Limnol. Oceanogr., 57(1), 2012, 113–123

doi:10.4319/lo.2012.57.1.0113

Ecosystem response to a salmon disturbance regime: Implications for downstream nutrient fluxes in aquatic systems

Sam J. Albers* and Ellen L. Petticrew

Geography Program and Quesnel River Research Centre, University of Northern British Columbia, Prince George, British Columbia, Canada

Abstract

Recent work in salmon spawning streams has shown that sediment resuspended during nest construction flocculates with salmon organic matter to form suspended composite particles characterized by increased size and settling velocities. In a river system, these flocs have the potential to interact with benthic biofilms, suggesting a mechanism for the incorporation of organic matter into aquatic food webs. Using the Horsefly River spawning channel in central British Columbia, the spatial scale of biofilm floc trapping was evaluated for a salmon disturbance regime, which consists of the active digging of redds, spawning, and carcass decay. We stocked two sequential enclosures in the spawning channel with sockeye salmon (*Oncorhynchus nerka*) and established one upstream control enclosure. Biofilms were sampled for chlorophyll *a*, trapped sediment, and marine isotope tracers (δ^{15}N and δ^{13}C). In the active-spawn period, biofilm abundance was reduced due to spawning disturbance, with isotope values indicating low utilization of marine-derived nutrients (MDNs). During the post-spawn period, downstream biofilm abundance exceeded pre-spawn values, indicating a near-field nutrient pulse with isotope values reflecting biofilm utilization of MDNs. At the same time, an increase in biofilm trapping efficiency occurred in concert with a significant increase in the in situ particle size of suspended sediment, suggesting that flocs were a temporary storage site of MDNs. The retention of MDNs over short spatial scales acts to retard the flushing of MDNs to downstream rearing lakes. The magnitude of these processes has ecological implications on the downstream lake's productivity, thereby influencing the success of future salmon stocks.

Rivers are typically discussed as ecosystems regulated by flow regimes with the transfer of nutrients and energy proceeding unidirectionally to downstream habitats (Vannote et al. 1980). While this early conceptual model incorporated the uptake of matter into the food web by invertebrates, disturbances generated by other organisms also have the potential to alter the flow of nutrients and energy through aquatic ecosystems and, therefore, the subsequent ecosystem response. Using a variety of plant-based examples, Viles et al. (2008) showed that an organismal-driven disturbance regime can have either a stabilizing or destabilizing spatial effect on an ecosystem. In addition to spatial variation, there is often a temporal lag between a disturbance and the ecosystem response. For example, the yearly migration of Pacific salmon (*Oncorhynchus* spp.) to their natal streams to spawn and die represents a salmon disturbance regime (Albers 2010) that elicits a spatially and temporally varied ecosystem response (Wipfli et al. 1998; Moore and Schindler 2008). Salmon can be considered fundamental biogeomorphic agents (Petticrew and Albers 2010) acting on physical and biological elements of ecosystems at local to regional scales (Corenblit et al. 2011). Salmon redd construction causes considerable disturbance as salmon resuspend sediment and biofilms from the benthic environment (Moore et al. 2007), which is coupled with the decaying bodies of salmon releasing significant amounts of marine-derived nutrients (MDNs), fertilizing local environments (Wipfli and Baxter 2010).

While several studies have related both increased suspended sediment fluxes (Moore et al 2007; Petticrew and Albers 2010) and increased biofilm growth (Wipfli et al. 1998; Moore and Schindler 2008) to salmon spawning activity, the processes connecting sediment, MDNs, and biofilm to ecosystem response have not been clearly identified. Rex and Petticrew (2008) identified a salmon-floc feedback loop whereby the overlap between salmon spawning and salmon decay provides ideal conditions for flocculation (the formation of flocs or aggregates of organic and inorganic matter). The digging of salmon redds combined with carcass decay provides favorable physical and biological conditions for fine resuspended sediment (< 63 μm), organic matter, and nutrients derived from salmon to aggregate into flocs, which act as a vector to transfer nutrients from the water column to the gravel bed (Petticrew et al. 2011). These larger aggregated particles can subsequently be delivered to gravel-bed gravels via increased settling rates and intergravel trapping. Once retained either in or on the gravel bed, these flocs increase the availability of organic matter and nutrients to the benthos (Wotton 2007; Petticrew et al. 2011). Currently, however, no satisfactory delivery mechanism for the transfer of MDNs to biofilms has been identified, and there have been no known attempts to relate suspended sediment, flocculation, and MDN-driven biofilm growth. Furthermore, the effect of these processes on the response of ecosystems to a disturbance regime is as yet unknown. The biological importance of aggregates formed in the water column is often overlooked (Wotton 2007), and we suggest that their role in MDN delivery to, and retention by, biofilms has not been sufficiently examined. The redistribution of MDNs and sediment by salmon at fine spatial scales (Moore et al. 2007) can have watershed-level

* Corresponding author: albers@unbc.ca

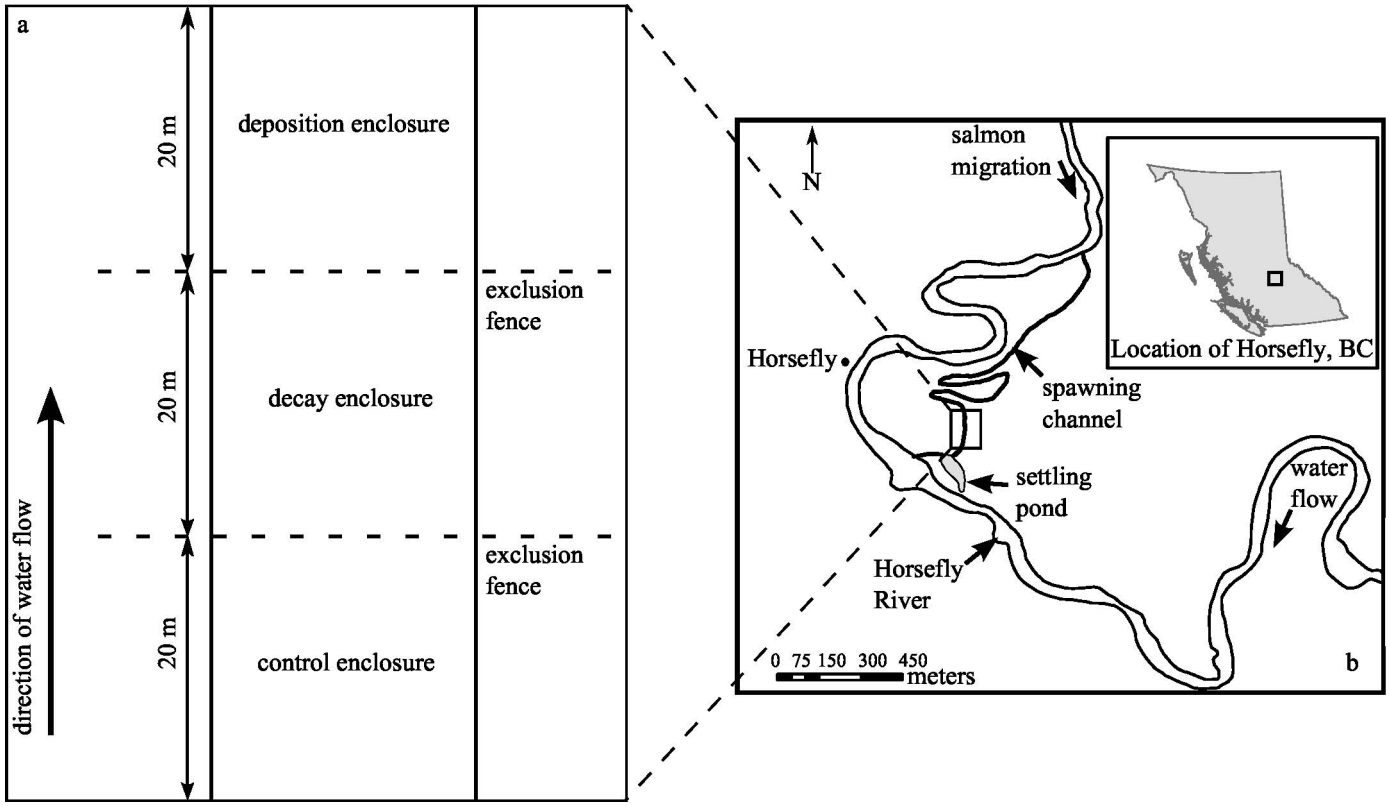

Fig. 1. Diagram of the Horsefly River spawning channel (HFC) and experimental reach. (a) Demonstrates the division of the HFC experimental reach into experimental enclosures. The exclusion fences prevented salmon from moving between enclosures. (b) The location of the HFC within British Columbia and position of the experimental reach within the HFC.

impacts as aggregated suspended particles alter the downstream flow of nutrients (Petticrew et al. 2011). As a result, the spatial patterns of MDN cycling remain only partially understood at both small and watershed scales. This spatial pattern of MDN transfer underlines the need to elucidate pathways of MDN processing.

Cycling of MDNs is particularly important in inland salmon habitats, where longer distances from the ocean diminish the marine connectivity but may increase the importance of MDNs (Johnston et al. 1997). The transfer of MDNs from spawning grounds to rearing habitats is particularly important in inland salmon systems because of the considerable residence time of juveniles (especially sockeye) in rearing lakes (~ 1 yr). A gravel bed potentially acting as a nutrient sink retarding nutrient transfer to downstream lakes in both space and time would have significant effect on juvenile salmon productivity. In inland British Columbia river systems connected to a downstream rearing lake, for example, the extent to which nutrients are flushed downstream, delivered in bursts, or retained closer to spawning grounds will dictate the spatial and temporal patterns of MDN-driven biofilm growth. Fertilization of lakes has a large impact on juvenile salmon productivity (Hyatt et al. 2004), suggesting that the magnitude, rate, and timing of an MDN input may have the same impact.

Substantial information has been gained on salmon spawning ecology from using both artificial stream-based studies (Rex and Petticrew 2008) as well as field observations (Moore and Schindler 2008). The Horsefly River spawning channel (HFC) in the Central Interior of British Columbia represents a unique experimental tool that incorporates the manipulability of an artificial stream with the realism of a natural habitat. Our objectives were to quantify changes in biofilm abundance and assess the mechanisms and nutrient sources associated with those changes. The interaction between sediment and biofilm was examined temporally and spatially in the context of a salmon disturbance regime. Salmon tend to reduce biofilm during the active-spawn period but increase it via fertilization during the post-spawn period (Moore and Schindler 2008). We hypothesized that the post-spawn period is (1) characterized by higher biofilm abundance and (2) driven by MDNs and that this increase is attributed to (3) in-stream floc formation via biofilm and sediment resuspension altering subsequent nutrient delivery to downstream channel beds.

Methods

Study site—The HFC is an artificial salmon stock enhancement stream that operates as a side channel off the main Horsefly River (52°19′N, 121°24′W) located within the Central Interior region of British Columbia, Canada (Fig. 1b). Water to the HFC is supplied from a settling pond that is directly connected to the Horsefly

River. Sockeye salmon enter the HFC via the Horsefly River, where they are confined and obliged to spawn inside the channel. Other resident fishes observed in the channel during the experimental period were a small number (< 10) of rainbow trout (*O. mykiss*), kokanee (*O. nerka*), and chinook salmon (*O. tshawytscha*). The west side of the channel was devoid of tree cover, while the east side of the channel had a 3-m strip of deciduous trees, which produced similar light levels across the entire experimental reach of the HFC. Because the HFC was constructed in part to provide uniform spawning habitat, differences in grain size between enclosures were negligible (mean surface area: 62.2 cm^2, standard deviation = 1.59).

In summer and fall of 2009, a portion of the HFC was converted into an experimental reach (Fig. 1a). The experimental reach was divided into three 20-m enclosures (Fig. 1a) using steel pole fences that limited salmon entry into a particular enclosure. We stocked the lower two sequential enclosures in the spawning channel with high densities of spawning sockeye salmon (*Oncorhynchus nerka*) and established one upstream enclosure as a spatial control. To determine the effects of spawning activity and salmon carcass decay on biofilm growth and downstream nutrient delivery, we removed post-spawn salmon from the downstream deposition enclosure while allowing decaying salmon to remain in the middle enclosure termed "decay." This enabled us to evaluate the effect of an upstream MDN source on an area that has experienced spawning disturbance. A small number of salmon escaped into the upstream enclosure, diminishing the spatial control. These fish, however, were removed from the upstream enclosure when possible, minimizing spawning activity and any potential die-off effects on the other two downstream enclosures. Channel enclosures are referred to throughout this paper as control (upstream), decay (middle enclosure), and deposition (third enclosure), as shown in Fig. 1a. A 1-m buffer around each fence was excluded from sampling and used for researcher movement through the channel. Prior to the start of the sampling period the channel bed was cleaned of sediment and biofilms, using a rake with 30-cm teeth mounted on a bulldozer.

Salmon enumeration and collection—Sampling began on 28 August 2009 and lasted until 26 October 2009. Sampling periods were defined based on salmon activity. Live and dead salmon densities were enumerated both visually and with a digital camera. The salmon were visually counted by two people. In instances in which the counts differed greatly (> 10 salmon), the salmon were recounted until a similar count was reached. When live salmon densities were too active to be accurately counted visually, a digital photograph was taken of the reach and salmon were counted at a later date. Salmon muscle tissue ($n = 4$) was sampled within 1 h of dying and analyzed for $\delta^{15}N$ and $\delta^{13}C$ (Pacific Centre for Isotopic and Geochemical Research, University of British Columbia).

Biofilm collection and characterization—Channel bed biofilms were collected in each enclosure from randomly sampled stones during the pre-spawn, active-spawn, and post-spawn periods. On every sample date, five stones were randomly collected from each of the three enclosures. Because of a small number of escapees into the upstream control enclosure, stones were collected in this enclosure where there had clearly been no salmon activity. Stone surface area was determined using a regression method (Graham et al. 1988). A second surface biofilm sample was collected on each sampling date in triplicate from each enclosure for stable isotope analysis.

Immediately after collection, stones were scraped with a toothbrush and rinsed with distilled water to remove all biofilm and sediment. The resultant slurry was filtered onto a pre-ashed, preweighed glass-fiber filter (GFF), protected from light, and frozen at −20°C until further analysis. Chlorophyll *a* (Chl *a*) was extracted from the slurry in 25 mL of 90% buffered acetone for 24 h at 4°C, and the extract was centrifuged at 3100 revolutions per minute for 20 min. Extracted Chl *a* was analyzed spectrophotometrically, correcting for pheophytins by acidification with HCl (American Public Health Association 1995). Any material left on the GFF after extraction and any material centrifuged into a plug in the above centrifugation step was combined, dried at 60°C for 12 h, weighed, ashed at 550°C for 2 h, and weighed again. The mass lost during the ashing step was defined as ash-free dry weight (AF dry wt), and the material left on the GFF was defined as the amount of inorganic sediment trapped by the biofilm.

Biofilms for stable isotope analysis were scraped from each stone in the same manner as described above, except the biofilm slurry was frozen in a microcentrifuge tube. Upon returning to the lab, samples were freeze-dried and analyzed for $\delta^{13}C$ and $\delta^{15}N$. Isotope ratios were determined as follows (Kline et al. 1990):

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

where R is the ratio of the heavy isotope to the light isotope. The standard for C is Peedee Belemnite and for N is air (Bilby et al. 1996).

Infiltration bags: collection and characterization—Sediment infiltration into the channel bed was assessed using modified infiltration bags, which allow for vertical and horizontal sediment delivery to a sample column of gravel (Rex and Petticrew 2006). Three 0.35-m holes were dug in each enclosure. Plastic frames covered with galvanized steel mesh (aperture 0.025 m) were placed in each hole. The plastic frames prevented outside stones from filling the hole, while the steel mesh allowed for normal water flow through the gravels. In each experimental enclosure, infiltration bags were placed at the base of three buckets and covered with gravel cleaned of sediment < 2 mm. Gravel was sampled weekly and replaced with clean gravel in the same position within the channel bed. For each weekly sampling date, gravels were rinsed through a 2-mm sieve to remove all < 2-mm sediment into a volumetrically calibrated bucket.

Fine sediment was sampled from this bucket by resuspending all the material collected from infiltration bags in a sample bucket, waiting 10 s, and subsampling the

top 10 cm of water. This method allows for larger particles to settle out and ensures that only fine sediment (< 70 μm) is sampled (Rex and Petticrew 2006; Petticrew and Albers 2010). Fine sediment from the infiltration bags was filtered onto GFF, dried at 60°C for 12 h, weighed, ashed at 550°C for 2 h, and weighed again. The response variable derived from this process was intergravel inorganic sediment.

Suspended sediment—Changes in suspended sediment particle size distributions were determined using laser in situ scattering transmissiometry (LISST, Sequoia Scientific). The LISST instrument measures the degree of diffraction when a laser is passed through a 60-mL sample chamber and provides a distribution of 32 size classes of particles ranging from 2 μm to 460 μm (Agrawal and Pottsmith 2000). Particle size of suspended sediment was examined using the LISST on 24 September 2009 and 14 July 2010. A particle size measurement with the LISST was taken on 14 July 2010 in the HFC to determine a background particle size distribution. Measurements taken on 14 July 2010 were conducted under similar channel conditions, and it is assumed that measurements taken on this day are representative of pre-spawn conditions in 2009. On both sample dates, the LISST was placed in the rear portion of the decay enclosure. The LISST was left in the channel for approximately 15 min and programmed to sample particles in 3-s laser bursts every 15 s.

Statistical analysis—Response variables were log- and square-root–transformed as required to meet the assumptions of parametric tests. Chl *a,* AF dry wt, intergravel inorganic sediment, and isotope ratios (δ^{13}C and δ^{15}N) were the response variables derived from analyzing biofilms. Null hypotheses were rejected at an α level of 0.05. All statistical analysis was conducted using R 2.11.1 (R Development Core Team 2010). All graphics were created using R 2.11.1 (R Development Core Team 2010) with the *ggplot2* (Wickham 2009) package. We acknowledge that this study is pseudoreplicated as we did not replicate our experimental unit, and, therefore, it has limited applicability to a broader scale. Replication at this scale was not possible due to practical constraints. We do feel, however, that the HFC is a representative inland salmon spawning bed, and thus conclusions derived here can reasonably be the basis for further experimentation in natural systems.

All biofilm response variables were analyzed with a two-way analysis of variance (ANOVA) using period and enclosure as fixed effects. Enclosures were considered to be adequately independent to merit an ANOVA approach, although we acknowledge that some temporal dependence may exist (Cak et al. 2008). The minimum adequate model for each parameter was determined by comparing the *F*-ratio of a full and reduced ANOVA model (Whittingham et al. 2006). Averaged data points from each sampling day served as replicates within each experimental period. A significant period $\times$ enclosure interaction indicated that a particular enclosure demonstrated a different temporal trend as the salmon run progressed. Linear contrasts of means were used to test specific hypotheses if a significant interaction was determined (sensu Mills and Bever 1998). Each contrast was compared both to its temporal and spatial controls. Contrasts were chosen to explore the simultaneous effect of disturbance and fertilization (i.e., the salmon disturbance regime) on biofilm abundance. To confirm that similar starting conditions existed, the contrasts tested for differences in the means in each enclosure at the outset of the experiment. Contrasts were coded according to the conditions for linear contrasts set out by Fox (1997).

Models without a significant interaction term had to be interpreted solely on their main effects. In this case, an effect of salmon was still inferred. Interpreting these main effects is more difficult, as the hypothesis tested only allows for the comparison of the marginal means. In the absence of a significant interaction term, pairwise multiple *t*-tests with Holm's *p*-value correction were used to compare mean differences for the main effects (Fox 1997) to avoid overly conservative estimates (Quinn and Keough 2002).

Intergravel inorganic sediment—All bivariate relationships were analyzed using Pearson's product moment correlation (Quinn and Keough 2002). The relationship between AF dry wt and Chl *a* of biofilms was analyzed using individual stone values. The relationship between inorganic sediment and Chl *a* was limited to post-spawn biofilms in the deposition enclosure. The correlation between intergravel inorganic sediment and surface Chl *a* from biofilms was a comparison between the weekly values of both parameters from all enclosures.

Particle size comparisons—LISST data were processed using a semiautomated macro with MS Excel (Microsoft) that calculated cumulative distributions, measures of central tendency as well as diagnostic parameters. These measures were averaged for the entire 15-min sampling period and used as the response variable. A Kolmogorov-Smirnov (K-S) test was used to compare mean background and active-spawn distributions (Siegel 1957), and cumulative distribution plots were used to examine grain coarsening patterns.

Results

Salmon—Live salmon densities reflected natural spawning conditions and historical usage of the HFC. Salmon numbers peaked on 12 September 2009 in both the decay and deposition enclosures. Peak die-off in the decay enclosure occurred on 7 October 2009, and this date was defined as the beginning of the post-spawn period (Fig. 2). Over the course of the experiment, some salmon were removed from the HFC either via dead pitching or black bear (*Ursus americanus*) consumption. Dead-pitching was done to maintain a natural density of a spawning stream. Stable isotope values for salmon carcass tissue sampled from the HFC were within the range of other reported values for sockeye and were comparable to studies conducted at similar latitudes and distances from the ocean (Johnston et al. 1997; McConnachie and Petticrew 2006).

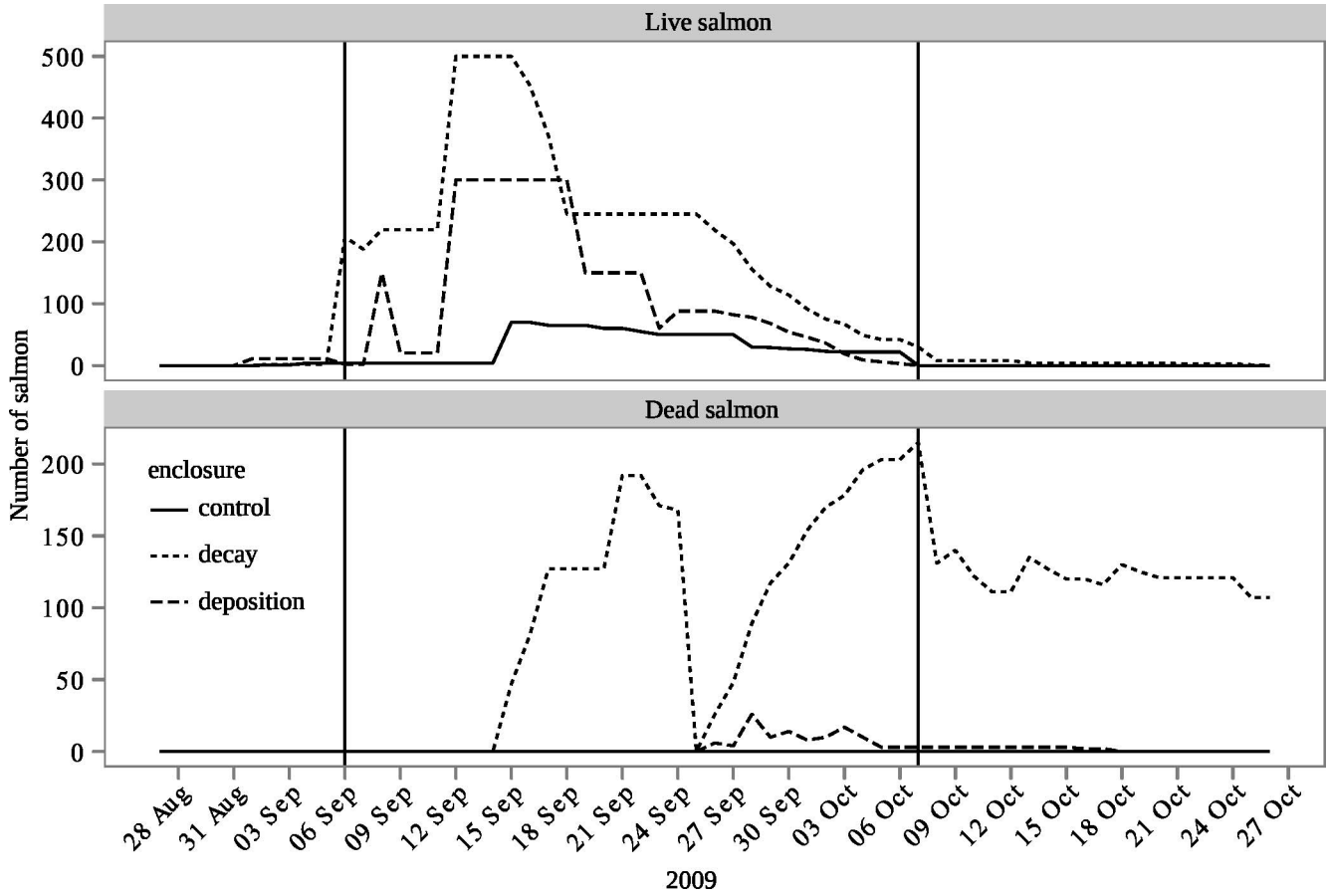

Fig. 2. Live and dead salmon counts in the HFC by experimental enclosure. Vertical solid lines indicate divisions of the experimental period.

Biofilm growth and trapping patterns—The enclosure × period interaction was significant for surface Chl *a*, AF dry wt, and inorganic sediment (Table 1). Mean AF dry wt values demonstrated a similar pattern as the Chl *a* values. During the active-spawn period, biofilms sampled from the decay enclosure were significantly reduced in Chl *a* (4.2×) and AF dry wt (3.2×) from the upstream control for the same time period (Fig. 3). Biofilms sampled from the deposition enclosure during the post-spawn period were higher in Chl *a* (2.7×) and AF dry wt (3.0×) than during the active-spawn period in the same enclosure. Post-spawn Chl *a* in the decay enclosure was not significantly different from that in the active-spawn period in the decay enclosure and the upstream control enclosure for the post-spawn period.

Inorganic sediment found in the biofilm samples followed a slightly different pattern than the two parameters described above. Like Chl *a* and AF dry wt, inorganic sediment trapped by biofilms in the decay enclosure during active spawning was reduced compared to the pre-spawn values in the same enclosure (3.0×) and the upstream

Table 1. Results from a two-way ANOVA of spatial (enclosure) and temporal (period) salmon treatments on Chl *a*, AF dry wt, or inorganic sediment. Interaction contrasts are separated by a vertical line (|). Contrasts are labeled by corresponding enclosure and the spawning period (C, control; Dcy, decay; Dep, deposition). Sum sq. = sum of squares.

Source of variation	df	Chl *a*		AF dry wt		Inorganic sediment	
		Sum sq.	p ($>F$)	Sum sq.	p ($>F$)	Sum sq.	p ($>F$)
Enclosure	2	2.288	0.009	1.779	0.008	0.338	<0.000
Period	2	3.309	0.002	4.060	0.000	0.866	<0.000
Enclosure×period	4	3.297	0.011	2.009	0.024	0.281	0.001
Dcy:active|C:active and Dcy:pre	1	2.424	0.002	1.337	0.006	0.212	<0.000
Dep:post|Dep:active and C:post	1	0.868	0.044	0.663	0.042	0.001	0.819
Dcy:post|Dcy:active and C:post	1	0.000	0.965	0.008	0.812	0.057	0.029
Starting conditions	1	0.005	0.878	0.002	0.916	0.011	0.300
Residuals	18	3.342		2.483		0.181	

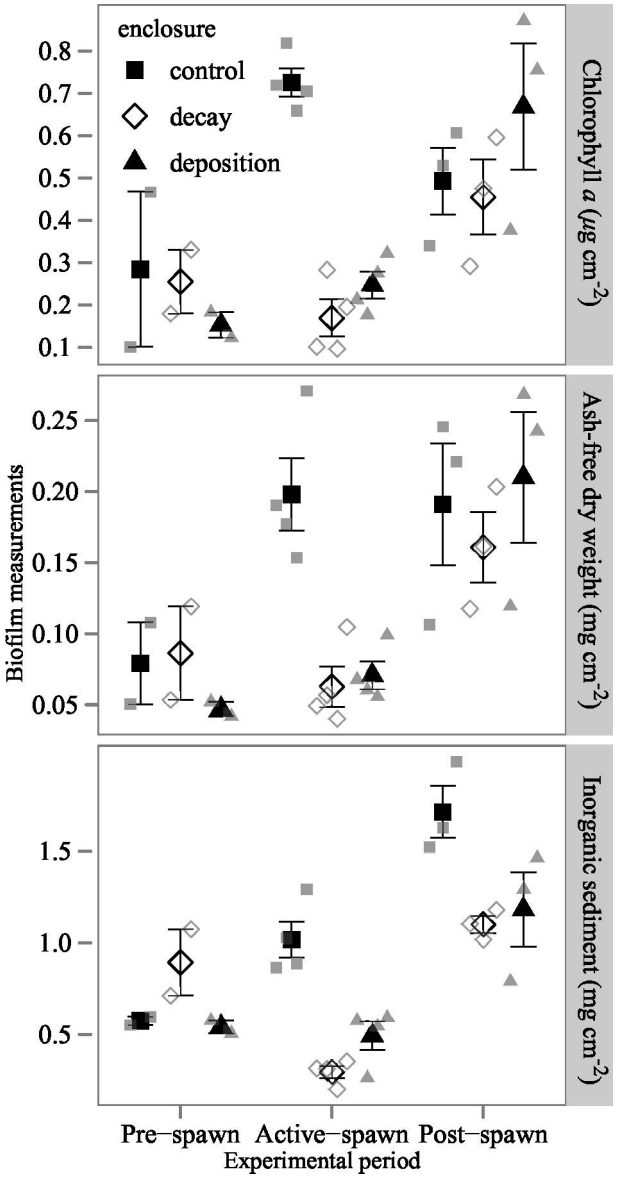

Fig. 3. Channel bed surface biofilm parameters tested in the HFC over the course of the salmon disturbance regime. Large black data symbols are mean values with error bars representing ± 1 standard error of the mean (SEM). Small gray symbols indicate raw data points. Data point shape refers to experimental enclosure.

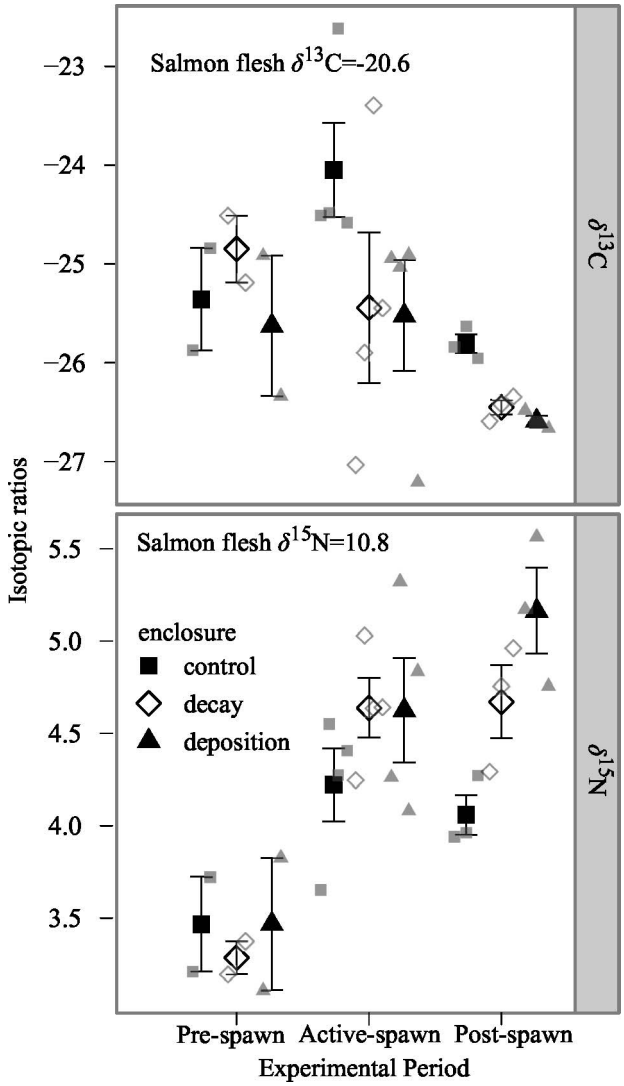

Fig. 4. Isotopic ratio of benthic biofilms for both carbon and nitrogen. Ratios moving toward salmon flesh values indicate a marine nutrient source, although $\delta^{13}C$ values can be confounded by photosynthetic processes (Staal et al. 2007). Isotopic ratios were calculated as per Eq. 1. Salmon flesh values were sampled from fresh salmon carcasses ($n = 4$). Large black data symbols are mean values ± 1 SEM. The small gray symbols are the raw data points.

enclosure during the same period (3.4×; Fig. 3). In contrast to measurements on the other two biofilm parameters, the difference in inorganic sediment was not significant in the deposition enclosure in the post-spawn period but was significant in the decay enclosure during the post-spawn period. Post-spawn inorganic sediment in the deposition enclosure was higher when compared to the active-spawn period in the decay enclosure (3.7×) but lower when compared to the upstream control during the same period (1.6×). Both values were contrasted in the same manner as above (Table 1).

Across both carbon and nitrogen isotope parameters tested, there was a significant effect of sample period ($\delta^{15}N$: $F_{2,\ 22} = 21.98$, $p = 0.001$; $\delta^{13}C$: $F_{2,\ 22} = 5.39$, $p = 0.012$; Fig. 4). Levels of $\delta^{15}N$ were significantly higher during the active-spawn ($p < 0.000$) and post-spawn ($p < 0.000$) periods than the pre-spawn temporal control. Levels of $\delta^{13}C$ were significantly greater during the active-spawn period than the post-spawn period ($p = 0.019$). Additionally, enclosure was a significant factor for $\delta^{15}N$ values ($F_{2,\ 22} = 3.69$, $p = 0.041$). Post hoc comparisons, however, revealed no significant differences in the mean amount of $\delta^{15}N$ across enclosures.

Suspended sediment size—The maximum vertical deviation (D_n) between mean particle size distributions measured on 14 July 2010 and 24 September 2009 was significant (K-

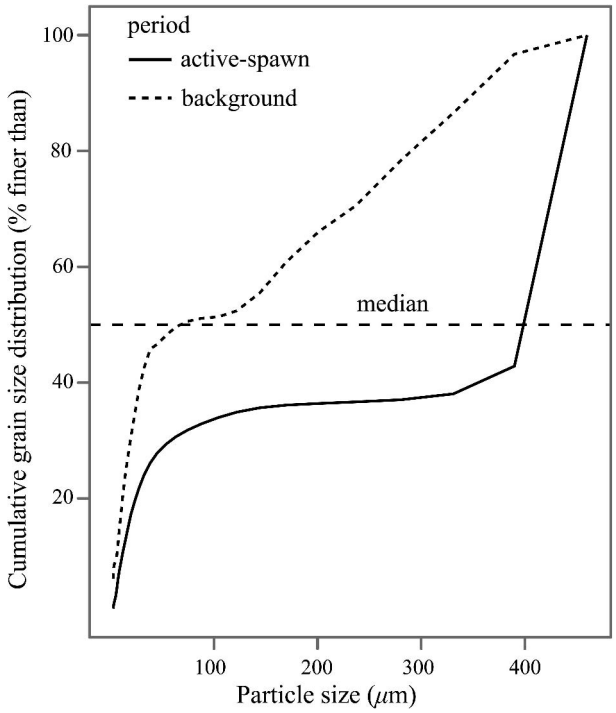

Fig. 5. Particle size distributions of suspended sediment in the HFC. Background suspended sediment particle sizes were sampled on 14 July 2010, while the active-spawn particle size was taken during the HFC study on 24 September 2009. Dashed horizontal line intersects the median of both distributions.

S test: $D_2 = 5312$, $p < 0.000$). The background proxy sample exhibited a greater proportion of smaller particles in the system. In contrast, the particle size characterization taken during the active-spawn period exhibited a greater proportion of larger particles (Fig. 5). A similar measurement taken in 2007 supports similar background conditions in the HFC and indicates that the 2010 measurement is typical of regular channel conditions (Hulsman and Wubben 2008).

Correlations—Surface biofilm Chl *a* in the deposition enclosure was significantly and highly correlated to deposition enclosure inorganic sediment trapped by surface biofilms (p-value < 0.000, $r = 0.815$; Fig. 6a) and AF dry wt (p-value < 0.000, $r = 0.926$; Fig. 6b). Intergravel inorganic sediment (the mass of inorganic material collected from the infiltration bags) and Chl *a* were significantly negatively correlated (p-value < 0.006, $r = -0.509$; Fig. 6c).

Discussion

As stated above, this experiment was pseudoreplicated, and all results should be interpreted with this caveat. The HFC is an artificial channel that provides a realistic natural habitat simulation with immense potential for experimental manipulation. The HFC mimics a natural stream in hydrologic conditions and habitat characteristics while allowing control of salmon densities. Due to the temporal and spatial scales required to test the selected hypotheses, a pseudoreplicated experimental design was required. This approach has a more limited ability to distinguish between selected treatment factors and other unmeasured variables (Hurlbert 1984). These results do, however, provide a reliable method to test whether biofilm abundance changes as noted by Moore and Schindler (2008) are driven by the mechanism of flocs acting as temporary MDN storage sites and delivery vectors as observed by Rex and Petticrew (2008).

The results presented here correspond well with other studies that have reported decreases in biofilm abundance from active salmon spawning followed by a post-spawn increase (Moore and Schindler 2008). Factors that influence biofilm abundance include the supply of light and nutrients in addition to hydrologic and physical disturbances (Peterson 1996). All biofilms in each enclosure were subject to similar light conditions because of similar tree cover and the same experimental flow conditions. Thus, temporal and spatial ecosystem response patterns (measured as biofilm abundance) were primarily driven by the disturbance from salmon redd construction and nutrients from salmon carcass decay. The salmon disturbance regime was characterized by two main periods—active- and post-spawn. The decay enclosure, during active-spawning, experienced lower biofilm abundance and increased sediment infiltration into the bed. The post-spawn period in the deposition enclosure was characterized by higher biofilm abundance and lower sediment infiltration.

Channel bed benthic response—Initial low pre-spawn biofilm abundance can be attributed to the preparatory channel cleaning and suggests young immature biofilms. The upstream control reflects natural biofilm growth patterns in the absence of salmon. Biofilms growing in the upstream control were noticeably thicker and more uniform than biofilms in the downstream enclosures (S.J.A. pers. obs.). The standing stock of surface Chl *a* and AF dry wt was significantly reduced in the decay enclosure during the active-spawning period and can be attributed to the physical reworking of gravels by salmon (Fig. 3).

The relation of post-spawn downstream Chl *a* and AF dry wt increases to decaying salmon carcasses suggests an MDN influence on biofilm growth in the deposition enclosure (Figs. 2, 3) rather than simply natural biofilm succession and growth (Peterson 1996). While biofilms in the decay enclosure recovered from the salmon disturbance primarily via natural succession, biofilms in the deposition enclosure received an added nutrient pulse of upstream decaying salmon. Hunt and Perry (1999) found that a strong correlation between AF dry wt and Chl *a* levels suggests an in-stream nutrient source, providing further evidence for our study that decaying upstream salmon are the source of the biofilm abundance increase.

It should be noted that Chl *a* values are very similar to values reported in the literature. In particular, the timing and magnitude of Chl *a* increases and decreases reported by Moore and Schindler (2008) are very similar to Fig. 3. Post-spawn increases in Chl *a* are also very comparable to values reported by Cak et al. (2008). Both of these studies

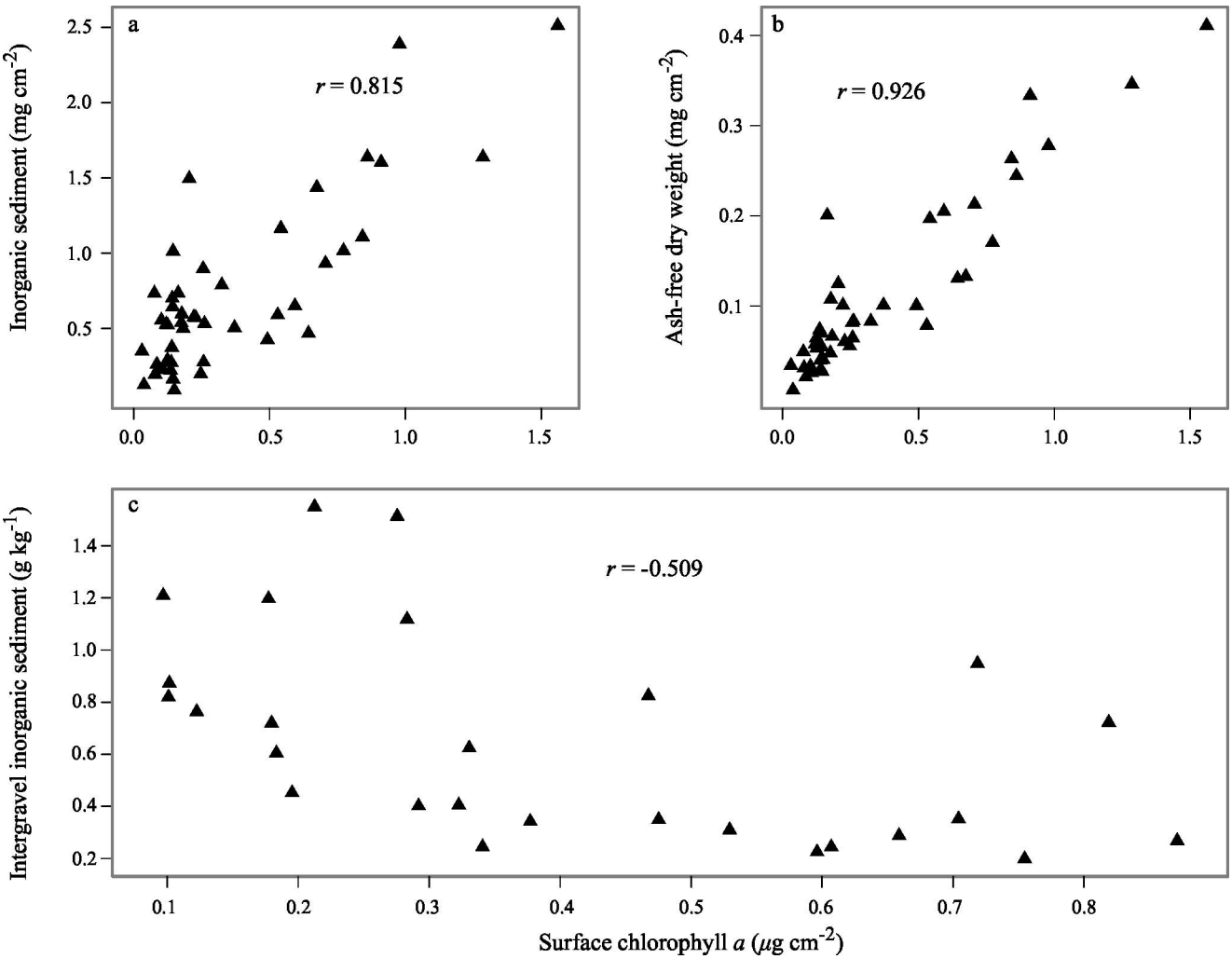

Fig. 6. Inorganic sediment and biofilms measurement comparisons. (a) Relationship between biofilm growth (Chl *a*) and inorganic sediment trapped by the biofilm from stones sampled in the deposition enclosure. (b) Bivariate relationship between two measures of biofilm growth. This suggests an in-stream nutrient source (Hunt and Perry 1999), which is likely salmon. (c) Chl *a* vs. intergravel inorganic sediment (collected from infiltration bags) that has deposited into the channel bed. Decreased surface biofilm abundance results in larger masses of fine sediment infiltrating into the channel bed (bag depth = 0.30 m). All p-values < 0.05.

were conducted in coastal environments, suggesting a common response across a range of habitats.

MDN utilization by biofilms—Decay and deposition enclosure biofilm δ^{15}N isotope ratios increased compared to the upstream control over the course of the salmon spawning event (Fig. 4). These results are consistent with other studies that have found increases in δ^{15}N values and indicate MDN sequestration by biofilms. The increase of δ^{15}N is statistically significant, indicating that the rapid growth of biofilm following the active-spawn decrease is primarily driven by marine-derived nitrogen. Lower salmon N values in inland systems may account for observed low biofilm δ^{15}N values (3.1–5.6‰), as reported values are generally higher (Bilby et al. 1996, δ^{15}N = 7.1‰).

The patterns of biofilm δ^{13}C isotopic enrichment are not typical of previous reports that used δ^{13}C as a tracer for MDNs (Kline et al. 1990; Bilby et al. 1996). The least negative (i.e., higher) δ^{13}C value in this study (−24.0‰) corresponded to the active-spawn period in the upstream enclosure, where there were few salmon present (Fig. 2). Interpretation of biofilm carbon isotope values is confounded by high variability in analytical results (France 1995) as well as by several processes that prevent establishing a clear relationship between MDNs and δ^{13}C ratios. First, variable discrimination of isotopes by biofilms at different stages of development tends to confound isotopic ratios (Peterson and Fry 1987; Kline et al. 1990). Older, thicker, more mature biofilms, for example, tend to have higher (less negative) δ^{13}C isotopic ratios because of well-developed internal carbon cycling processes (Staal et al. 2007), which was the case in the salmon-free upstream control. Second, typically in studies that use the prevalence of marine isotopes to infer a salmon nutrient source, the degree of δ^{13}C fractionation is usually assumed to be small. This assumption, however, may not be warranted, as some

fractionation usually occurs in the uptake of $\delta^{13}C$ by algal-dominated biofilms, resulting in elevated $\delta^{13}C$ biofilm values (Peterson and Fry 1987; Bilby et al. 1996). Lastly, the patterns of $\delta^{13}C$ may suggest alternative sources of carbon for biofilms. A decrease in $\delta^{13}C$ during the post-spawn period coupled with the rapid biofilm growth during the same period suggests that biofilms may have been using other sources of carbon. Nutrient leaching from upstream macrophytes may have contributed to an isotopic signature that did not directly represent salmon influence.

Infiltration and trapping—These results present a novel mechanism of MDN delivery to benthic biofilms. Sediment is often overlooked in discussions of salmon spawning ecology. This omission is usually attributed to the fact that the relevant studies use a fisheries rather than a geomorphological approach (Kondolf 2000; Corenblit et al. 2011; but *see* McConnachie and Petticrew 2006; Moore et al. 2007). The results of this experiment indicate that sediment plays a crucial role in two ecological processes outlined below, highlighting the importance of abiotic factors within the salmon disturbance regime. Furthermore, the inorganic sediment component of biofilm analysis is rarely reported alongside measures like Chl *a* and AF dry wt. These findings highlight important insights that can be gained from using this information.

Redd construction resuspends a broad range of particle sizes of sediment into the water column (Rex and Petticrew 2008). Coarsening of this suspended sediment suggests the presence of either flocculated particles or aggregates in the water column (Fig. 5). Increases in downstream surface inorganic sediment are closely related to increases in biofilm abundance (Fig. 6a), which suggests that the two processes are operating in concert.

First, it seems clear from the data presented here that flocs are forming in the decay enclosure and settling over a small spatial scale in the deposition enclosure (Fig. 5). Second, increased biofilm growth may be facilitating particle trapping via extracellular polymeric substances and fine sediment trapping interactions (Romani and Sabater 2000). The biofilm abundance increase is likely being driven by MDNs (Fig. 4), which suggests a positive feedback loop whereby biofilm mass increase allows for greater subsequent nutrient enrichment. Rather than shading biofilms, flocs and particle aggregates appear to act as MDN storage sites and a mechanism of inorganic sediment and salmon nutrient delivery. Biofilm abundance increases and $\delta^{15}N$ values suggest a rapid downstream ecosystem response. Lastly, this floc settling mechanism is also supported by an increase in the size of suspended particles in the water column during active-spawn (Fig. 5). Increased settling rates of MDN particles generated in the decay enclosure as a result of flocculation would explain the near-field (< 20 m) biofilm response (Droppo et al. 1997).

This work identifies a previously unreported effect of salmon redd construction. Rapid growth of biofilms after redd construction appears to aid gravel-bed surface sequestration of MDNs, suggesting an interaction between biogeomorphic disturbance and ecosystem response. A significant negative relationship between intergravel inorganic sediment and biofilm growth indicates that greater biofilm abundance in the post-spawn period decreases infiltration of sediment into the channel bed (Fig. 6c). Alternately, low biofilm abundance from redd construction (Fig. 3) is accompanied by higher sediment infiltration (Fig. 6c). This suggests intergravel storage of MDNs during the active-spawn periods when biofilm abundance is low and channel bed surface storage when biofilm abundance is high. A growing surface biofilm layer may facilitate rapid MDN uptake as photosynthetic activity is diminished deeper in the gravel bed (Gibert and Deharveng 2002). This idea corresponds well with a subsurface increase in organic matter film seen by Petticrew and Arocena (2003) in response to MDNs and suggests that bacteria are processing sediment stored within gravels during active-spawn (Petticrew and Albers 2010). These results reinforce the role that salmon play as self-regulators of their habitats, as the temporal and spatial conditions of spawning and die-off can dictate the location and degree to which MDNs are incorporated back into their natal ecosystem.

Implications—The identified pattern of ecosystem response, in response to salmon spawning and die-off, is indicative of the high benthic resiliency to the salmon disturbance regime as biofilm abundance quickly rebounded from low active-spawn levels. Several recent studies have highlighted the localized negative effects of redd construction on aquatic habitats (McConnachie and Petticrew 2006; Moore and Schindler 2008), but this ignores the potential for the transfer of nutrients from upstream spawning sites, where localized negative effects occur, to the benefit of downstream ecosystems. The results presented here indicate that this downstream effect can be seen over short distances ($\sim$ 20 m). Moreover, our results identify a potential delivery vector for downstream benthic MDN delivery in the form of flocs. Flocs are known to form (Rex and Petticrew 2008) and occur (McConnachie and Petticrew 2006) in salmon-bearing streams. This study, however, represents the first known attempt to link the growth patterns of biofilms to the formation of flocs in the water column and the consequent trapping of sediment and nutrients. The patterns of sediment trapping presented here highlight the importance of this link and identify the trapping ability of biofilms, suggesting that biofilms capture and utilize flocs as an MDN source. As a basal portion of benthic food webs, biofilms often structure benthic resilience and resistance, ultimately determining overall system stability (DeAngelis et al. 1990). The interaction between MDNs, sediment, flocs, and biofilms are presented here, highlighting the importance of intergravel and gravel-bed MDN storage patterns to the ecology of salmon bearing streams.

These patterns of nutrient incorporation suggest that salmon indirectly, through processes discussed above, divert nutrient flow towards near-field benthic habitats. This diversion acts as a stabilizing force (Viles et al. 2008), provided the nutrients are utilized in the ecosystem. Floc formation acts as the mechanism for this diversion, and increased biofilm growth indicates an ecosystem response.

Viewed from a biogeomorphic perspective as an organismal-driven physical process (Corenblit et al. 2011), these results indicate that salmon, via the salmon disturbance regime, aid in creating and maintaining suitable habitat for subsequent generations.

Acknowledgments

Thanks to Rob Little for his enormous efforts in the field. Thanks to Fisheries and Oceans Canada staff Roy Argue, Doug Turvey, and Jason Hwang for support with the Horsefly spawning channel. Thanks to the community of Horsefly for a wide range of support and interest. Thanks to Rick Holmes and Bill Best of the Quesnel River Research Centre (QRRC). This is contribution number 12 in the QRRC publication series. This research was supported by a Natural Science and Engineering Research Council Discovery grant and Forest Renewal British Columbia Landscape Ecology funding to E.L.P. and the Peace-Williston Aquatic Research Award to S.J.A. We also thank two anonymous reviewers whose feedback and suggestions improved the scope and clarity of the manuscript.

References

Agrawal, Y. C., and H. C. Pottsmith. 2000. Instruments for particle size and settling velocity observations in sediment transport. Mar. Geol. **168:** 89–114, doi:10.1016/S0025-3227(00)00044-X

Albers, S. J. 2010. The salmon disturbance regime: Effects on biofilm, sediment and water. M.Sc. thesis. Univ. of Northern British Columbia.

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1995. Standard methods for the examination of water and wastewater, 18th ed. American Public Health Association and American Water Works Association and Water Pollution Control Federation, Washington, D.C.

Bilby, R. E., B. R. Fransen, and P. A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: Evidence from stable isotopes. Can. J. Fish. Aquat. Sci. **53:** 164–173, doi:10.1139/f95-159

Cak, A. D., D. T. Chaloner, and G. A. Lamberti. 2008. Effects of spawning salmon on dissolved nutrients and epilithon in coupled stream-estuary systems of southeastern Alaska. Aquat. Sci. **70:** 169–178, doi:10.1007/s00027-008-8090-5

Corenblit, D., and others. 2011. Feedbacks between geomorphology and biota controlling Earth surface processes and landforms: A review of foundation concepts and current understandings. Earth-Sci. Rev. **106:** 307–331, doi:10.1016/j.earscirev.2011.03.002

DeAngelis, D. L., P. J. Mulholland, J. W. Elwood, A. V. Palumbo, and A. D. Steinman. 1990. Biogeochemical cycling constraints on stream ecosystem recovery. Environ. Manage. **14:** 685–697, doi:10.1007/BF02394718

Droppo, I. G., G. G. Leppard, D. T. Flannigan, and S. N. Liss. 1997. The freshwater floc: A functional relationship of water and organic and inorganic floc constituents affecting suspended sediment properties. Water Air Soil Pollut. **99:** 43–53, doi:10.1007/BF02406843

Fox, J. 1997. Applied regression analysis, linear models, and related methods. Sage Publications.

France, R. 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol. Oceanogr. **40:** 1310–1313, doi:10.4319/lo.1995.40.7.1310

Gibert, J., and L. Deharveng. 2002. Subterranean ecosystems: A truncated functional biodiversity. Bioscience **52:** 473–481, doi:10.1641/0006-3568(2002)052[0473:SEATFB]2.0.CO;2

Graham, A. A., D. J. McCaughan, and F. S. McKee. 1988. Measurement of surface area of stones. Hydrobiologia **157:** 85–87, doi:10.1007/BF00008813

Hulsman, H., and L. Wubben. 2008. Salmon spawning and sediment dynamics in the Horsefly River in British Columbia. M.Sc. thesis. Universiteit Utrecht, Utrecht, Netherlands.

Hunt, A. P., and J. D. Perry. 1999. Algal-bacterial relationships in streams with varying doc concentration and source, p. 34–42. *In* C. W. Keevil, A. Godfree, D. Holt, and C. Dow [eds.], Biofilms in the aquatic environment. The Royal Society of Chemistry.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. **54:** 197–211, doi:10.2307/1942661

Hyatt, K., D. McQueen, K. Shortreed, and D. Rankin. 2004. Sockeye salmon (*Oncorhynchus nerka*) nursery lake fertilization: Review and summary of results. Environ. Rev. **12:** 133–162, doi:10.1139/a04-008

Johnston, N., J. MacDonald, K. Hall, and P. J. Tschaplinski. 1997. A preliminary study of the role of sockeye salmon (*Oncorhynchus nerka*) carcasses as carbon and nitrogen sources for benthic insects and fishes in the "Early Stuart" stock spawning streams, 1050 km from the ocean. Fisheries Project Report No. RD55, British Columbia Ministry of Environment, Lands and Parks, Victoria, British Columbia.

Kline, T. C., J. J. Goering, O. A. Mathisen, P. H. Poe, and P. L. Parker. 1990. Recycling of elements transported upstream by runs of Pacific salmon: I. δ^{15}N and δ^{13}C evidence in Sashin Creek, southeastern Alaska. Can. J. Fish. Aquat. Sci. **47:** 136–144, doi:10.1139/f90-014

Kondolf, G. M. 2000. Assessing salmonid spawning gravel quality. Trans. Am. Fish. Soc. **129:** 262–281, doi:10.1577/1548-8659(2000)129<0262:ASSGQ>2.0.CO;2

McConnachie, J. L., and E. L. Petticrew. 2006. Tracing organic matter sources in riverine suspended sediment: Implications for fine sediment transfers. Geomorphology **79:** 13–26, doi:10.1016/j.geomorph.2005.09.011

Mills, K., and J. Bever. 1998. Maintenance of diversity within plant communities: Soil pathogens as agents of negative feedback. Ecology **79:** 1595–1601, doi:10.1890/0012-9658(1998)079[1595:MODWPC]2.0.CO;2

Moore, J. W., and D. E. Schindler. 2008. Biotic disturbance and benthic community dynamics in salmon-bearing streams. J. Anim. Ecol. **77:** 275–284, doi:10.1111/j.1365-2656.2007.01336.x

———, ———, J. L. Carter, J. Fox, J. Griffiths, and G. W. Holtgrieve. 2007. Biotic control of stream fluxes: Spawning salmon drive nutrient and matter export. Ecology **88:** 1278–1291, doi:10.1890/06-0782

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18:** 293–320, doi:10.1146/annurev.es.18.110187.001453

Peterson, C. G. 1996. Response of benthic algal communities to natural physical disturbance, p. 375–402. *In* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe [eds.], Algal ecology: Freshwater benthic ecosystems. Academic Press.

Petticrew, E. L., and S. J. Albers. 2010. Salmon as biogeomorphic agents: Temporal and spatial effects on sediment quantity and quality in a northern British Columbia spawning channel. Int. Assoc. Hydrol. Sci. Publ. **337:** 251–257.

———, and J. M. Arocena. 2003. Organic matter composition of gravel-stored sediments from salmon-bearing streams. Hydrobiologia **494:** 17–24, doi:10.1023/A:1025416904982

———, J. F. Rex, and S. J. Albers. 2011. Bidirectional delivery of organic matter between freshwater and marine systems: The role of flocculation in Pacific salmon streams. J. N. Am. Benthol. Soc. **30:** 779–786, doi:10.1899/10-133.1

Quinn, G., and M. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge Univ. Press.

R Development Core Team. 2010. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing [accessed 2011 October 11], Available from http://www.R-project.org

Rex, J. F., and E. L. Petticrew. 2006. Pacific salmon and sediment flocculation: Nutrient cycling and intergravel habitat quality. Int. Assoc. Hydrol. Sci. Publ. **306:** 1–8.

———, and ———. 2008. Delivery of marine-derived nutrients to streambeds by Pacific salmon. Nat. Geosci. **1:** 840–843, doi:10.1038/ngeo364

Romani, A., and S. Sabater. 2000. Influence of algal biomass on extracellular enzyme activity in river biofilms. Microb. Ecol. **40:** 16–24. doi:10.1007/s002480000041

Siegel, S. 1957. Nonparametric statistics. Am. Stat. **11:** 13–19, doi:10.2307/2685679

Staal, M., R. Thar, M. Kühl, M. C. M. Van Loosdrecht, G. Wolf, J. De Brouwer, and J. Rijstenbil. 2007. Different carbon isotope fractionation patterns during the development of phototrophic freshwater and marine biofilms. Biogeoscience **4:** 613–626, doi:10.5194/bg-4-613-2007

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. Cushing. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. **37:** 130–137, doi:10.1139/f80-017

Viles, H. A., L. A. Taylor, N. E. A. Carter, and D. Chaput. 2008. Biogeomorphological disturbance regimes: Progress in linking ecological and geomorphological systems. Earth Surf. Proc. Land. **33:** 1419–1435, doi:10.1002/esp.1717

Whittingham, M. J., P. A. Stephens, R. B. Bradbury, and R. P. Freckleton. 2006. Why do we still use stepwise modelling in ecology and behaviour? J. Anim. Ecol. **75:** 1182–1189, doi:10.1111/j.1365-2656.2006.01141.x

Wickham, H. 2009. ggplot2: Elegant graphics for data analysis. Springer [accessed 2011 October 11]. Available from: http://had.co.nz/ggplot2/book

Wipfli, M. S., and C. Baxter. 2010. Linking ecosystems, food webs, and fish production: Subsidies in salmonid watersheds. Fisheries **35:** 373–387, doi:10.1577/1548-8446-35.8.373

———, J. Hudson, and J. Caouette. 1998. Influence of salmon carcasses on stream productivity: Response of biofilm and benthic macroinvertebrates in southeastern Alaska, USA. Can. J. Fish. Aquat. Sci. **55:** 1503–1511, doi:10.1139/f98-031

Wotton, R. 2007. Do benthic biologists pay enough attention to aggregates formed in the water column of streams and rivers? J. N. Am. Benthol. Soc. **26:** 1–11, doi:10.1899/0887-3593(2007)26[1:DBBPEA]2.0.CO;2

Associate editor: Alexander D. Huryn

Received: 08 March 2011
Accepted: 30 August 2011
Amended: 01 September 2011