Delivery of marine-derived nutrients to streambeds by Pacific salmon

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Marine fish that migrate to freshwater rivers to spawn deliver substantial quantities of marine-derived nutrients to terrestrial and freshwater environments[1,2]. These nutrients support riparian vegetation[3], terrestrial organisms[4], benthic macroinvertebrates[5], algae[6] and other fish populations[1]. Although it is known that the quantity of nutrients delivered to these environments is influenced by the number of spawning salmon[7], little is known about the mechanisms of nutrient delivery. Here, we present a pathway for nutrient delivery and retention in a Pacific salmon stream, which depends on the aggregation of inorganic and organic particulate matter[8]. We verify the existence of this pathway in the controlled environment conditions of a recirculating flume, replicating the hydrologic conditions of a stream environment. We show that the addition of salmon organic matter and clay to the flume increases the formation of organic–inorganic aggregates in the water column, and the transfer of these aggregates to the stream bed. We find that the formation of these aggregates is associated with an increase in the concentration of bacteria in the stream bed. We suggest that bacterial aggregation of salmon organic matter and inorganic particulate matter delivers nutrients to streambeds, and thus plays an integral role in nutrient cycling in Pacific salmon streams.

Pacific salmon exert considerable control on the environmental conditions of their natal streams. Spawning salmon can create as much bedload movement during redd construction as occurs during spring floods[9]. Redd creation modifies the streambed by increasing its roughness and decreasing the clay and silt content[10]. This study extends those findings by identifying how the resuspension of fine sediment ($<63\,\mu$m) during redd creation enhances nutrient binding and retention in spawning streams.

Spawning activities provide optimal conditions for flocculation because the fine sediment resuspended during redd creation is available for aggregation with organic matter released by spawning and dead salmon[11]. Organic material released from salmon supports surface-living bacteria, which have extracellular polymeric substances (EPS) capable of generating flocs by attaching salmon organic matter particles to silt and clay resuspended during redd excavation. EPS are recognized as a prominent component and a factor in formation of freshwater benthic biofilms and non-salmon organic-matter flocs[12–14].

Flocs are known to comprise a significant portion of the suspended sediment load and to vary in their composition as both inorganic and organic sources and hydrodynamic conditions change over the year[8]. Salmon-derived flocs play a role in nutrient cycling because they show a distinctly different structure and composition than their component parts. That is, the floc has a different particle size, shape and density from either the salmon organic matter or silt and clay alone. These structural modifications can enhance sedimentation onto, and infiltration into, the streambed, and consequently delivery of the attached organic material including marine-derived nutrients to benthic food webs.

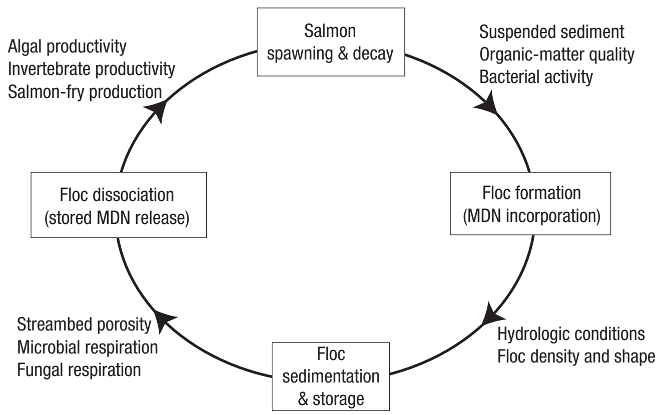

Figure 1 The salmon–floc feedback loop. The model presented includes factors that may regulate each stage (boxes) of the loop during the delivery and cycling of marine-derived nutrients (MDN) in natal Pacific salmon streams.

To test the occurrence of this feedback loop in Pacific salmon streams, we traced salmon organic matter and marine-derived nutrients through the series of stages the feedback loop requires, namely floc formation, floc sedimentation and storage, and floc dissociation (Fig. 1). These stages were generated within the controlled environment of a recirculating flume (see the Methods section) using field-verified ranges of water velocities and depths, salmon tissue areal density and total suspended solid concentrations from the O'Ne-eil Creek watershed, a productive Pacific salmon watershed within British Columbia's central interior[15]. Water-column and gravel-bed samples were drawn from the flume during consecutive baseline, clay, salmon, and salmon-plus-clay exposures, to identify flocculation processes during these different treatments.

Similar to field observations in O'Ne-eil Creek, suspended-sediment particle-size distributions in the flume were significantly different between treatments (F-test statistic $= 20.86$, d.f. $= 3, 124$, $p < 0.01$). Some inorganic particulate aggregates were observed in the water column during the baseline and clay exposure periods, as identified by the proportion of suspended material

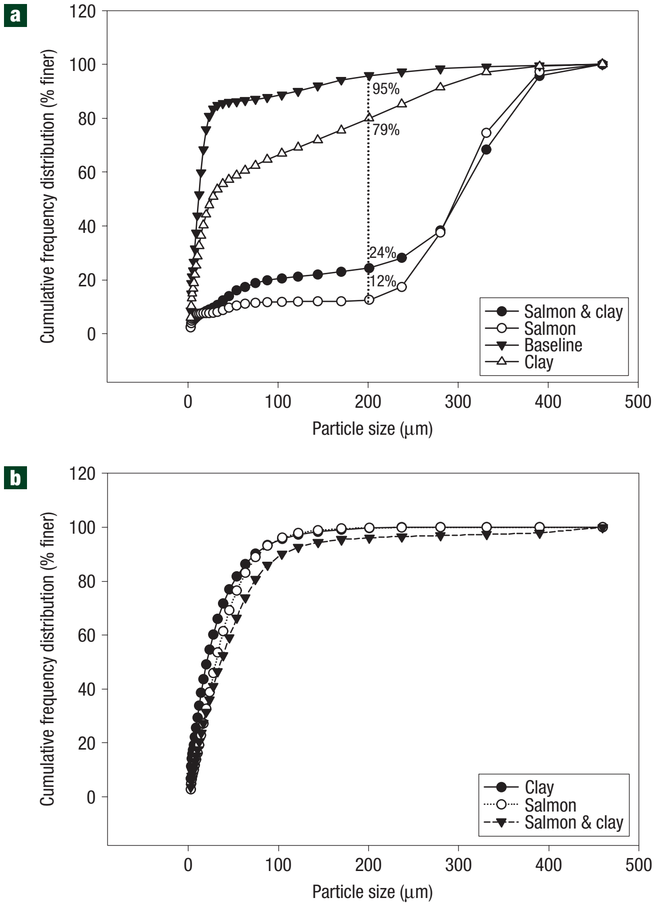

Figure 2 Cumulative grain-size distributions for (a) *in situ* suspended sediment and (b) gravel-stored sediments as determined by a 12 h settling study. *In situ* suspended sediments forming owing to flocculation in the water column are to the right of the dotted line in **a** because stock solutions were filtered with a 200 μm screen. The salmon and salmon-plus-clay treatments show significantly larger flocculated particles than observed during the baseline and clay-only treatments.

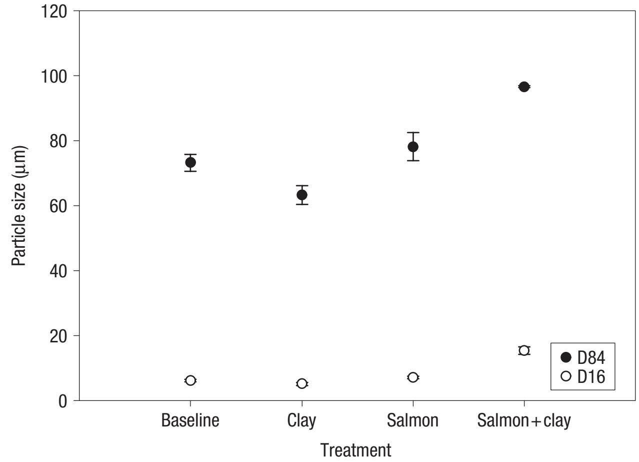

Figure 3 Mean effective particle size for the d_{84} and d_{16} of gravel-stored fine sediment following each treatment. The mean of three samples per treatment, with the error bars representing one standard error of the treatment mean. Both tails of the distribution (that is, d_{16} and d_{84}) show a significant increase in particle size following the addition of salmon plus clay.

between 63 and 200 μm (approximately 5 and 21% respectively) (Fig. 2a). More importantly, following the introduction of salmon organic matter there was a significant increase in the proportion of particles generated that were larger than 200 μm. The proportion of particles smaller than 200 μm changed from 95% to 79% during the baseline and clay exposures, through to 12 and 24% in the salmon and salmon-plus-clay exposures, indicating the formation of large (>200 μm) particles when salmon organic matter was added. This agrees with field observations in O'Ne-eil Creek, which identified that the largest suspended particles occurred when both dead salmon and actively spawning salmon were present[15–17]. The recirculating-channel simulation confirms that stage one of the feedback loop, floc formation, occurs in the water column in the presence of salmon organic matter plus clay.

Once formed in the water column, these large salmon-organic-matter-based flocs infiltrated into the flume's gravel-bed matrix. Settling studies of gravel-stored sediment collected from the flume's gravel-bed show that approximately 20% of the fine sediment in the salmon-plus-clay bag samples was 75 μm and larger, compared with approximately 11 and 10% in the salmon and clay bag samples respectively (Fig. 2b). These results correspond to field observations from O'Ne-eil Creek[17], where gravel-stored sediments were also found to be smaller than suspended flocs, presumably owing to the breakage or compaction of suspended flocs as they infiltrate into the smaller interstitial gravel-bed spaces, where ambient hydrologic pressure is higher. Further, although based on a single sample for each treatment, there was a trend of increasing settling rate of captured sediment following the addition of salmon organic matter plus clay. Settling-velocity analysis of these captured sediments identified that particles between 49 and 96 μm (which incorporates the 75 μm particle size above) settled fastest following the salmon-plus-clay exposure (approximately $0.82\,\mathrm{cm\,s^{-1}}$ for salmon plus clay and $<0.3\,\mathrm{cm\,s^{-1}}$ for all other samples).

The effective particle-size distribution (EPSD) of gravel-stored fine sediment, which includes both the inorganic and organic particulate components, significantly increased following the addition of salmon plus clay for both tails of the grain-size distribution, d_{84} and d_{16} ($F = 23.48$, d.f. $= 3, 8$, $p < 0.01$; $F = 44.80$, d.f. $= 3, 8$, $p < 0.01$, Fig. 3). In contrast, there was no corresponding increase in the absolute particle-size distribution (APSD), which measures the dispersed sediment particle size. This indicates that the large suspended sediment flocs observed in the water column (Fig. 2a) during the salmon-plus-clay treatment were incorporated into the streambed rather than there being an increase in the size of inorganic particulate matter sampled. This supports the assertion that salmon organic matter facilitates flocculation and floc delivery during the spawning period. Further, once formed, these large salmon-organic-matter flocs are intercepted by the streambed, and despite forces that break or compact them these organic-material-based flocs remain larger than their inorganic aggregate counterparts. This corroborates electron microscopy observations of gravel-stored fine-sediment samples following the salmon spawning period in O'Ne-eil Creek, which found that postspawning flocs had an organic film-like covering that contributes to floc strength[16]. The strength and resistance to disaggregation afforded by the film enhances its retention within the streambed and its subsequent entry into benthic and microbial food webs. These experimental data confirm stage two of the proposed loop, namely that flocs forming in the water column are stored on or in the streambed.

Bacteria attached to salmon organic matter aid in floc formation via EPS that adhere the salmon organic matter and clay components similar to EPS processes observed in the formation of non-salmon-organic-matter-based flocs[13,14]. These floc-residing bacteria may increase benthic bacterial numbers and are at least partially responsible for salmon-organic-matter mineralization. The flume study showed that there were significant differences in the concentration of gravel-bed bacteria between treatments, with the lowest being observed during the baseline, clay and salmon exposures and the highest concentrations being observed during the salmon-plus-clay exposure ($F = 67.71$, d.f. $= 3, 8$, $p < 0.01$). Attached bacterial numbers exceed the free-floating, unattached bacterial counts because they reside on the flocs (Fig. 4), which settle faster and/or remain trapped in the gravels. The role of attached bacteria in forming flocs with inorganic particulate matter is supported by the absence of a significant increase in gravel-bed bacterial concentration following the addition of salmon organic matter alone. Further, large flocs observed in the water column during the salmon exposure were less dense than the salmon-plus-clay treatment owing to their lower inorganic content, and did not enter the gravel-bed as readily (for example Fig. 2b). These lower levels of gravel-stored 'salmon-only' particles are associated with significantly lower concentrations of benthic bacteria, which increase dramatically following the salmon-plus-clay treatment, concurrent with the significant increase in EPSD (Fig. 3). Stage two, floc sedimentation, is shown here to be mediated by the presence of salmon organic matter and its associated bacterial populations.

The addition of salmon-organic-matter-based flocs to the flume's gravel-bed resulted in the delivery and storage of marine-derived nutrients to the gravel-bed. Nutrient enrichment of the gravel-bed and floc dissociation was found to occur in the presence of salmon plus clay. Specifically, there was a significant decrease in the carbon-to-nitrogen ratio of captured gravel-bed sediment from approximately 15:1 to 10:1 ($F = 30.87$, d.f. $= 3, 4$, $p < 0.01$) following the addition of salmon plus clay. These values align with field information from O'Ne-eil Creek, which identified a decrease in the carbon-to-nitrogen ratio in suspended particulate matter from more than 15:1 to approximately 11:1 at the same time that the salmon nitrogen ($\delta^{15}N$) signal peaked[15]. Similarly, in the recirculating flume, the delivery of salmon organic matter to the streambed is identified by nitrogen enrichment, and it confirms that flocs generated from salmon organic matter observed in the water column are delivered and retained within the gravel-bed of the recirculating flume. Stage three, MDN enrichment and release, can be identified by the shift in the carbon-to-nitrogen ratio of gravel-bed sediment samples.

The biophysical mechanisms of nutrient and organic-matter cycling through marine and freshwater environments are necessary prerequisites to understanding the ecology and ensuring the sustainability of the resources they provide. Similar to ref. 18, this work highlights the importance of microbial communities in local energy and nutrient utilization processes, but here we identify the important role they play in delivering and retaining marine-derived materials to freshwater streambeds through the process of flocculation. This investigation has demonstrated that salmon-organic-matter-based flocs formed in the water column and settled onto the streambed in the presence of clay and attached bacteria. Once captured in streambed interstices, these nutrients and organic matter stimulate microbial community development and enter the benthic food web. Our results indicate that the floc feedback loop proposed here is an ecologically important mechanism for delivering and retaining marine-derived nutrients for later cycling in natal Pacific salmon streams.

Fish-habitat-restoration activities that singularly focus on physical habitat modification or natal-stream fertilization may be unsuccessful unless recognition is given to the active role that returning salmon stock play in contemporaneously resuspending fine inorganic particulate matter and adding decay-related nutrients and organic material to the streambeds they modify during spawning[19]. The ecological response of streams to spawning salmon is variable[20]and the same can be expected with salmon carcass additions or their analogues. Salmon restoration will require a more complete understanding of the biophysical interactions between spawning Pacific salmon and their natal streams. It requires further investigation and quantification of marine-derived nutrient cycling pathways and residence time in natal streams. The floc feedback loop exemplifies this by uncovering a suite of interactions not previously acknowledged and it specifically highlights the important role bacterial EPS play in delivering nutrients to the streambed.

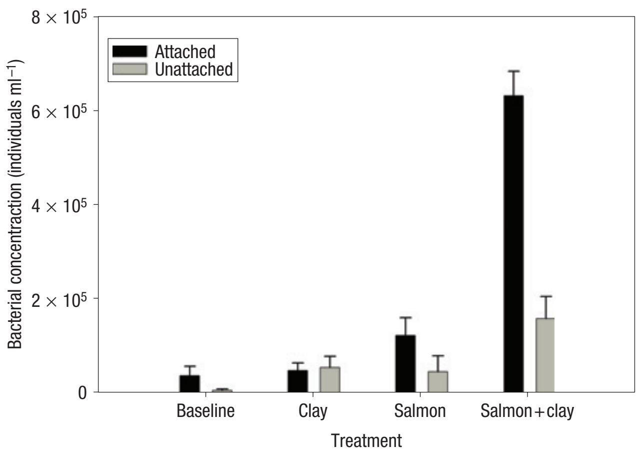

Figure 4 Treatment means for attached and unattached bacterial concentrations from gravel-bed sediment collected in the recirculating flume. The mean of three samples per treatment, with the error bars representing one standard error of the treatment mean.

METHODS

Flocs were generated in a recirculating flume (length 30 m; width 2 m; depth 2 m) located at the Quesnel River Research Center in Likely, British Columbia. The flume replicated hydrologic conditions observed during previous field investigations in O'Ne-eil Creek, British Columbia[17]. The flume's slope was less than 0.01 mm^{-1} and it was seeded to a depth of 0.3 m with washed gravel and cobble between 1 and 10 cm, which covers the substrate size range cited as optimal for Pacific salmon spawning[21]. The gravel-bed was manipulated to create riffle bars for infiltration bag sampling that were approximately 10 cm higher than lower areas. The flume was filled with groundwater devoid of salmon organic matter or suspended sediments. Water was recirculated through the flume at a depth of 20 cm and a velocity range of 5–10 cm s^{-1} using a 30 l s^{-1} centrifugal pump.

Kaolin clay was added to the channel at a concentration of 5 mg l^{-1}, which is similar to that observed in O'Ne-eil Creek. The kaolin clay size distribution's mode was 2 μm, which is similar in magnitude to the to the 8 μm mode of lacustrine sediment around O'Ne-eil Creek. Salmon organic matter was collected by decanting the liquid fraction from 6 kg of pink salmon (*Oncorhynchus gorbuscha*) that had rotted for three weeks and then screening it through a 200 μm Nitex screen to ensure that large organic particles were not added to the flume. Six kilograms of salmon tissue corresponds to 100 g m^{-2} of tissue to gravel-bed area, which is the lower range of concentrations observed in O'Ne-eil Creek during previous studies[22]. Sediment and salmon organic matter were added to the flume in sequence to assess each material's effect on flocculation, sedimentation and gravel-bed enrichment. Specifically, following a baseline period, clay was added, followed by salmon organic matter and then salmon organic matter plus clay.

Water-column particle size, gravel-stored sediment settling studies, and gravel-stored sediment EPSD and APSD data were collected using a LISST-ST (ref. 23). The LISST-ST collects particle-size information using laser diffraction and transmissometry[24]. Water-column particle-size measurements were collected *in situ* by orienting the LISST sensing area perpendicular to the flow at a distance of 10 cm above the flume's gravel-bed. Flume water flow ensured that the same sediment sample was not measured more than once and prevented settling of sediment onto the lower optic[23]. *In situ* samples were collected at a 3 s frequency for 1 h and particle size was measured into 32 size classes that are logarithmically spaced in the range of 2–460 μm. Gravel-stored sediment settling studies were conducted over a 12 h period using a 30 cm settling column and logarithmic sampling frequency similar to ref. 24. Gravel-stored sediment samples were measured for EPSD and APSD using eight size classes (0–3.5, 3.5–6.8, 6.8–13.1, 13.1–25.5, 25.5–49.4, 49.4–95.8, 95.8–185.8 and 185.8–460 μm). EPSD data were collected by analysing untreated sediment samples. APSD data were collected by dispersing the EPSD sample with a chemical dispersant (Calgon—sodium hexametaphosphate and sodium carbonate anhydrous) followed by physical dispersion using a Misonix Inc. Sonicator, Ultrasonic Processor XL 2020.

Gravel-bed sediments were collected by infiltration bags[25,26]. Fifteen bags were buried to a depth of 25 cm in a column of reference gravel consisting of flume material that was screened to remove sediment of less than 2 mm. Three randomly selected bags were removed following two to three days exposure to selected treatments (that is, baseline, clay, salmon or salmon plus clay). During retrieval, bags were brought up through the column of reference gravel capturing both the reference gravel and other material that settled during the exposure period. These samples were sieved through a 2 mm screen and subsamples of the liquid fraction were collected for absolute particle size determination, bacterial enumeration and carbon-to-nitrogen ratio.

Bacterial enumeration was completed using an oil immersion lens of a BX-50 microscope and a direct count method[27] using samples stained with the Syto9 nucleic-acid stain (Molecular Probes). Samples were filtered at low pressure on 1.0 and 0.1 μm Sudan Black stained filters. Attached bacteria were enumerated on the 1.0 μm filter, whereas unattached bacteria were contained on the 0.1 μm filter. Carbon-to-nitrogen ratio was determined by elemental analysis of sediment samples collected on pre-ashed (550 °C) glass-fibre filters. Carbon-to-nitrogen ratio was determined using the Dumas principle of complete and instantaneous oxidation of the sample by flash combustion using a FISON NA-1500 Elemental Analyzer (Milan, Italy)[28].

Analysis of variance (Systat 11) was used to test for treatment effects on normally distributed data for suspended sediment and gravel-stored sediment particle size as well as bacterial counts. Percentage-finer data were arcsine transformed before analysis. Post hoc comparisons were made using Tukey's HSD test[29]. Data are provided as mean ± standard error.

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Author contributions

This work represents the PhD focus of J.F.R. with the supervision and assistance of E.L.P. Conceptual planning of the experiment derives from earlier work by E.L.P. and J.F.R. in O'Ne-eil Creek. The manuscript was written by J.F.R. and revised and edited by both authors.

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