) was generally higher downstream of waterfalls and logjams prior to the arrival of salmon, but depressed when salmon were present.

Results from comparisons among streams were similar to the within-stream analyses. The isotopic patterns indicated that biofilm $\delta^{15}N$ increased with a 4-year mean salmon density in both seasons and while there was a strong negative effect of salmon on autumn chlorophyll *a*, there was a weaker yet positive relationship in the spring. Lower autumn algal biomass in streams that had more salmon was likely due to increased scour as seen in other studies (Moore *et al.*, 2004; Moore & Schindler, 2008). However, the tendency for increased algal biomass in the spring, as shown by both analyses, may be explained by two competing hypotheses: top-down or bottom-up control.

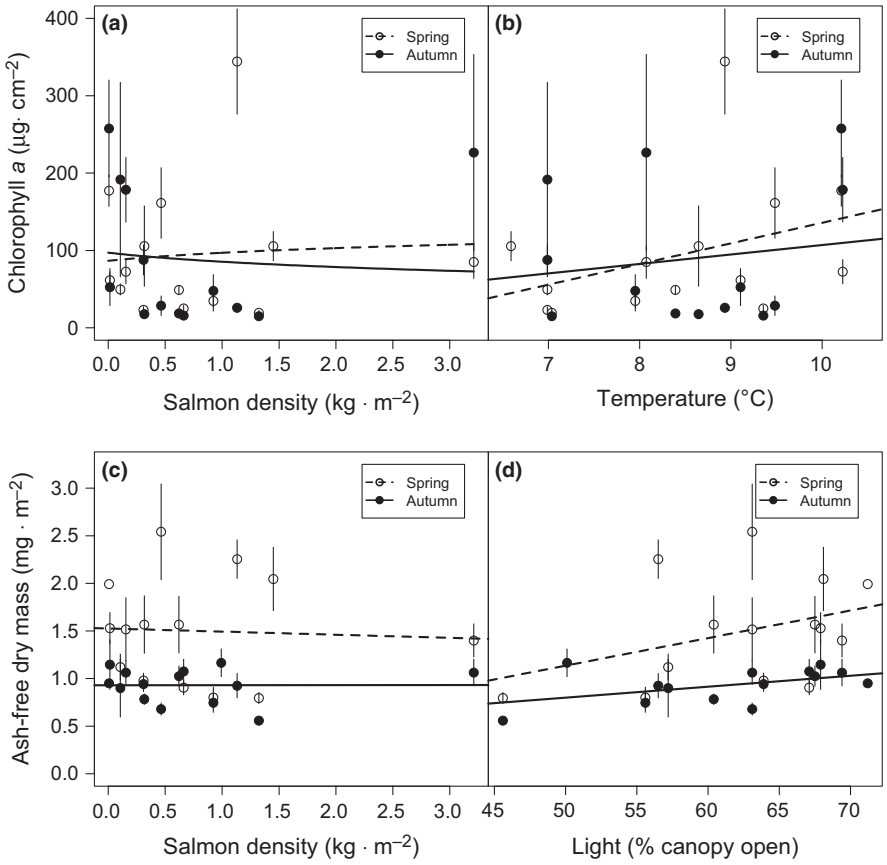

Fig. 7 Bivariate plots of chlorophyll *a* versus (a) salmon density and (b) temperature, and AFDM versus (c) salmon density and (d) light. Salmon density was $\log_{10}$-transformed for both analyses, whereas temperature and percentage canopy open were not. Model lines reflect the $\log_{10}$-transformed (a and c) and linear (b and d) relationships. Each data point represents a different stream.

If biofilm were controlled from the top-down, a salmon subsidy to resident fish could trigger a trophic cascade (Polis, Anderson & Holt, 1997; Moulton *et al.*, 2010), whereby increased predation on grazing insects could release biofilm from grazing pressure. This hypothesis could explain the positive correlation between salmon and spring biofilm biomass. Indeed, Swain *et al.* (2014) showed that prickly and coast range sculpins (*Cottus asper* and *C. aleuticus*, respectively) in the same study sites fed primarily on benthic invertebrates in the spring before switching almost exclusively to salmon eggs in the autumn.

Alternatively, if biofilm were controlled from the bottom-up, salmon-derived nutrients would need to be retained in these systems from autumn to spring and the streams would need to be nutrient-limited (Marczak *et al.*, 2007). Many coastal streams in this region are nutrient-limited (Gende *et al.*, 2004), including the sites we studied, as shown by low pre-spawn dissolved inorganic nitrogen and soluble reactive phosphorus. We calculated that in these streams, salmon can contribute a substantial amount of nitrogen annually compared with the nitrogen content in biofilm. For example, salmon can import up to 53 g m^{-2} of nitrogen in low years and over 266 g m^{-2} of nitrogen in high years, while nitrogen in biofilm ranges from 0.05 to 0.69 g m^{-2} (median = 0.26 g m^{-2}) in the same sites. These numbers are based on recorded salmon counts for all sites, and the percentage nutrients by wet salmon weight calculated by Gende *et al.* (2004). These nutrients are delivered in a mineralised form that is particularly useful to primary producers (Tiegs *et al.*, 2011). Though dissolved nutrients were low in the spring, biofilm δ^{15}N and δ^{13}C remained enriched in salmon spawning sections and were correlated with a 4-year mean salmon biomass density. This nutrient legacy may be due to either nutrient recycling within the biofilm mat (Hill & Middleton, 2006) or nutrient retention over winter (Kline *et al.*, 1990; Bilby *et al.*, 1996; Verspoor *et al.*, 2010). Large wood, low flow and deep pools within our sites facilitate salmon carcass retention in streams for several weeks to months (Minakawa & Gara, 2005; Strobel, Shively & Roper, 2009). Carcass transfer by predators [e.g. bears (*Ursus* spp.) and wolves (*Canis lupus*)] into the riparian zone can also prolong the release and uptake of salmon-derived nutrients in terrestrial and freshwater environments (Reimchen, 2000; Hocking & Reynolds, 2012). Nutrients from salmon carcasses on land can flow back into the stream via flooding and surface runoff (Willson,

Gende & Marston, 1998), leach into the hyporheic zone (O'Keefe & Edwards, 2002) or enrich leaf litter, invertebrates and vertebrates (Hocking & Reimchen, 2002; Christie, Hocking & Reimchen, 2008), which can end up back in streams as decaying matter.

The current study cannot tease apart whether it is solely bottom-up or top-down effects controlling biofilm in these streams. However, evidence from Swain *et al.* (2014) and post hoc analyses suggests that it is probably a combination of the two processes. For example, biofilm carbon : nitrogen (C : N) decreased in both seasons with salmon density, suggesting bottom-up control is also in effect (see Figure S1 in Supporting Information), but we recognise we cannot rule out the effect of grazers on biofilm C : N in the current study (Jaramillo & Detling, 1988).

Effects of habitat on biofilm isotopes and biomass

Catchment size had the largest and most consistent effect on both biofilm isotopes and biomass, specifically $\delta^{13}C$ and chlorophyll *a*. There were also effects of invertebrate grazers, alder trees in the riparian area, stream temperature, gradient and soluble reactive phosphorus but their effects were not as consistently strong as catchment size. This is consistent with the hypothesis that carbon requirements increase with catchment size due to a decrease in $CO_{2(aq)}$ and higher *in situ* production in larger streams and rivers (Finlay, 2001). We did not acid-fumigate our samples and so it is possible that carbonates (e.g. diatoms) may have contributed, in part, to the positive relationship between $\delta^{13}C$ and catchment size. However, it seems unlikely that carbonates would be the sole reason for the positive relationship between $\delta^{13}C$ and salmon particularly when other studies have documented similar results between carbon isotopes and salmon density.

Given our findings, we suggest including catchment size in future biofilm analyses because it is an important determinant of the total potential productivity of a system, including invertebrate grazers and predators (Kiffney & Roni, 2007), and consequently has potential implications regarding land use. For example, this study was performed in the remote and relatively pristine Great Bear Rainforest, a temperate rainforest with a land-use agreement between coastal First Nations and the province of British Columbia (Price, Roburn & MacKinnon, 2009). While 2.1 million hectares (33%) of the Great Bear Rainforest are protected from commercial activity (e.g. forestry and hydro-electric power projects), limited land-use plans exist in this area (Price *et al.*, 2009; Council, Nations & Lands, 2012). Changes to upstream catchments could alter the important interplay between salmon and biofilm and nutrient cycling controlling stream food webs (Tiegs *et al.*, 2008; Levi *et al.*, 2011).

Our combination of within-stream and among-stream comparisons shows a dual role for salmon as both as a nutrient subsidy and a mechanism of disturbance of biofilm. What is most interesting is that even though disturbance by salmon results in a decrease in biofilm during spawning, salmon-derived nutrients from previous years are linked to an increase in both isotopes and algal biomass in salmon spawning reaches, prior to the arrival of salmon. Salmon-derived nutrients could be eliciting a trophic cascade or simply enhancing basal biomass.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Mean biofilm carbon : nitrogen (C : N) versus salmon density with 95% confidence intervals.

Table S1. Top models with ΔAICc <2 and the confidence set used for model averaging.

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