



Organic matter composition of gravel-stored sediments from salmon-bearing streams

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Key words: fine sediment, gravel bed storage, organic matter, settling rates, biofilms, flocculation

Abstract

The objective of this project was to evaluate the changing composition and structure of the sediment-associated organic matter (OM) stored in the gravel bed of highly productive salmon-bearing streams and, determine if the OM changes affect the morphology and settling rates of the sediment. In July of 2001, a dozen infiltration gravel bags were buried in the channel bed of O'Ne-eil Creek in northern British Columbia (Canada) to collect fine sediment and the associated organic matter for chemical and morphological analysis. The bags were removed over a 10 week period which incorporated summer low flows, salmon spawning, salmon die-off and the onset of autumn low flow conditions. Our results indicate two visibly different structures in the organic matter film overlying the mineral material of the flocs. A web-like structure was noted during mid-spawn while a film-like covering was observed in pre-spawn and post-fish periods. The strength of the film-like covering is surmised to be associated with the larger gravel-stored floc sizes noted at these times. Chemical analysis of these biofilms indicated higher metal complexation properties during the spawning periods as opposed to before or after salmon were present. The changing OM contributions were associated with changes in floc size, density and settling rates. The physical disturbance to the gravels associated with spawning salmon was also correlated with altered characteristics of the gravel-stored flocs.

Introduction

Organic matter loadings to streams increase the probability of gravel bed storage of sediment as organic compounds aggregate with inorganic fine sediment (<63 μm) to generate flocs with modified settling rates (Droppo, 2001). Increased aggregate storage in intergravel spaces physically impedes interstitial flow while the decay of organic matter increases oxygen demand. Therefore, intergravel storage of organic and inorganic aggregates can be detrimental to benthic organisms and problematic for the success of incubating fish eggs. Studies in pristine fish-bearing streams indicate that gravel-stored aggregates, or flocs, exhibit faster settling rates than flocs suspended in the water column (Petticrew & Droppo, 2000) and that the flocs are notably larger following spawning periods when massive fish die-offs occur. While the physical activity

of fish cleaning the gravels to prepare for spawning reduces the storage of fine sediments, the effect of the instream organic matter contribution from the extensive fish die-off on sediment composition and storage has not been evaluated. The observed nutrient burst associated with salmonid die-off (Johnston et al., 1998) and its effect on the generation of biofilms (Wold & Hershey, 1999) are presumed to be of importance in the creation of instream aggregates which have the potential to be stored in the gravel bed. To date, the changing composition of the organic matter associated with gravel-stored sediments and the related effects of this on settling properties has only been speculated upon (Petticrew & Droppo, 2000). In this paper, we evaluate the chemical and morphological changes in organic matter associated with fine grained sediment stored in the intergravel pores of a productive salmon bearing stream before, during and following the

spawning period as a means of determining the role of the fish in floc formation. As well we investigate morphological characteristics (settling rates, density, size) of the gravel-stored fines to determine if any changes are associated with the activities of the returning fish.

O'Ne-eil Creek is a highly productive sockeye salmon (*Oncorhynchus nerka*) stream in the Takla Lake area of northern interior British Columbia (Canada). The lower 1.8 km of this 20 km stream is intensively used for sockeye spawning with annual returns varying between 1000 and 53 000 over the last 20 years (Petticrew, 1996). In 2001, a total of 13 893 salmon returned to spawn in O'Ne-eil Creek. Our sample riffles were located between 1400 and 1550 m from the stream mouth, where approximately 200 fish were noted on the riffles at the peak of spawn. The stream width was between 10 and 12 m and water depths were between 25 and 40 cm.

Methods

On July 13, 2001 twelve infiltration gravel bags were buried in ~25 cm holes dug into the gravel bed of O'Ne-eil Creek following the methods of Lisle & Eads (1991). The folded bags were covered by cleaned gravel which was washed through a 2 mm sieve using stream water. Ropes were attached to the bag for retrieval purposes allowing collection of both gravel and infiltrated finer sediment (<2 mm). The bags were removed in pairs over the following 10 weeks to coincide with the fish activities: pre-spawn (PS) – July 17; early spawn (ES) – July 28; mid-spawn (MS) – August 3; die-off (DO) – August 12 and 16. In late September when all visible evidence of fish carcasses was gone from the stream a final set of bags were retrieved, these are identified as post-fish (PF) samples.

When the infiltration bags were removed from the stream, the finer, infiltrated sediment was separated from the gravel by washing with distilled water through a 2 mm sieve. The large gravel material was kept for standard sieve size analysis. The infiltrated sediment (<2 mm), which was washed into a bucket, was stirred to re-suspend all grain sizes. The material was settled for 10 s to allow removal of sand sized material from the top layer of water. A 250 ml sub-sample of sediment was taken from this top layer of water, to allow the collection of the fine-grained particles (silt and clay) and slower settling, large composite particles (flocs or aggregates). The <2 mm sediment sample in the bucket was taken back to the laboratory and settled,

dried, weighed, ashed and sieved to obtain grain size curves. The fine-grained sub-sample was returned to the lab and used for organic matter and image analysis.

Morphology and elemental composition of the organic matter

The morphology (structure) and elemental composition of the organic samples were investigated using a PhilipsTM XLS 30 scanning electron microscope (SEM) equipped with EDAXTM energy dispersive system (EDS). Preparation of the samples included air-drying on a SEM tin stub, and sputter-coating with Au for 60 s. The Au-coated samples were observed under the SEM for morphology of the organic matter stored in the gravel beds. The elemental composition of this organic matter was semi-quantitatively determined from an energy dispersive spectrum collected for 400 s from at least five points on observable films of organic matter on the surface of the mineral material. The semi-quantitative chemical composition was estimated using ZAF, a standard-less energy dispersive technique, where the estimates of the chemical composition were corrected for factors including Z (atomic number), A (absorption), and F (fluorescence) for each element of interest.

We characterized the changes in organic matter composition of the gravel-stored sediments in two ways using the ZAF results. First, the apparent total acid content (ATAC) of the organic matter was calculated using Equation (1), where the ATAC equals the potential cation exchange capacity as measured by the amount of metals adsorbed onto the organic matter (McBride, 1994):

$$\text{ATAC (moles}_+k g^{-1}) = \Sigma(((\text{Wt}\%_M * 1000) / \text{MW}_M) * n_+), \quad (1)$$

where $\text{Wt}\%_M$ = weight percentage of the metal from SEM-EDS, MW_M = atomic weight (g) of the metal, n_+ = oxidation number of the metal.

Secondly, the affinity of organic matter for each metal was estimated from the relative metal saturation of the cation exchange sites as given by:

$$\text{Metal saturation (\%)} = (mM^{n+} / \text{ATAC}) * 100, \quad (2)$$

where M^{n+} = metal of interest adsorbed on exchange sites, m = moles of metals expressed as single positive charge (moles₊)

Functional groups in the organic matter

Another technique used to assess the changes in the composition of the organic matter over the period of study involved a CentaurusTM microscope attached to a Nexus 670TM Fourier Transform Infra-red (FT-IR) spectrometer. This allowed us to investigate the changes in functional groups of the organic matter stored in the gravel beds. We used a ZnSe Attenuated Total Reflection (ATR) objective as an accessory to the microscope. The ATR technique is non-destructive and allows the analysis of a small area ($10\ \mu\text{m} \times 10\ \mu\text{m}$) of a sample. The infrared beam penetrates the organic matter to a depth of $0.66\text{--}2.0\ \mu\text{m}$ (at $1000\ \text{cm}^{-1}$) and allowed us to determine the functional groups in the film of organic matter. Several drops of the 250 ml fine sediment sub-sample were placed on a glass slide and the microscope stage was raised until the sample just touched the ZnSe ATR objective. For each sample, we collected an infrared spectrum from 64 scans ranging from wavenumber 4000 to $675\ \text{cm}^{-1}$ (wavelength $(\lambda) = 2.5\text{--}15\ \mu\text{m}$). We also air-dried the samples on a reflective sample holder to allow for a comparison of wet and dried samples.

Floc morphology and settling rates

The fine sediment sub-sample, depleted of sand-sized particles, was introduced into a settling tube ($1.51 \times 0.14 \times 0.06\ \text{m}$) filled with filtered creek water (13.4 l). Time-lapsed digital images of settling particles were taken using a Firewire-based CCD, controlled by an Intel PC running Northern Eclipse (Empix Imaging) software. For each gravel bag, 100–250 individual particles were identified and tracked between chronological images to determine settling velocities and densities (Petticrew & Droppo, 2000). Images taken during the settling process were also used to size a larger population (1000–2500 particles) of fine sediments. The number, area, perimeter, roundness, elongation, diameter and volume of each particle were determined using the image analysis software.

Statistical analyses

To determine the influence of salmonid spawning and die-off on the composition and structure of the organic matter stored in gravel beds, the main factors in the statistical analyses were based on chronological groupings of the data into five classes: pre-spawn (PS); early spawn (ES), peak or mid-spawn (MS), die-

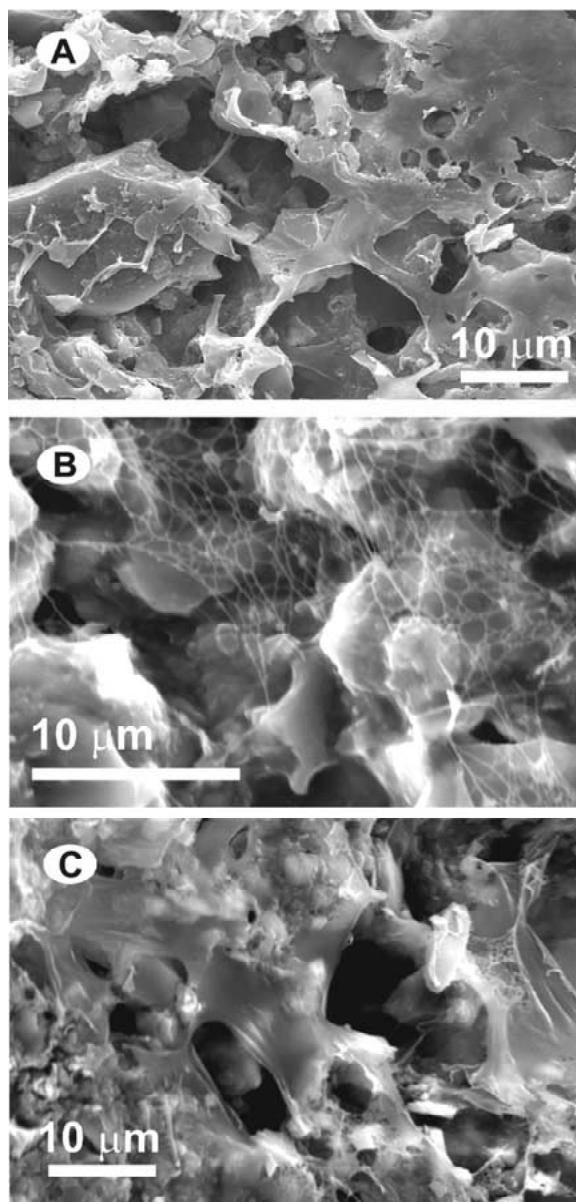


Figure 1. Electron micrographs of (a) pre-spawn (b) mid-spawn and (c) post-fish samples of fine sediment collected from interstitial waters of a gravel bed river.

off (DO), and post-fish (PF). Data for the structure and chemical composition of the organic matter were analyzed using one-way ANOVA using StatisticaTM Version 5 (Statsoft Inc., 1995). *Post hoc* comparison of significantly different means was made using planned LSD test statistics. Because of large sample sizes ($n = 92\text{--}204$) in each group of settling properties, non-normally distributed data were not transformed.

Results

Structure and composition of gravel-stored organic matter

The results of the electron microscopy indicate that the morphology of the organic matter stored in gravel beds exhibits two main types of structures. The PS and PF samples exhibit a ‘film-like’ structure while the MS samples have a ‘web-like’ structure. These structures coat the fine clay and silt-sized inorganic components of the flocs (Fig. 1a–c). The ‘film-like’ coating in pre-spawn samples shows a tendency to curl to strands ~ 1.0 – $5.0 \mu\text{m}$ in diameter (Fig. 1a) while the ‘film-like’ structure in post-fish samples is more extensive and coats larger surface areas of the inorganic flocs ($\sim 50 \mu\text{m}^2$ in Fig. 1c). The less dense, web-like pattern of organic matter in the mid-spawn sample is composed of small strands ($\sim 0.1 \mu\text{m}$ in diameter) enclosing many inorganic particles (Fig. 1b). While not shown in this image the MS samples also exhibit some regions of ‘film-like’ structures.

Chronological changes in the elemental composition of the gravel-stored organic matter, as determined from the film and web-like structures of the samples, are noted in the semi-quantitative estimates from the scanning electron microscope (Table 1). The C contents follow the order DO > PF > PS > MS, while mid-spawn samples have the highest contents of monovalent cations (Na^+ and K^+). Except for Cu^{2+} , MS and DO samples have higher divalent cations (Mg^{2+} , Ni^{2+} , and Cu^{2+}) than PS and PF samples. The contents of Al^{3+} decreases in the MS and DO samples while Si^{4+} exhibits the opposite trend. The apparent total acid content is significantly lower in MS and DO samples as compared to PS and PF samples (Table 2). The affinity for the adsorption of divalent cations as shown by the divalent metal saturation shows $\text{Cu}^{2+} \geq \text{Ni}^{2+} > \text{Mg}^{2+}$ for PS, DO and PF samples while for MS samples, $\text{Cu}^{2+} > \text{Mg}^{2+} > \text{Ni}^{2+}$ is observed (Table 2).

Functional groups in organic matter

Our second method of assessing changes in the organic matter composition (IR spectra), also showed differences over the time period of sampling (Fig. 2). Infrared spectra from ‘wet’ and ‘air-dried’ samples showed IR absorption bands in the following regions (wavenumber): 1170 – 950 cm^{-1} ($\lambda = 8.5$ – $10.5 \mu\text{m}$) indicating stretching of C–O in polysaccharides as

well as Si–O of silicates; 1470 cm^{-1} ($\lambda = 6.8 \mu\text{m}$) and 720 – 730 cm^{-1} ($\lambda = 13.9$ – $13.7 \mu\text{m}$) for C–H bending; 1660 – 1630 cm^{-1} ($\lambda = 6.0$ – $6.1 \mu\text{m}$) for the C=O stretching bonds of amide group, quinone and/or H-bonded conjugate ketones; 1700 cm^{-1} ($\lambda = 5.9 \mu\text{m}$) for the C=O stretching vibration from free carboxylic acid and from esters; 3230 – 3377 cm^{-1} ($\lambda = 8.5$ – $10.5 \mu\text{m}$) for the OH-stretching of water; and 2900 cm^{-1} ($\lambda = 3.4 \mu\text{m}$) for aliphatic C–H stretching (Stevenson, 1994; Ambles, 2001; Frimmel, 2001).

Changes in the composition of organic matter with time are noticeable by the decreased transmittance at 1094 cm^{-1} ($\lambda = 9.1 \mu\text{m}$) wavenumber in ES compared to PS and PF samples in IR spectra from ‘wet’ samples. This reflects increases in the polysaccharide and silicate structures in the ES as compared to the end points of our sampling period (PS and PF). Another difference among these samples is evident in the decreased transmittance at 1700 cm^{-1} ($\lambda = 5.9 \mu\text{m}$) wavenumber in PS and PF compared to ES samples in IR spectra of air-dried samples. This indicates an increase in carboxyl groups during the active spawning relative to the pre and post-spawning periods.

Settling properties of flocs

The mean diameter of the settling particles was significantly larger during the PS and the PF periods. Smaller diameter flocs were noted for the three weeks that fish and/or fish carcasses were observed in the stream (Table 3). Note that the particle diameter of the PS and PF samples are statistically similar but their settling rates and densities are significantly different. From the full data set it is apparent that later in the season, similarly sized flocs settle faster and have a higher particle density. The MS particles exhibit the smallest average size over the full sample period but settle significantly faster and are significantly denser than particles observed to be of similar size collected during the period when fish are present in the stream. Also presented in Table 3 is the proportion of inorganic sediment $< 63 \mu\text{m}$ trapped in the gravels relative to the total amount of infiltrated ($< 2 \text{ mm}$) sediment. The largest proportions of $< 63 \mu\text{m}$ inorganics accumulate in the gravels in the pre and post fish period. Values are lower during the period when fish are cleaning the gravels for spawning, but increase during the period of die-off.

Table 1. Mean (and standard deviation) of the semi-quantitative estimates* of the elemental composition (weight%) of biofilm observed on flocs collected at various stages of fish activities ($n = 5$)

	C	Na ⁺	K ⁺	Mg ²⁺	Ni ²⁺	Cu ²⁺	Fe ³⁺	Al ³⁺	Si ⁴⁺
Pre-Spawn (PS)	66.7a (6.80)	1.41a (1.22)	0.29b (0.16)	0.20ab (0.23)	2.80c (0.83)	3.01b (1.70)	3.61b (1.29)	9.77a (1.80)	0.41a (0.11)
Mid-Spawn (MS)	64.5a (1.6)	4.16b (0.63)	0.37c (0.12)	1.90c (0.46)	2.05b (0.19)	7.40c (0.85)	3.79b (0.62)	1.42b (0.33)	2.16c (0.34)
Die-Off (DO)	72.1b (1.8)	0.65a (0.30)	0.20ab (0.06)	0.37a (0.22)	3.64d (0.65)	4.44bc (1.98)	5.81c (1.25)	2.3b (1.00)	1.17b (0.59)
Post-Fish (PF)	67.3ab (2.4)	0.96a (0.99)	0.11a (0.02)	0.41b (0.54)	0.94a (0.21)	11.3a (1.92)	1.65a (0.30)	9.1a (1.44)	0.44a (0.27)

In each column, means followed by similar letter are not significantly different ($p > 0.05$).

* Using energy dispersive system (SEM-EDS).

Table 2. Mean (and standard deviation) total acid content (moles kg^{-1}) and metal saturation (%) of the organic matter observed on flocs collected at various stages of fish activities. Data collected using energy dispersive system (SEM-EDS) ($n = 5$)

	Acid	Na ⁺	K ⁺	Al ²⁺	Mg ⁺²	Ni ²⁺	Cu ²⁺	Fe ³⁺	Si ⁴⁺
Pre-Spawn (PS)	16.11a (1.57)	4.05a (3.64)	0.46b (0.25)	66.97a (5.77)	1.01a (1.17)	5.94b (1.81)	5.67a (2.65)	12.26b (4.71)	3.65a (0.97)
Mid-Spawn (MS)	13.20b (1.70)	13.77b (1.77)	0.72b (0.19)	11.84b (1.69)	11.94b (1.81)	5.31b (0.61)	17.57b (1.27)	15.43b (1.80)	23.42c (1.80)
Die-Off (DO)	10.57c (1.13)	2.68a (1.25)	0.49a (0.12)	23.99c (9.98)	3.04a (1.87)	11.81c (2.51)	12.98b (4.86)	29.69c (6.49)	15.41b (6.73)
Post-Fish (PF)	16.31a (2.32)	2.51a (1.32)	0.19a (0.04)	62.06a (3.49)	1.92a (2.30)	1.95a (0.28)	22.7b (4.92)	5.44a (0.76)	3.76a (1.98)

In each column, means followed by similar letter are not significantly different ($p > 0.05$).

Discussion

Enhanced microbial and algal growth has been observed in streams as a result of salmon carcass-derived nutrients in a variety of locations and with a range of fish types (Richie et al., 1975, Wold & Hershey, 1999). Johnston et al. (1998) worked in three Takla area streams and found that salmon density was significantly correlated to the amount of isotopic carbon in stream epilithion, indicating the utilization of salmon-derived carbon by instream organisms. In this study, we observe that the presence or absence of fish is associated with changes in the accumulation/drying pattern of organic film on mineral surfaces (i.e., 'web-like' in MS samples compared to 'film-like' in PS and PF samples) in gravel beds. These structures are likely the results of differences in chemical composition of organic matter present in the stream. The decomposition of fish carcasses will deliver a pulse of proteinaceous and carbohydrate materials to the stream. Schulten &

Schnitzer (1998) found that proteinaceous materials comprise up to 40% of humic substances found in soils and sediments, so it is not difficult to surmise that the material delivered from the die-off of salmon would be incorporated into the sediments. Evidence of this is the higher amount of C=O bonds associated with carboxylic acids and/or esters found in the early spawn samples. These temporal changes in organic matter composition may be influencing the organic film formation on mineral surfaces, thereby resulting in the contrasting appearance of 'web-like' and 'film-like' structures.

Other properties of the organic matter influenced by the addition of organic compounds from fish carcasses include 'complexation properties'. The higher affinity of MS samples for Mg²⁺ compared to Ni²⁺ could be due to the higher amounts of R-COOH groups from the organic compounds added to the river during the decomposition of fish carcasses. Mg²⁺ is a 'hard' acid that preferentially binds with 'hard' base

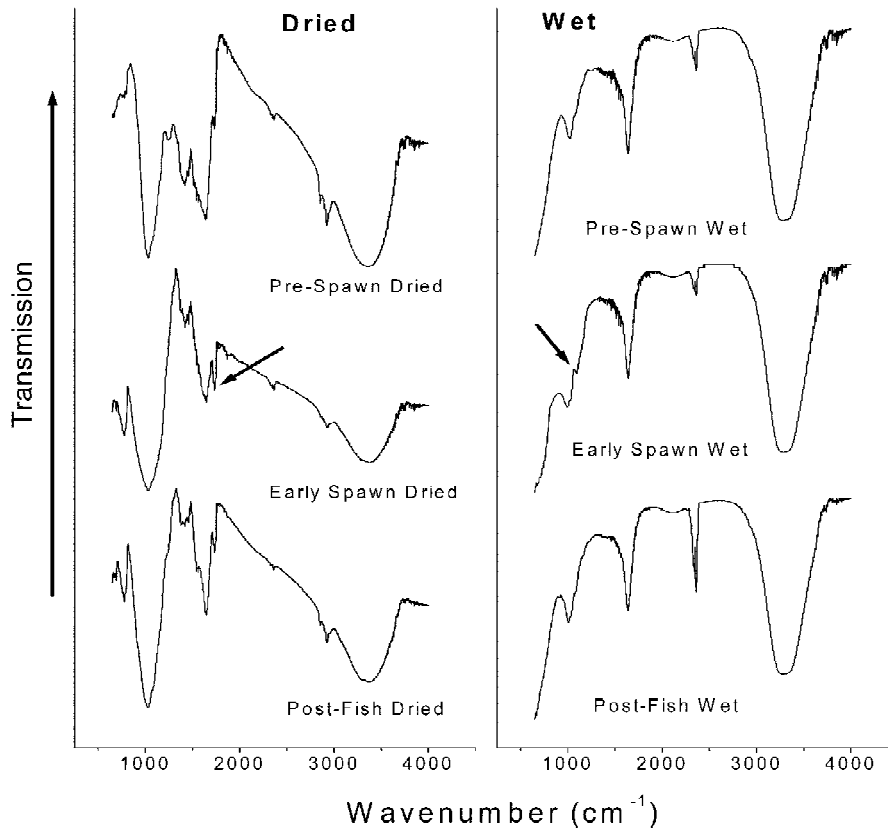


Figure 2. Infrared spectra for fine sediment samples collected from interstitial waters of a gravel bed river during pre-spawn, early spawn and post-fish periods. Observed differences between sample times are highlighted by arrows. Both wet and air-dried samples are presented.

such as R-COO^- while Cu^{2+} and Ni^{2+} are ‘softer’ acids that bonds with ‘soft’ bases (McBride, 1994). Recently in the soils literature, Pignatello (1998) elucidated the importance of nanoporosity in organic matter ‘hole-filling’. Although, Pignatello (1998) stressed the role of nanopores in the adsorption of organic compounds, these pores could also adsorb (or trap) metal ions whose ionic radii are in the picometer range and are much smaller than the $\sim 0.5\text{--}1.0$ nm nanopores. In addition, Pignatello (1998) added that that adsorption of di- and trivalent cations (e.g., Mg^{2+} , Al^{3+} and Fe^{3+}) increased the condensation of organic matter into dense organic polymer mesh. This same process may be responsible for the variable structures (web versus film) we observed. This is supported by the pattern of changing metal saturation associated with the cation exchange capacity of the OM we evaluated. Recall that the lowest values were during MS and DO when web-like structures existed and the highest values were during PS and PF when denser organic film persisted.

The influence of fish activities on both the structure and composition of organic matter stored in gravel beds of salmon-bearing streams is manifested in the chemical, morphological and settling properties of the flocs. Both the physical activity of spawning, when the fish dig their redds and the addition of a large pulse of organic matter from the carcasses of dying salmon seems to influence the size, appearance and chemical content of gravel-stored sediment. The PS and PF gravel-stored flocs are more abundant and larger than during the period when fish are present in the stream. The resuspension of gravel-stored sediment by fish during the cleaning of the spawning sites could be expected to break apart loosely bound flocs to reduce the average size. The pulse of organic matter introduced to the stream from the decay of $>13\,000$ fish in this stream would have contributed tremendous amounts of OM to the system potentially reducing floc density. Relative amounts of carbon indicate the highest amounts during die-off (DO) when the lowest particle densities are noted. Electron microscope images of PS,

Table 3. Mean (and standard deviation) particle diameter (mm), settling rate (mm s^{-1}) and particle density (g cm^{-3}) of flocs collected at various stages of fish activities

Activities	<i>n</i>	Particle diameter (mm)	Settling rate (mm sec^{-1})	Particle density (g cm^{-3})	Percent weight of inorganics <63 μm *
Pre-Spawn (PS)	92	0.332b (0.109)	2.170a (1.38)	1.047a (0.033)	5.75
Early Spawn (ES)	202	0.261a (0.112)	2.117a (0.816)	1.092b (0.068)	3.48
Mid-Spawn (MS)	102	0.244a (0.089)	2.449b (1.03)	1.116c (0.079)	1.79
Die-Off (DO)	153	0.262a (0.095)	2.122a (0.989)	1.032a (0.048)	4.34
Post-Fish (PF)	204	0.316b (0.157)	3.677b (1.59)	1.121c (0.033)	4.85

n = number of observations.

In each column, means followed by similar letter are not significantly different ($p > 0.05$). * Note that $n = 2$ for all samples except die-off when $n = 4$.

MS and PF samples showed that the biofilm coating the inorganic particles is more extensive in PS and PF samples potentially reflecting stronger floc structure. The 'web-like structure' of biofilm in MS may not be as effective as 'film-like' in maintaining floc structure. The combination of the physical movement of the gravels by spawning fish and the less dense biofilm may result in weaker floc structure and therefore smaller average floc sizes during the period when fish and carcasses are present.

It is useful to note that particle size analysis (by Coulter Counter) of the disaggregated, inorganic particle populations of these same fine-grained samples have constituent particle sizes <73 μm . This means that the sediment sub-sample taken with an effort to remove sands was effective and that the particles between 73 and 800 μm which were observed in the settling tube and used for chemical analysis are all in fact composite structures. These flocs which remained in the sampled surface water have settling rates less than sands as their densities are decreased due to increases in organic matter and/or modification of their porosity and shape. Given the maximum size of the constituent particles is 73 μm and the maximum size of the observed flocs is $\sim 800 \mu\text{m}$, a floc factor of ~ 11 is calculated for the gravel-stored flocs.

Conclusions

A temporal evaluation of the organic matter of gravel-stored flocs indicated that changes in the physical structure and the chemical composition were influenced by the activity of salmon in the gravel bed stream. The effect of both the changing organic matter

contributions and composition as well as the physical activity of the spawning salmon was to alter the size, density and settling rate of the gravel-stored flocs in O'Ne-eil Creek.

Acknowledgements

The assistance of Rebecca and Jennifer McConnachie, Matthew Riley, Leslie Chamberlist and Tauqeer Waqar in field and/or laboratory work is appreciated. This work was supported by National Science and Engineering Research Council grants to both authors, the Canada Research Chair Program to JMA and a grant from Fisheries Renewal British Columbia to ELP.

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