The Flocculation Feedback Loop: Delivery of Marine Derived Nutrients in Pacific Salmon Streams

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The complicated behavior of the world we see around us is merely "surface complexity arising out of deep simplicity." John Gribbin, Deep Simplicity

Abstract

Pacific salmon contribute significant quantities of marine derived nutrients (MDN) to their natal streams. Post-spawning carcasses fertilize and stimulate stream productivity as they decay. Until now, there has been no complete description of a nutrient delivery mechanism for natal streams. The salmon-floc feedback loop proposed here is a positive feedback system that delivers salmon derived nutrients and organic matter to the streambed where they can be retained and metabolized by benthic food webs to stimulate stream productivity and provide sustenance for juvenile salmon.

The three stages of the salmon-floc feedback loop were verified using the controlled environment of flumes and field verified concentrations of salmon organic matter and inorganic particulates. Stage one, floc formation was found to occur in the water column in the presence of salmon organic matter as well as salmon organic matter and clay. During the salmon organic matter treatments the particle size distribution of suspended sediment shifted toward larger particles indicating the formation of flocs. Stage two, floc sedimentation was identified by an increase in the effective particle size distribution of fine sediments in the gravel bed after the addition of salmon organic matter and clay. Water column flocs settled or were sequestered on the flume bed by advective and intergravel flow through porous, raised, gravel bed sections. Stage three, floc dissociation and nutrient release was identified by the increase in nutrient, bacterial, and biochemical oxygen demand levels of fine sediments that were captured and retained within intergravel pores. Floc formation and streambed delivery/retention requires sufficient quantities of organic matter from decaying salmon, sufficient but not excessive inorganic sediment levels, bacterial populations, low-

flow stream conditions, porous raised gravel bed areas, and hyporheic exchange to be an effective MDN vector.

The verification of the salmon-floc feedback loop is an important step toward further exploring nutrient cycling processes within salmon streams and investigating other similarly complex biophysical relationships between spawning Pacific salmon and their natal streams.

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Chapter 1: The Salmon-Floc Feedback Loop

Pacific salmon play a significant role in the nutrient cycle of their natal watersheds because they deliver substantial quantities of marine derived nutrients during spawning events (Bilby *et al.*, 1996; Naiman *et al.*, 2002; Schindler *et al.*, 2003). The MDN delivered can support riparian zone vegetation and terrestrial organisms (Drake *et al.*, 2006; Hocking and Reimchen, 2006), benthic macroinvertebrates (Chaloner *et al.*, 2002), algae (Johnston *et al.*, 2004), and fish populations (Naiman *et al.*, 2002). The quantity of MDN delivered to spawning streams varies annually with the mass of spawning salmon that return. For example, Finney *et al.* (2000) identified that spawning salmon contribute a range of 25-75% of the annual nitrogen load in southeastern Alaskan streams. In addition to the annual variability of MDN imported to natal stream by spawning salmon, there is a mass balance relationship between the MDN imported by spawning salmon and the mass of nutrients exported by departing smolts (Moore and Schindler, 2004; Moore *et al.*, 2007).

Watersheds with declining populations of salmon can experience a net loss in nutrients because the nutrients contained in departing smolts exceeds the amount of MDN imported by spawning salmon (Scheurell et al, 2005). The export of nutrients from salmon bearing watersheds can lead to lower overall watershed productivity as well as a decreased capacity to produce salmon (Scheurell *et al.*, 2005). Salmon enhancement programs target this relationship in watersheds experiencing salmon population declines by depositing salmon carcasses, salmon carcass analogues, or other fertilization techniques to subsidize the decrease in MDN and increase stream productivity and salmon numbers (Kohler *et al.*, 2008; Wipfli *et al.*, 2004). Although the complex relationships between MDN import and nutrient

removal by juveniles are gradually being identified, there has been no description of the underlying biophysical processes that control the delivery of MDN in natal streams. Given the importance of maintaining Pacific salmon habitat, there is a clear need for a mechanistic understanding of the processes that deliver and redistribute salmon organic matter (SOM) and MDN in natal streams to enhance resource management activities.

This dissertation presents and verifies the salmon-floc feedback loop, a conceptual model for MDN delivery and retention in streambeds of Pacific salmon natal streams (Figure 1). This first chapter proposes a series of processes that potentially allow the loop to operate in spawning salmon streams. The following three chapters (2 to 4) provide the results of experiments designed to test the occurrence and outcomes of these processes. The loop is initiated by the digging of salmon redds during spawning and the concomitant die-off and decay of spawners which provide in-stream sources of fine sediment (< 63 μm) and organic matter respectively. This dissertation focuses on the three stages following spawning salmon decay to determine the fate of MDN released to the stream from carcasses.

In Figure 1, the first stage of the salmon-floc feedback loop after spawning salmon decay suggests that MDN is incorporated into flocs formed by the combination of salmon organic matter (SOM) and fine-grained inorganic sediment. SOM originating from post-spawn carcasses and suspended fine sediment, available at ambient levels or suspended by spawning activity, have the potential to combine in the water column through flocculation. Flocculation refers to the group of physical, chemical, and biological processes that joins inorganic sediments with organic materials in aquatic environments (Droppo *et al.*, 1997). If

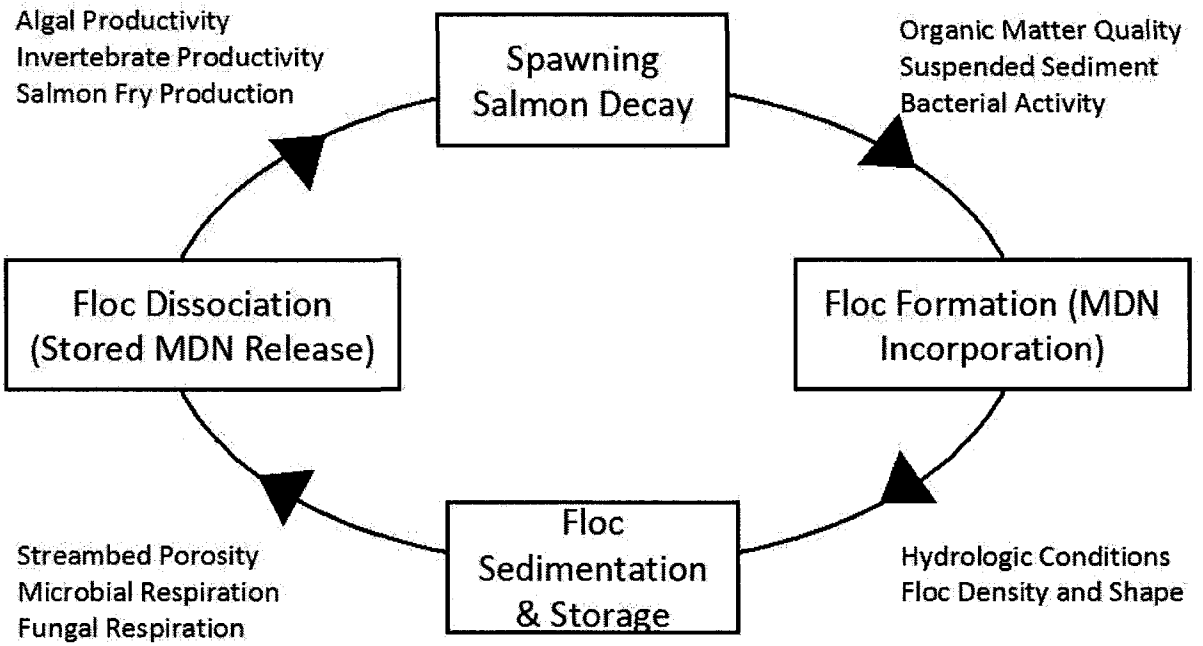

Figure 1: The salmon-floc feedback loop (Rex and Petticrew, 2008). Stages of the loop are boxed while environmental factors influencing each stage are shown along the pathways. This study focuses on the three stages following spawning salmon decay, namely floc formation (stage 1), floc sedimentation and storage (stage 2) and floc dissociation (stage 3).

formed in the water column, SOM-based flocs are predicted to exhibit larger sizes and higher settling rates than their individual component parts. Settling and storage of SOM-based flocs on, or in, the streambed is presented as stage two of the salmon-floc feedback loop. Gravel-bed deposition and storage of SOM-based flocs is expected to provide benthic microbes the opportunity to metabolize the flocs, mineralizing and releasing MDN during stage three of the loop, floc dissociation and MDN release. MDN metabolism and release should stimulate stream productivity and thereby juvenile salmon production. By providing sustenance for future spawners the link to the preliminary stage of the feedback loop, spawning salmon decay, can be completed.

1.1 Pacific Salmon MDN and Redd Creation

Spawning Pacific salmon provide the most significant contribution of externally derived organic material and nutrients to their natal streams on an annual basis because they gain up to 95% of their body mass during their time at sea (Willson, 1997). The MDN spawning salmon contribute represents a net gain of nutrients to natal watersheds as long as the mass of smolts produced following spawning is less than the mass of spawning salmon returned and retained within the watersheds (Scheurell *et al.*, 2005).

SOM-based flocs are expected to form in the water column due to the collision of SOM and suspended sediment, but also due to the presence of bacteria that bind SOM and suspended sediment together with extra-cellular polymeric substances (EPS). The presence of salmon redds should enhance streambed capture of salmon-floc bound MDN because the physical structure of redds increases streambed roughness and surface water down-welling

(Tonina, 2005). Increased down-welling will also increase the probability of water-borne flocs entering the streambed.

During redd construction, spawning salmon remove fine sediments from the streambed at the redd site (Bjornn and Reiser, 1991; Malcolm *et al.*, 2004). Streambed excavation by the female salmon disturbs bed sediments, the finest of which (silts and clays) remain in suspension and flow downstream while the coarser sediments, such as sands and small gravels, settle out below the redd near the tailspill (Figure 2, Quinn, 2005). The quantity of streambed material moved during spawning can be substantial, rivaling spring freshet when spawning populations are high (Poirier, 2004; Hassan *et al.*, 2008).

1.2 The Salmon-Floc Feedback Loop: Research Questions

The salmon-floc feedback loop proposed here emphasizes the floc as a MDN delivery agent to the streambed. Once settled, MDN within flocs can be retained, mineralized by benthic food webs, and later used to support juvenile salmon. To identify the likelihood of the salmon-floc feedback loop occurring in Pacific salmon streams, this project poses three research questions, each one addressing a stage of the feedback loop. The preliminary stage, spawning salmon decay is not tested here, because it has been observed that carcasses remaining in the stream add decay products to the stream over time (Naiman *et al.*, 2002; Johnston *et al.*, 2004). The three research questions addressed are:

- Do SOM-based flocs form in the water column in the presence of salmon decay products and inorganic particulate matter?
- Do SOM-based flocs settle or otherwise become entrained within gravel beds?

  Do SOM-based flocs enrich the streambed and are these nutrients available to benthic

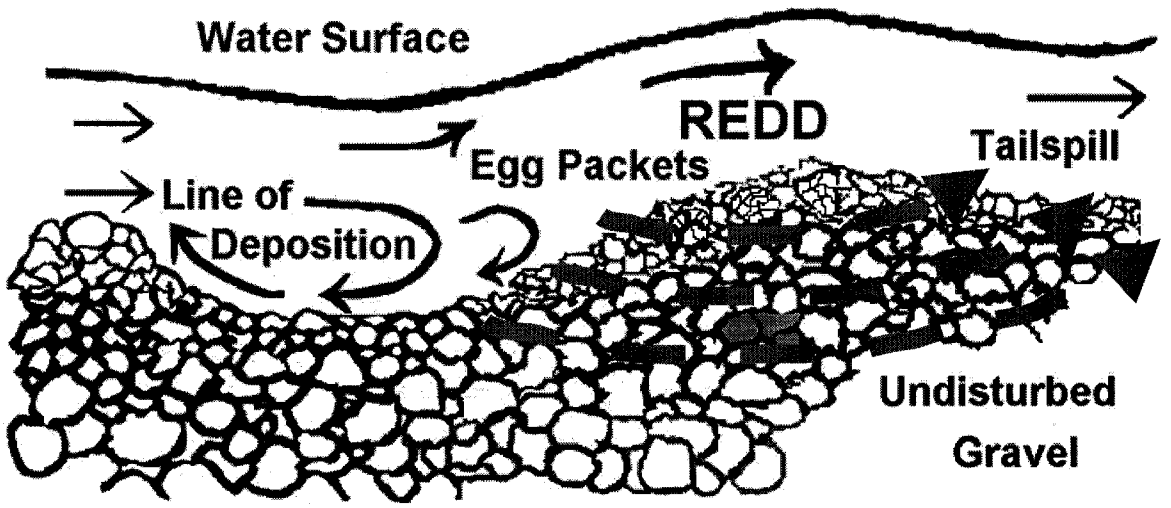

Figure 2: Gravel bed cross-section showing a redd with egg packets and down-welling flow at the head of the redd and upwelling at the tailspill as identified by arrows around the egg packets. (Image adapted from Washington Department of Fish and Wildlife).

foodwebs?

To test the possibility of this feedback loop occurring in Pacific salmon streams, I experimentally traced salmon decay products through the series of stages the loop requires, namely: floc formation, floc sedimentation, gravel bed retention, and MDN addition to the gravel bed. These stages were generated within the controlled environment of flumes using field-verified ranges of water velocity, salmon density, and total suspended solids concentrations observed during previous field studies in O'Ne-eil Creek, a productive Pacific salmon watershed within the central interior of British Columbia (McConnachie and Petticrew, 2006; Petticrew and Rex, 2006). Flume treatments included baseline/control, clay, salmon, and salmon + clay exposures to identify flocculation processes under the different conditions.

The three stages of the floc feedback loop were studied over five sampling seasons using different combinations of parameters (Table 1). Accordingly, individual floc stages and their corresponding experiments are presented as separate chapters in this thesis. Chapter two presents data evaluating water column-based floc formation in 2006 and 2007 (Stage 1). Chapter three presents results for floc sedimentation and interception studies using data collected from 2004 to 2007 (Stage 2). Chapter four presents information on floc dissociation and MDN release using data collected from 2004 to 2008 (Stage 3). The fifth and final chapter provides conclusions and summary statements. Due to technique and sampling program similarities between salmon-floc stages, the methods sections within chapters three and four will refer to chapter two's method descriptions rather than repeating the same text.

The salmon-floc feedback loop presented here has been discussed in other publications based upon individual program year findings. Specifically, floc sedimentation and MDN release from 2004 were presented in Rex and Petticrew (2006). The salmon-floc feedback loop including floc formation, sedimentation, and MDN storage for the 2007 program were presented in Rex and Petticrew (2008). Finally, the floc dissociation and MDN release from the nitrogen study in 2008 were presented in Rex and Petticrew (2009).

Table 1: Salmon-floc feedback loop stages, corresponding study parameters and their location within the thesis.

Stage	Chapter	Parameters Measured	Sample Years
One Floc Formation & MDN Incorporation	Two	Water Column Temperature, Turbidity and Conductivity Suspended Sediment Particle Size Bacterial Enumeration	2006-2008 2006-2007 2006-2007
Two Floc Sedimentation & Storage	Three	Total Suspended Solids Particle Size Analysis Particle Settling Velocity Carbon:Nitrogen Ratio Bacterial Enumeration	2004-2007 2004, 2006-2007 2006-2007 2004-2008 2006-2007
Three Floc Dissociation & MDN Release	Four	Biochemical Oxygen Demand Intergravel Dissolved Oxygen Nitrogen Forms Carbon:Nitrogen Ratio	2004-2007 2004-2007 2008 2004-2008

Chapter 2: Stage 1 Floc Formation and MDN Incorporation

2.1 Background

Flocs comprise a significant portion of the suspended sediment load of most rivers and vary in their composition as inorganic and organic matter sources change over the year (Droppo *et al.*, 1997). In natal salmon streams, spawning periods provide optimal conditions for floc formation because redd creation increases fine sediment re-suspension and transport (Kondolf, 2000) at the same time that SOM is released from the carcasses of post-spawn salmon. SOM-based flocs have the potential to form in the water column due to the collision of SOM and suspended sediment. Floc formation should be enhanced beyond the physical process of collision because bacteria that bind SOM and suspended sediment together with extra-cellular polymeric substances (EPS) should be abundant during salmon carcass decay.

Bacteria and algal cells produce EPS to provide protection, attachment, flotation, and to aid their locomotion (Wotton, 2004). Although EPS composition varies between organisms and environments, they are predominantly composed of carbohydrates that swell upon contact with water (Wotton, 2004). EPS are common in aquatic environments (Wotton, 2004; Passow, 2002) and their glue-like properties have been found to play an important role in floc formation (Droppo *et al.*, 1998; Droppo, 2001).

Bacteria enhance floc formation by attaching their EPS to both organic and inorganic particulate matter (Droppo, 1998; Wotton, 2004). Examination of the internal and external structure of contaminated sediments identified that floc skeletal structure was due to EPS from internally residing bacteria (Droppo, 2004). This finding indicates that bacteria and

their EPS can have a controlling influence on floc structure. EPS can also strengthen a floc by embedding particles, permeating the void space between particles, and offering additional ionic bonding sites and cross linkages (Gerbersdorf *et al.*, 2008).

EPS often originate from bacterial and algal populations that are utilizing organic matter (Wotton, 2007). Given the labile nature of SOM, water column bacteria levels and their associated EPS are expected to increase following the return of spawning salmon (Mitchell and Lamberti, 2005). Decaying salmon tissue will support surface living bacteria, some of which should have EPS capable of generating flocs by attaching the SOM particles to the stream's suspended silts and clays.

To determine if flocs form in the water column in the presence of salmon and clay (research question 1, Chapter 1) and to address the relationship between floc formation and bacterial activity, I investigated the effect of salmon and clay mixtures on suspended sediment particle size in the water column of a re-circulating flume and the corresponding bacterial levels in collected sediments. Water-column-based flocculation of salmon and clay mixtures will lead to an increase in particle size over that of the other stock solutions while changes in bacterial number in the sediment will indicate their participation in SOM-based flocculation.

2.2 Methods

Two different conditions associated with the delivery of SOM were simulated here, namely active spawning and post-spawning. Active spawning encompasses the full period of redd construction which includes the time when early return salmon are decaying in the

stream while later spawners are still building redds. Post-spawning occurs during the decay of the late spawning carcasses after the period of redd construction. The active spawning period was simulated by exposing SOM to higher suspended sediment (TSS) concentrations (~5 mg l^{-1}) than post-spawning concentrations (~0.5 mg l^{-1}) because active redd construction increases TSS levels (Kondolf, 2000; Petticrew, 2005). Both the active spawning and post-spawning scenarios contribute to the feedback loop but are generated by different conditions.

2.2.1 Study Site Description

Flumes were constructed from decommissioned concrete salmon fry rearing channels at the Quesnel River Research Center in Likely, British Columbia. This is a University of Northern British Columbia (UNBC) research facility that was once a Department of Fisheries & Oceans (DFO) salmon hatchery. Two types of flume configurations were used during the study including a single re-circulating and four flow-through channels (Figure 3).

Only one re-circulating channel was set-up for use in 2004, 2006, 2007, and 2008 because it required the use of a large pump and tubing (Figure 3). Experimental treatments of clay, salmon, and salmon + clay were sequentially added to this re-circulating channel following a baseline period to establish background conditions during which no additions were made to the channel.

The floc formation study detailed here was conducted in the re-circulating channel in 2006, 2007, and 2008. As such, only that flume configuration and experimental conditions are described in this chapter. One flume was used for this part of the study because there

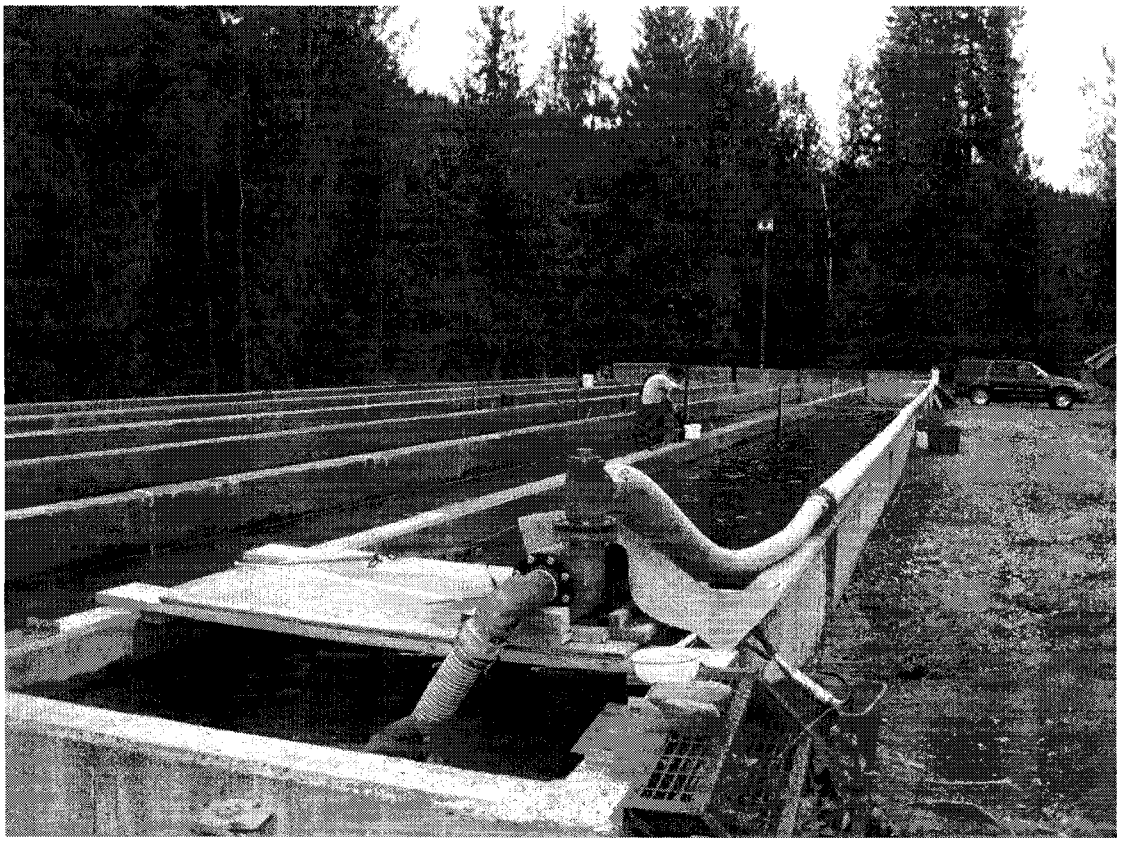

Figure 3: Upstream view of the re-circulating channel in 2006. In the foreground is the Gould's pump used to re-circulate water to the header tank. The LISST-ST was placed at mid-length of the channel near the staff gauge in the channel and Rubbermaid outside the channel. The flow-through flumes are to the left of the re-circulating one.

was one Laser-In-Situ-Scattering and Transmissometry Probe (LISST-ST). The sequential treatment design for the re-circulating channel suited multiple deployments of a single unit better than the simultaneous treatment exposure design used for the four flow-through channels.

The 2 m deep re-circulating channel contained approximately 1 m of fine gravels, sands, and some clay. This substrate could not be removed prior to study of floc formation in the water column because there was no equipment available to do so at the beginning of the study nor was there access for heavy equipment into the channel. Instead of removing it, this material was topped with 20-25 cm of clean gravel and small cobble ranging in size from 1-10 cm (intermediate or b- axis width) in 2006 and a further 10-15 cm of the same sized material in 2007. The grain size of this topcoat material was selected because it is within the preferred gravel size range of spawning Pacific salmon (Bjornn and Reiser, 1991). Topcoat gravels were screened and pressure washed using sediment free groundwater prior to their placement in the channel, further agitation and pressure washing occurred within the channel before each set of experiments to remove stored fine sediment.

The re-circulating flume was designed to replicate the general hydrologic conditions observed at O'Ne-eil Creek rather than specific channel morphologic conditions (i.e. no riffle pool complexes with large woody debris (LWD) placement were created). Flume slope was less than 0.01 m m^{-1} with a water depth of 20-25 cm and a surface water velocity between 5 and 10 cm s^{-1}. The channel was filled with approximately 18,000 liters of aerated groundwater that did not contain SOM or suspended sediments. Water was re-circulated

using a Gould's centrifugal pump at approximately 1800 l min^{-1}. The gravel bed was manipulated to create five 'riffle bars' that were approximately 10 cm higher than the rest of the gravel bed and were 1 m long. The LISST-ST was placed on one of these riffle bars in the center of the flume to measure *in-situ* particle size. To minimize edge effects due to hydraulic drag from the flume walls, the LISST-ST was placed so that the sample window was in the middle of the channel (Figure 4). Stock solutions of clay, salmon, and salmon + clay were introduced to a stock bucket at the head of the channel. The stock bucket was a 72-litre Rubbermaid plastic container with a 200 cm^2 grid of sixteen 0.6 cm holes in the front and rear central portion of the container. The upstream grid was not screened but the downstream grid was screened with 200 μm Nitex to prevent large particles from leaving the stock bucket.

2.2.2 Stock Solution Preparation

Stock solutions were composed of lab grade kaolin clay and SOM derived from rotting 6 kg of Pink salmon (*O. gorbuscha*). Kaolin clay was added to the flumes to a final concentration of approximately 0.5 or 5 mg l^{-1} (Table 2), corresponding to post-spawning and active spawning TSS concentrations from O'Ne-eil Creek (Petticrew, 2005). Prior to its introduction to the re-circulating channel the clay solution was disaggregated using an ultrasonic probe (Misonix Inc, Sonicator, Ultrasonic Processor XL 2020, 10 minute exposure at amplitude setting 4, ~ 220 watts). The kaolin clay size distribution had a mode of 2 μm, which is similar in magnitude to the lacustrine sediment around O'Ne-eil Creek that had a mode of 8 μm. SOM was collected by decanting the liquid fraction of 6 kg of Pink salmon that had rotted for a three-week period in a 20-litre bucket. The 6 kg of salmon tissue set to

Figure 4: Downstream view in the re-circulating flume during *in-situ* monitoring of the baseline water column condition using the LISST-ST. The arrow identifies the direction of water flow through the LISST-ST sample window (gravel bucket in foreground has a diameter of 20cm).

Table 2: Total SOM and kaolin clay additions to the re-circulating channel in 2006, 2007, and 2008.

Year	SOM		Kaolin Clay	
	Mass (grams)	Concentration (mg l^{-1})	Mass (grams)	Concentration (mg l^{-1})
2006 (post-spawn) [1]	118[2]	3.3	18[3]	0.5
2007 (active spawn)	141[2]	3.9	180[3]	5.0
2008 (post-spawn)	378	21	9	0.5

[1] Post-spawn simulation is based on a TSS concentration of ~0.5 mg l^{-1}, and active spawn a TSS of ~5 mg l^{-1}

[2]Concentration based on two equivalent exposures during the salmon and salmon + clay periods.

[3]Concentration based on two equal exposures of 9 and 90 grams respectively during the clay and salmon + clay exposure periods.

rot corresponds to the lower range of salmon tissue observed in the spawning reaches of O'Ne-eil Creek on a streambed area basis (i.e. 100 g m^{-2}) during previous field investigations (McConnachie and Petticrew, 2006; Petticrew and Rex, 2006).

The proportion of liquid salmon decayed from the 6 kg of tissue varied yearly due to differences in decay rates as might be expected under natural conditions. The 2006 and 2007 SOM additions similar but the 2008 value is considerably higher. The difference between 2006-07 and 2008 is likely due to salmon tissue preparation. In 2006-07 salmon were cut into steaks for rotting while in 2008 they were filleted. Filleted salmon may decay more efficiently due to their larger surface area. The combination of stock solutions, or the amounts of SOM and clay used in 2006 and 2008 corresponded to post-spawning conditions (i.e. clay ~0.5 mg l^{-1}) while in 2007 it represented active spawning (i.e. clay ~ 5 mg l^{-1}).

The study period extended between August 18 and September 5 in 2006 and 2007 and August 8 to August 24 in 2008. This timing coincides with the early sockeye (*O. nerka*) and Chinook salmon (*O. tshawytscha*) run in the Quesnel River so the experimental channels experienced the same ambient air temperatures and solar radiation conditions as local salmon streams of similar channel width and depth. Sediment and SOM were added to the re-circulating flume in a sequence to assess the effect of each material on floc formation. In 2006 and 2007, the treatment sequence included a baseline period where no treatment mixtures were added to the channel, followed by a clay treatment, a salmon treatment, a salmon + clay treatment, and a recovery period. Clay, salmon, and salmon + clay treatment samples were collected two days after the addition of the specified treatment mixture. The

recovery sample was collected three days after the salmon + clay sample to determine if concentrations of SOM in the gravel bed were declining. The 2008 sampling program included a baseline, salmon + clay treatment sample, and three recovery samples over a two-week period to identify SOM decline over a longer period than studied in 2006 and 2007.

2.2.3 Sampling Program

To assess the influence of each treatment on floc formation, general water quality conditions were measured and two intensive sampling programs were initiated. The first intensive sampling was a water column particle-sizing program conducted in 2006, 2007, and 2008 using the LISST-ST to quantify suspended sediment particle size distributions. The second program was the flume-bed bacterial enumeration in 2006 and 2007 to assess the relationship between water column flocculation and gravel-bed bacterial enrichment. Bacteria levels from gravel-bed sediments were used as a proxy for their involvement in SOM-based floc formation and subsequent delivery to the flume bed as it was easier to collect bulk samples of flocculated sediments on, or in the gravels, than in the water column.

Pseudoreplication is inherent to this sampling program as well as those presented in Chapters 3 and 4 because several gravel samples are drawn from a single flume but the samples are treated as being statistically independent (Hurlbert, 1984). Pseudoreplicaiton increases the likelihood of a Type I error (rejection of the null hypothesis when it is true) during the analysis of the bacterial samples presented here as well as the other bulk sample data presented in Chapters 3 and 4. Although there is a risk of Type I errors occurring, these sub-samples are treated here as replicates for the purpose of this dissertation.

2.2.3.1 Water Column Turbidity and Conductivity

Water quality conditions were measured at the mid-length and mid-depth of the re-circulating channel in 2008 using a Yellow Spring Instrument 6920 multi-parameter probe. The water column was continuously monitored on a 15-minute interval for temperature (range -5 to 70 °C, accuracy 0.15 °C and resolution 0.01 °C), conductivity (range 0 to 100 ms cm^{-1}, accuracy 0.5% and resolution 0.1 ms cm^{-1}), and turbidity (range 0 to 1000 nephlometric turbidity units (NTU), accuracy 2% and resolution 1 NTU). In 2006-2007 YSI probes were installed in the four flow-through channels and were not available for the re-circulating channel. Instead, daily measurements of turbidity and conductivity were collected along with a continuous recording of water temperature.

2.2.3.2 Suspended Sediment Structure: LISST Sampling

The LISST-ST measures suspended sediment size over 32 size classes ranging from 2 μm to 460 μm. It fires a laser into a known sample area and measures the scatter of laser light on to a series of concentric ring detectors (Agrawal and Pottsmith, 2000). Each ring is calibrated to receive light reflected at a specific angle, where large particles reflect laser light at a small angle while smaller particles reflect at a larger angle (equipment specifications and sampling protocols are provided in Appendix 1). The LISST-ST was mounted on Perspex plastic blocks near the YSI probe approximately halfway down the length of the flume. It was positioned with the 10 cm sample orifice perpendicular to flow 12 cm off the channel bottom.

Prior to its placement in the channel, the LISST-ST probe was calibrated with

ultrapure water to ensure that the background scatter of the instrument was within allowable factory calibration limits. This calibration file was then used to correct field data for background scatter. It was observed during the baseline period that the groundwater used in these flumes was indistinguishable from the laboratory and factory calibration traces with ultra-pure water indicating very low particle content in the channel's water column before stock solutions were added. LISST-ST measurements for each exposure period commenced at the time stock solutions were introduced to the stock bucket at the head of the channel and continued for 72-minutes at a frequency of 1 Hz (n = 4320 samples).

Prior to statistical analysis, LISST-ST data were processed using MS Excel™ macros that verified proper probe functioning and calculated central tendency measures for sample comparison (Williams, 2006). To determine the influence of treatments on the particle size distribution of suspended sediments in 2006 and 2007, a single two-sample Kolmogrov-Smirnov test (K-S test, SYSTAT 12 ™) was used to compare the average grain size distributions (i.e. average from 4320 sample distributions; Siegel, 1956) for each treatment within each sample year.

2.2.3.3 Bacteria Samples

Bacteria samples were collected from wet-sieved gravel-bed samples in 2006 and 2007 (n = 10 and 15 infiltration bag samples each year, respectively). Bacterial samples were collected from the wash water of infiltration bag gravel samples (description in *s.* 3.2.3.1) that were washed with distilled water through a 2 mm sieve. Fine sediments in the wash water were gently re-suspended and a sub-sample was collected with a 20 ml sterile

scintillation vial within 10 seconds of the sediment re-suspension. Bacterial samples were fixed within 30 minutes of collection using a 10% phosphate buffered glutaraldehyde solution in a 9:1 ratio with the sample volume (i.e. 18 ml of sample to 2 ml of fixative) in 2006. In 2007, the Live-Dead Bac kit from Molecular Probes™ was used and preservation included fixation with 2% formaldehyde following a 15 minute incubation with 5-cyano-2, 3-ditolyl tetrazolium (CTC) (Smith *et al.*, 1994)

Bacterial enumeration was completed within an eight-week period from sample collection and followed the modified direct count procedure of Droppo *et al.* (1996). Sudan black stained, 1 μm and 0.1 μm filters were used to operationally define attached bacteria (1 μm) and unattached bacteria (0.1 μm) that had been stained with Acridine Orange or Syto 9™ (Molecular Probes Inc.). Both stains are effective nucleic acid stains (Neu *et al.*, 2002). Once samples were stained and filtered, filters were mounted on slides using low-fluorescence immersion oil (Type Cargille DF). Bacteria were enumerated using a BX-50 fluorescence microscope equipped with a 100-W mercury lamp, B filter package (DM-580 dichromic mirror and a 0-590 filter), BG-36 excitation filter and a 0-515 barrier filter. An Olympus UVFL 100 oil-immersion objective was used to provide a total magnification of 1200X. In accordance with standard procedures (APHA, 1998) a minimum of 200 bacteria were enumerated on each filter and/or a minimum of 20 fields of view. Attached and unattached bacteria levels were compared across treatments each year using a two-way analysis of variance (ANOVA, Sokal and Rohlf, 1995) in SYSTAT 12™ with form (attached and unattached) and treatment (baseline to recovery) as factors. When significant differences were identified by ANOVA, post-hoc multiple comparisons were made using Tukey's

honestly significant difference test because it is a conservative multiple comparison technique (Quinn and Keough, 2002).

2.3 Results

2.3.1 Water Column Turbidity and Conductivity

The average daily temperature varied over the monitoring period within and between years because water within the re-circulating channel is open to ambient heating and cooling. Daily ranges were typically less than 4 °C (Figure 5). Average daily temperature was highest in 2008 (Table 3) due to higher ambient air temperatures and direct solar radiation compared to 2006 and 2007. Average daily turbidity values show minimal turbidity increases each year (Table 3). Although turbidity values do show some spikes on a daily basis in 2008 most were less than 3 NTU (Figure 5). Given the sporadic nature of the turbidity increases in 2008 and their closeness to the resolution of the probe, they are not considered to represent a trend of increasing turbidity. Conductivity in contrast, generally shows an increase over the sequence of treatments in 2006 and 2007. Conductivity increased by 25% and 10% over baseline conditions in 2006 and 2007, respectively (Table 3). The increase in conductivity in these years is positively associated with temperature as well as the addition of SOM. The liquid fraction of SOM added to the re-circulating flume includes electrolytes, so it was expected to increase water conductivity levels.

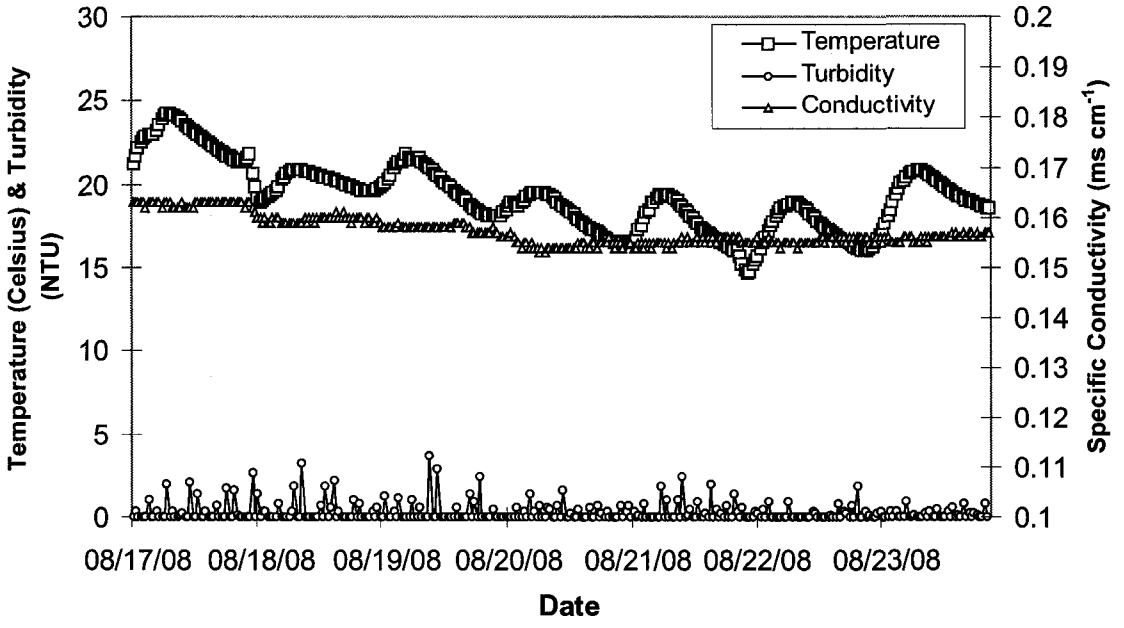

Figure 5: Water quality data for the re-circulating channel from August 17 to 23, 2008 after the addition of salmon + clay.

Table 3: Average daily water quality conditions in the re-circulating channel in 2006, 2007, and 2008. (Temperature for each year and conductivity and turbidity for 2008 based on 15-minute samples while turbidity and conductivity in 2006 and 2007 are based on once-daily samples over the two-day treatment period. Standard error is provided in brackets if significant digits were similar or less than the average).

	Water Temperature (Celsius)			**Turbidity (NTU)**			**Specific Conductivity (ms cm^{-1})**		
	2006	**2007**	**2008**	**2006**	**2007**	**2008**	**2006**	**2007**	**2008**
Baseline	14.1 (0.5)	13.0 (1.2)	18.4 (0.2)	0.4	0	0.3	0.144	0.119	0.178
Clay	12.5 (0.9)	13.1 (0.7)		0.7	0		0.162	0.121 (0.001)	
Salmon	12.6 (1.7)	14.2 (0.9)		0.1	0		0.188	0.127 (0.001)	
Salmon + clay	14.4 (2.3)	14.0 (0.5)	19.2 (0.1)	0.9 (0.1)	0.2 (0.2)	0.3 (0.04)	0.178 (0.003)	0.134 (0.001)	0.162

2.3.2 Suspended Sediment Structure: LISST Sampling

This section focuses on data gathered in 2006-2007 because an in-channel algal bloom compromised the 2008 water column particle-sizing program. Similar blooms were not observed in 2006 or 2007, possibly due to the lower temperature and mass of SOM added during those years (Tables 2 and 3).

In both 2006 and 2007, the suspended sediment particle size distribution significantly changed following the addition of SOM and the salmon + clay mixtures (Figure 6). There was a larger proportion of > 200 μm particles generated in 2007 when higher concentrations of clay and SOM were used to simulate active spawn conditions (Table 2). The 2006 cumulative grain size distribution exhibits a more modest change when post-spawn conditions were simulated (Figure 6). In 2006, the proportion of particles larger than 200 μm was lowest in the baseline sample (1%) compared to the other samples (K-S test $p < 0.01$), while clay (4%) was slightly higher than baseline and statistically lower (K-S test $p < 0.01$) than the salmon (16%) and salmon + clay samples (6%), which were not statistically different (Figure 6). The 2007 cumulative distribution curves show more exaggerated differences between exposures than the 2006 data (Figure 6).

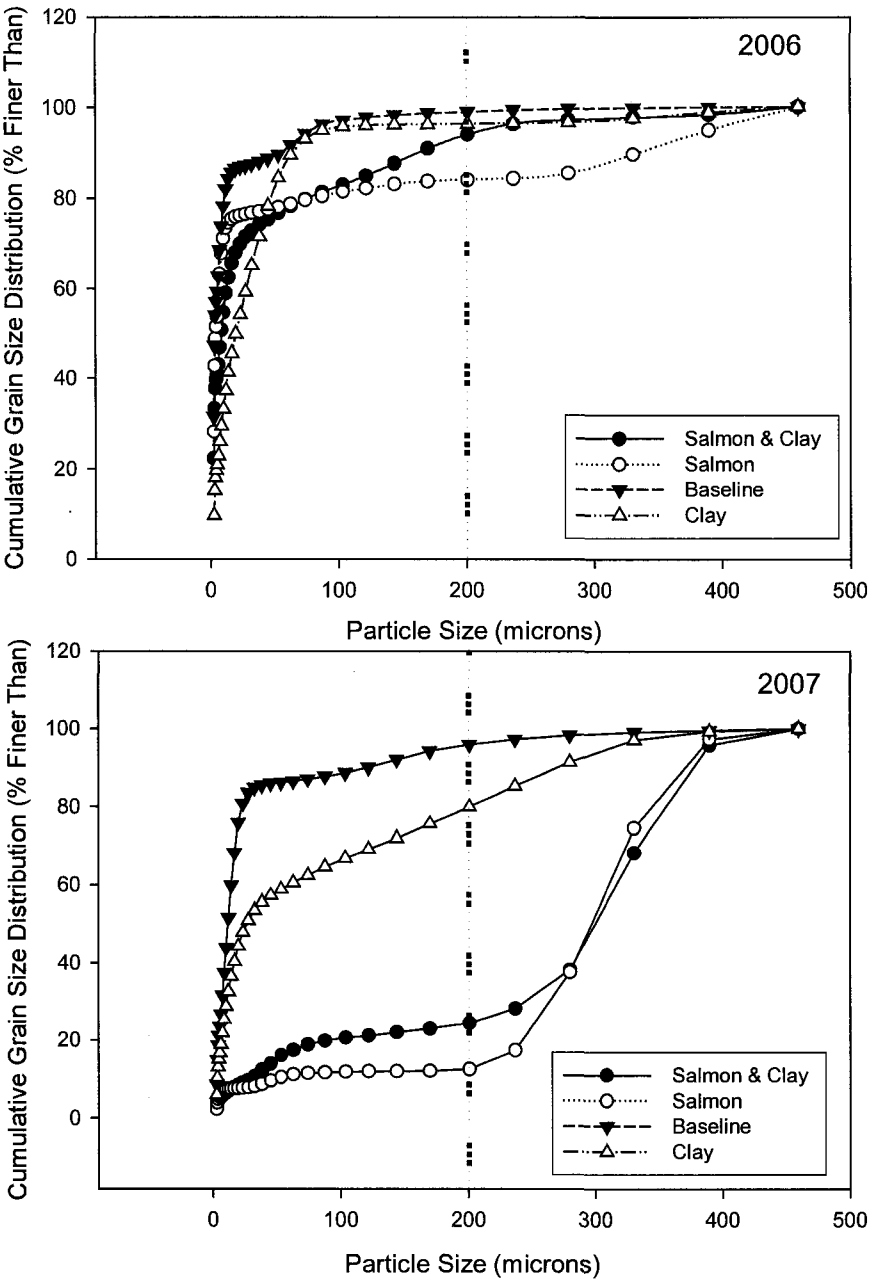

Figure 6: Average suspended sediment particle size distribution curves during each treatment period (72 minute exposure, n=4320). Particles to the right of the vertical line at 200 μm line formed in the water column because stock materials added to the channel were screened at 200 μm.

The proportion of suspended sediments >200 μm significantly increased from baseline (4%) to clay (20%) (K-S test $p < 0.01$), followed by salmon + clay (76%) and salmon (87%), which were not statistically different. There was an increase in the proportion of particles > 200 μm from 2006 to 2007, but the relative rank remained the same across years. The baseline and clay samples had a 4 and 5-fold increase in particles > 200 μm from 2006 to 2007, but they remained significantly lower than the salmon and salmon + clay samples, which had a 5 and 12-fold increase in the proportion of > 200 μm from 2006 to 2007. Ambient environmental conditions and SOM additions were generally similar between 2006 and 2007. One exception is the water temperature during the 2007 SOM exposure, which was ~ 2 °C warmer that the 2006 SOM exposure. The primary and designed difference between these years was the amount of clay added, namely post-spawning (0.5 mg l^{-1}) conditions in 2006 and active spawning (5.0 mg l^{-1}) in 2007.

2.3.3 Bacteria Levels

Bacterial levels positively responded to the addition of salmon and salmon + clay in 2006 and 2007 (Figures 7 and 8). In 2006, significant differences were found in bacterial levels between treatments ($F_{4, 10} = 15.9, p < 0.01$). Specifically, the baseline and clay samples had lower total bacterial levels than the salmon, salmon + clay, and recovery samples (Figure 7). The salmon and recovery samples were similar as were the salmon + clay and recovery samples (Figure 7). Further, there were significantly more attached bacteria than unattached bacteria ($F_{1, 10} = 197.5, p < 0.01$), which was observed consistently across all treatment conditions ($F_{4, 10} = 14.9, p < 0.01$).

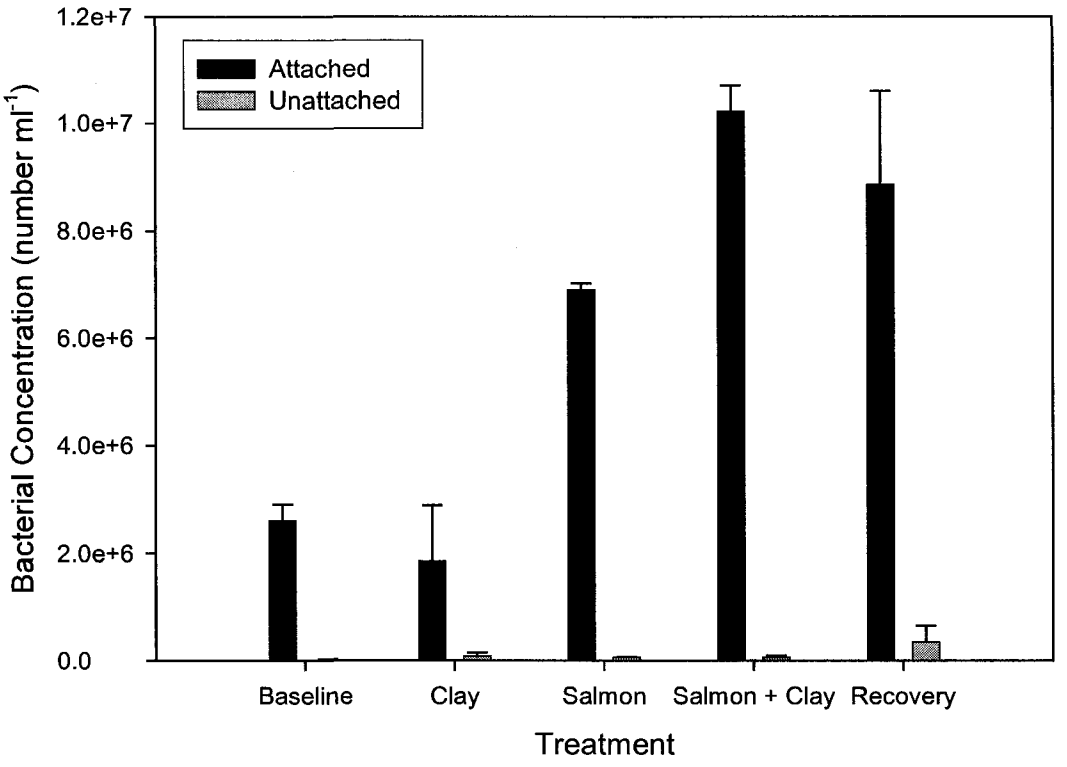

Figure 7: Average attached and unattached bacterial concentration from gravel bag samples collected in 2006. Error bars represent one standard error of the mean (n=2 infiltration bags per treatment).

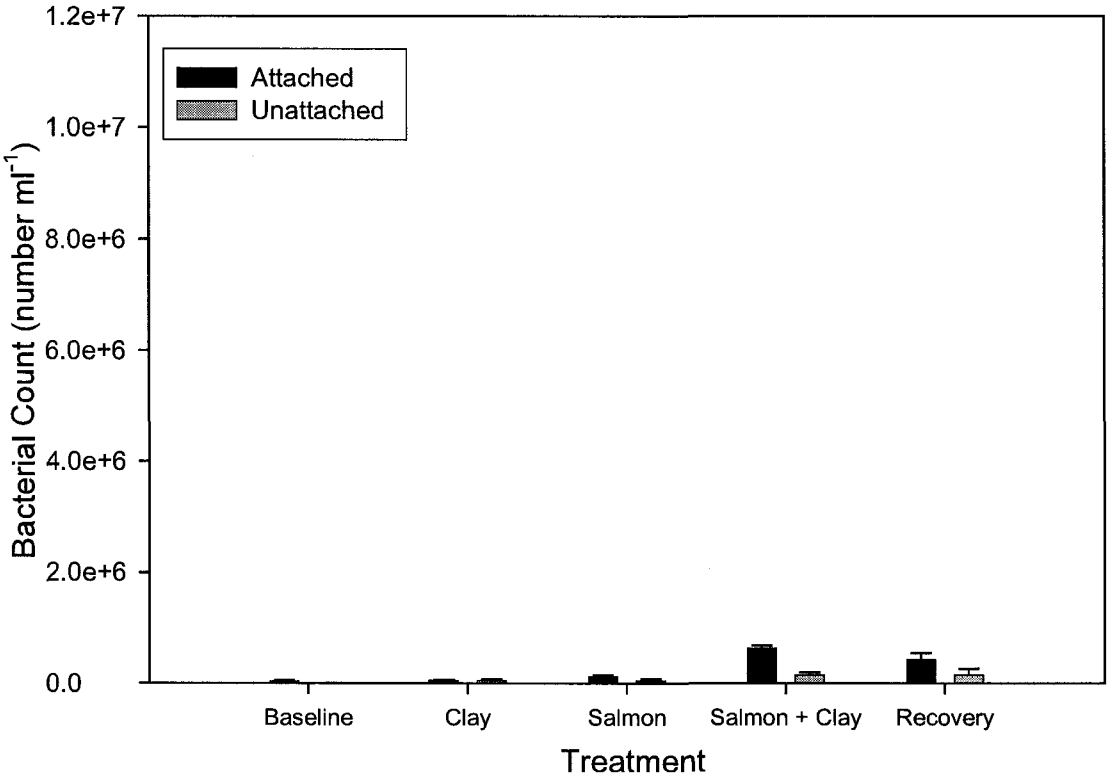

Figure 8: Average attached and unattached bacterial concentration from gravel bag samples collected in 2007. Error bars represent one standard error of the mean (n=3 infiltration bags per treatment).

Bacteria levels were an order of magnitude lower in 2007 than those observed in 2006. Despite this difference between years, the general pattern remains the same, with higher bacterial counts following the addition of SOM. There were significantly different bacteria levels between treatments in 2007 ($F_{4, 23} = 12.1, p < 0.01$) with the baseline, clay, and salmon samples being similar to each other and the salmon + clay and recovery samples also being similar (Figure 8). As in 2006, unattached bacteria concentrations were significantly lower than attached forms for salmon + clay and recovery ($F_{1, 23} = 17.2, p < 0.01$). Again, similar to 2006, the significant interaction effect ($F_{4, 23} = 4.6, p < 0.01$) indicates that a positive relationship exists between SOM and the presence of attached bacteria but not unattached bacteria.

2. 4 Discussion

The first stage of the salmon-floc feedback loop, floc formation and MDN incorporation, was verified by the re-circulating flume studies of 2006 and 2007. The LISST-ST data from 2006 and 2007 demonstrate that flocs > 200 μm formed in the water column during the salmon and salmon + clay exposure periods. There also appears to be an increase in particles < 200 μm. Flocs < 200 μm may be forming in the flume, however the use of a 200 μm Nitex screen in the stock-bucket precludes a definitive statement on smaller floc formation. The generation of smaller flocs, which can dominate the particle size distribution, is likely dependant upon the sources of organic and inorganic material. For example, Droppo *et al.* (1998) identified that the majority of flocs observed in the Mackenzie River Delta ranged between 12 and 40 μm. Flume-bed bacterial concentrations followed a

similar pattern as suspended particle size distributions by showing significant increases following the addition of salmon + clay.

The average water quality conditions in the re-circulating channel in 2006-2008 showed diurnal temperature changes and an increase in average daily temperature of ~2 °C during the SOM exposure in 2007 as well as increases in conductivity (2006-2007), the latter of which may be influenced by temperature and SOM additions. Ambient conditions during the re-circulating study were planned to reflect natal streams in the Likely, British Columbia region that have a similar width and depth. Averaged data from 2006-2007 and the continuous data from 2008 indicate that water temperatures were below lethal limits for most salmonids (Bjornn and Reiser, 1991).

The differences in the suspended sediment particle size distributions observed between 2006 and 2007 may be due to a combination of factors including temperature, clay concentration, and SOM concentration. The 2007 particle size distributions for clay, salmon, and salmon + clay had more particles > 200 μm than in 2006. Although they were not statistically compared because they were generated under different experimental conditions (i.e. active spawning and post-spawning) it is important to highlight some factors that may account for the difference.

Floc formation of particles >200 μm during the SOM exposure may have been enhanced in 2007 compared to 2006 due to higher temperatures (~2 °C) in 2007. Temperature increases enhance bacterial activity and floc formation within the range of 0-19

°C (Lau, 1990). Further, the 2007 additions of SOM were slightly larger and the addition of clay was substantially higher because it was supposed to reflect active spawning conditions with a higher suspended sediment concentration. Higher clay concentrations are known to increase floc formation (Milligan and Hill, 1998). The clay concentrations used in this study, < 0.5 mg l^{-1} and ~ 5.0 mg l^{-1}, are similar in magnitude to those observed in O'Ne-eil Creek. Petticrew (2005) observed increased suspended sediment concentrations from < 1 mg l^{-1} in the pre-spawn period to > 11 mg l^{-1} following the arrival of close to 27,000 spawning sockeye salmon and to 4 mg l^{-1} during the arrival of close to 11,000 spawning sockeye (*O. nerka*) in O'Ne-eil Creek. The increase in the proportion of flocs larger than 200 μm in 2007 may be due to the higher concentration of clay but the influence of SOM cannot be underestimated. As noted during salmon only exposures in both 2006 and 2007 the presence of SOM alone increased suspended particle size as organic particles combined likely due to bacterial EPS as identified by Wotton (2004). Although these large organic flocs form in the water column, they are not sequestered to the flume bed as readily as salmon + clay flocs as shown by bacterial counts.

Despite the disparate suspended sediment particle size distributions of 2006 and 2007, the pattern remains the same, namely that significantly more particles > 200 μm are generated in the water column when salmon or salmon + clay are present. Given that no particles > 200 μm were added to the flume this is an important finding, because it demonstrates that flocs formed before they reached the LISST-ST halfway down the channel from the stock buckets and during each circulation of the re-circulating channel. Further, they formed in sufficient quantity to alter the size distribution of suspended particles when

the lower range of field observed quantities of salmon tissue (i.e. 100 g m^{-2}) was mixed with sediment concentrations similar to post-spawning (< 1 mg l^{-1}) and active spawning periods (~5.0 mg l^{-1}). This in-channel floc generation was a result of both bacterial EPS and water column physico-chemical interactions. Flocs either maintained their structure despite the shear created by the centrifugal pump or floc parts that were broken apart during re-circulation formed new flocs prior to passing through the LISST-ST sample window. Kranck and Milligan (1980), working in saline water, identified that macro-flocs up, to 10 mm in diameter, formed quickly in water velocities up to 0.25 cm s^{-1}.

Gravel-bed bacterial numbers increased following the addition of SOM but were highest following the addition of salmon + clay indicating that these flocs were more effectively delivered than flocs formed by SOM alone. Using bacterial counts as a proxy for EPS involvement in floc formation, the findings from both 2006 and 2007 indicate that bacteria play a significant role. The number of attached bacteria was significantly higher in both years compared to unattached forms. These results reflect the literature on non-SOM flocs and biofilms where bacterial EPS are involved in accessing other organic sources and forming biofilms (Droppo, 2004; Battin *et al.*, 2005; Bhaskar and Bholse, 2005; Strauss, 2005; Wotton, 2007). Further, field study in O'Ne-eil Creek identified that suspended sediments had higher levels of attached bacteria during the post-spawn period than during the salmon-free low flow period (McConnachies, 2003). The role of bacterial EPS in generating flocs is further supported by lab-based flocculator studies which identified that flocs did not form in a salmon + clay mixture following treatment with azide, a bacterial growth inhibitor (Arkinstall, 2005).

Chapter 3: Stage 2 Floc Sedimentation and Storage

3.1 Background

Flocs are an integral part of the sediment load because they facilitate the movement of inorganic and organic matter through the watershed (Droppo, 2000; Wotton, 2004). The sediment load refers to the mass of suspended and bedload sediments passing a point in a watershed over a set period of time (Leopold, 1994). Suspended sediments are typically < 63 μm in size while bedload sediments are often > 63 μm, however there is some exchange between the forms (Knighton, 1998). Flocs will shift between sediment load types as a function of floc characteristics, hydrologic conditions, and streambed roughness. To understand how flocs move from the suspended load to the bedload these relationships must be clarified.

Suspended sediment is the fine inorganic and organic particulate matter that is held in the water column while bedload refers to particulates associated with the riverbed (Leopold, 1994). Suspended sediment includes fine sand, silt, and clay the latter two of which behave like cohesive sediments (Partheniades, 1975). Cohesive sediments by definition tend to bind together to form inorganic aggregates (Droppo, 2000). Inorganic aggregates differ from flocs because they do not have an organic component. Bedload generally consists of heavier materials on or within the streambed that can either be momentarily suspended and transported downstream by saltation or alternatively slid along the immobile bed surface (Leopold, 1994).

Suspended sediments are entrained in the bedload due to settling and interception by

the gravel bed. Settling is regulated by ambient hydrologic conditions and/or the suspended sediment's structure, composition, and density. Suspended sediments settle as a function of gravity, particle shape and density, stream discharge, and fluid viscosity. If shear velocities above the streambed surface are less than the settling velocity of the particle it will settle out of suspension but if shear is greater, the particle will not settle (Knighton, 1998). Decreased velocities can occur as a result of an increase in the hydraulic radius of a channel or decrease in slope such as at transition locations between riffle/run areas and pools or wider braided channels (Knighton, 1998). Streambed topography and porosity also play a role because elevated bed areas such as salmon redds, enhance surface water movement through porous gravel bed areas (Bjornn and Reiser, 1991). Altered flow patterns that increase velocity across elevated streambed areas also increases intergravel flow and the opportunity for particle capture as identified by Tonina and Buffington (2007).

Suspended sediment structure and composition will change as a result of flocculation (Droppo, 2000). Flocculation causes fine sediments (<63 μm) to form larger particles, which have higher settling rates than their individual components (Liss *et al.*, 2005). Thomas *et al.* (2001) identified that ^{14}C labeled medium-sized particulate organic matter (107-250 μm) had shorter travel distances in first and second order streams than fine (53-106 μm) or very fine particulate organic matter (<53 μm) during high flow conditions. Further, as particle size increases so does the likelihood of retention in the streambed matrix as it moves through intergravel pores. Brunke (1999) found that more than 72% of mobile matrix fines were smaller than 100 μm while less than 50% were less than 30 μm.

Floc formation during the salmon spawning period should provide an effective mechanism for delivering MDN to the streambed because flocs are larger than their component inorganic suspended sediments and they have a modified particle size, shape, and density compared to SOM alone. Floc settling velocity is positively correlated with increasing floc size (Liss *et al.*, 2005) as well as density in accordance with Stokes Law (Equation 1).

(Equation 1) $v = 1/18 \{((g*d^2)*(\rho_f - \rho_w))/\mu\}$

Where:

v = terminal settling velocity,
g = gravitational constant
d = diameter of particle
ρ_f = wet density of the particle
ρ_w = density of water
μ = viscosity of water

Stokes Law does not directly predict the settling rate of a floc because flocs are not spherical (Liss *et al*, 2005), but it does provide an approximate settling rate. Floc settling rates are also influenced by both shape and orientation in the water column. Spherical flocs settle fastest, followed by cylindrical, needle-like, and disc-like shapes (Lerman, 1979). Further, denser flocs can be expected to settle more quickly than less dense flocs that have higher porosity (Liss *et al.*, 2005).

Settling rates for flocs observed in O'Ne-eil Creek ranged between 2 and 9 mm s^{-1} (Petticrew, 2005). Interestingly, these settling rates are relatively low when compared to in-stream flow velocities of 5-10 cm s^{-1} also noted in O'Ne-eil Creek (Petticrew and Rex, 2006) and used in the flume (Chapter 2). Given the difference between floc settling rates observed in natural systems and the stream flow velocities in the creeks and experimental flumes used

in this study, streambed topography likely plays an important role in the capture and storage of flocs.

To determine if SOM-based flocs that formed in stage 1 of the salmon-floc feedback loop settled onto or were intercepted by the flume's gravel bed (research question 2, Chapter 1), I investigated the effect of SOM and clay mixtures on quantity, size, bacterial number, and carbon to nitrogen ratios of gravel bed sediments in experimental flumes. If SOM-based flocs are entrained into the gravel bed of the flume they should alter the particle size distribution of fine sediments in the flume bed toward large grain sizes as well as increase the nitrogen content and bacterial levels of those gravels.

3.2 Methods

3.2.1 Flume Description

Two flume types were used during this sampling program, including the single re-circulating flume detailed in Chapter 2 (*s.* 2.2.2.1) and four flow-through channels. The floc sedimentation and interception study was conducted in one or both types of flumes each year from 2004 to 2008 (Table 4). Flow-through experiments were initiated in 2005 using the single flume, without the re-circulating pump, but again with sequential treatments. Flow-through studies continued in 2006 and 2007 but used the four adjacent flumes at the same time with treatments added simultaneously (Figure 9). The single flume used in the preliminary study of 2004 as a re-circulating system with sequential treatments was also used for re-circulating channel studies in 2006, and 2007.

Table 4: Total SOM and clay additions to the flow-through and re-circulating channels from 2004-2008.

Year	Flume Type and Number	Surface Gravel[1]	Treatments and Type	Sediment Type[2]	Dosage SOM (g)	Dosage Clay (g)
2004	Re-circulating (1)	Rounded Fluvial	Baseline			
			Clay	Takla		83
			Salmon + Clay		7143[3,4]	83
2005	Flow-through (1)	Rounded Fluvial	Baseline			-
			Clay	Kaolin		90
			Salmon		393[4]	-
			Salmon + Clay	Kaolin	674[4]	90
2006	Re-circulating (1)	Rounded Fluvial	Baseline		-	-
			Clay	Kaolin	-	9
			Salmon		59	-
			Salmon + Clay	Kaolin	59	9
	Flow-through (4)	Angular Crush[4]	Control		-	-
			Clay	Kaolin	-	9
			Salmon		118	-
			Salmon + Clay	Kaolin	118	9
2007	Re-circulating (1)	Rounded Fluvial	Baseline		-	-
			Clay	Kaolin	-	90
			Salmon		70	-
			Salmon + Clay	Kaolin	71	90
	Flow-through (4)	Rounded Fluvial	Control		-	-
			Clay	Kaolin	-	90
			Salmon		118	-
			Salmon + Clay	Kaolin	118	90
2008[5]	Re-circulating (1)	Rounded Fluvial	Baseline		-	-
			Salmon + Clay	Kaolin	378	9

[1]Surface gravel consisted of 5-10 cm b-axis rounded gravel except for angular gravel in flow-through channels.
[2]Takla sediment gathered near O'Ne-eil Creek or lab grade kaolin clay was used as the sediment source.
[3]Salmon addition in 2004 is 119.05 g/m^2
[4]Salmon addition in 2004 and 2005 was not screened to remove larger salmon particles, mass dropped in future years to target the <200 μm fraction solely.
[5]2008 study focused on tracing nitrogen through the water column and gravel-bed.

The re-circulating channel conditions were previously described so this section focuses on the flow-through systems. Four flow-through channels were prepared so that each of the applicable treatment stock solutions (control, clay, salmon, and salmon + clay) could be added simultaneously to separate channels. Prior to the addition of stock solutions each channel had a baseline period during which only pumped groundwater flowed through the channel. The clay, salmon, and salmon + clay channels were each 30 m long, 2 m wide, and 2 m deep. The control channel, to which no treatment was applied, was only half-length (15 m) because of limited gravel available to fill the channel.

In 2006, when the four flow-through channels were initially set up they were completely emptied of storage material consisting of gravel and sand (approximately 640 tonnes) that was replaced with washed 1.8 cm (intermediate or b-axis) crushed gravels supplied by Mount Polley Mine. Due to the lack of available rounded gravels to topcoat the angular crush, experiments were run using these gravels in 2006. Although angular gravels are not similar to the streambed surface gravels of most salmon spawning streams, it was suitable for the 2006 study because the 1.8 cm angular gravel used is among the most effective grain size and shape for trapping fine sediments (Meehan and Swanston, 1977). In 2007, washed fluvial gravels and cobbles were available to cover these angular gravels. The new layer of rounded gravels was approximately 30 cm deep and was composed of material ranging in size from 1-10 cm similar to the re-circulating channel.

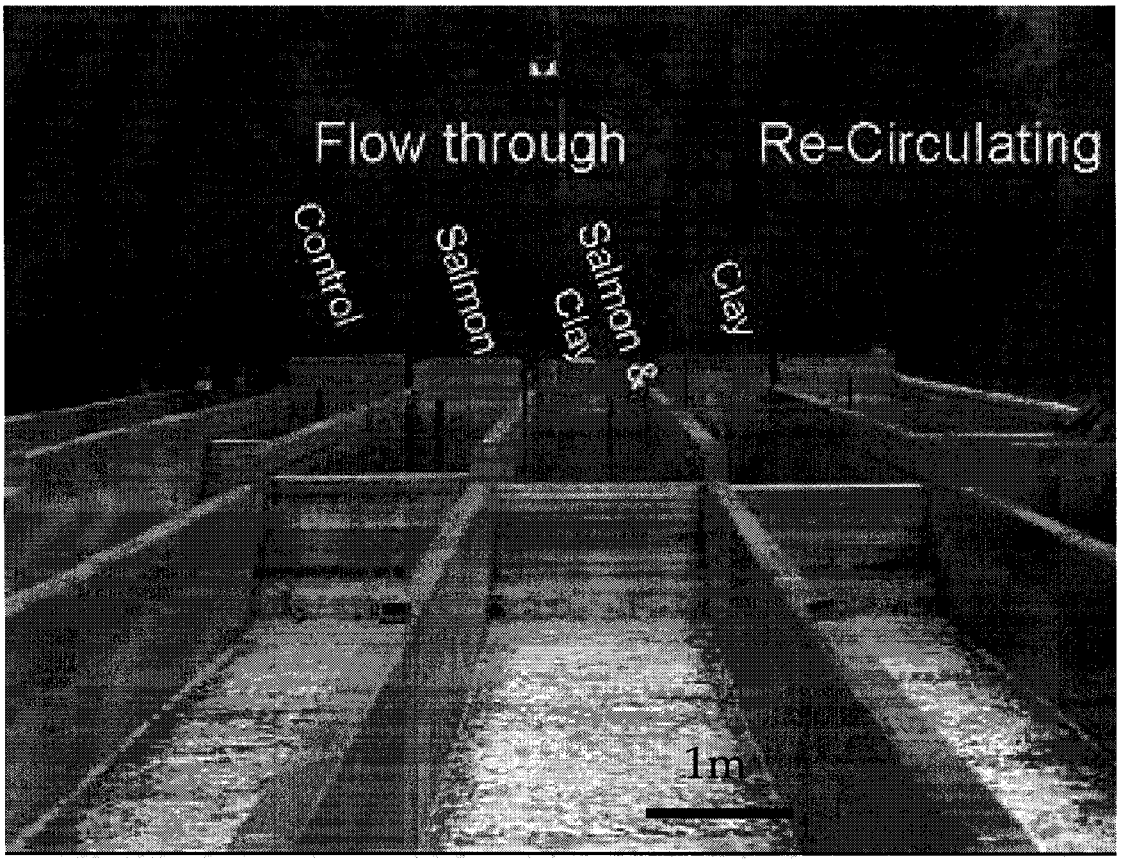

Figure 9: Upstream view of the four flow-through channels and the re-circulating flume conjointly used in 2006 and 2007.

The re-circulating and flow-through flumes were designed to replicate the general hydrologic conditions observed at O'Ne-eil Creek rather than specific channel morphologic conditions (i.e. no riffle pool complexes with large woody debris (LWD) placement were created). Flume slope was less than 0.01 m m^{-1} with a water depth of 20-25 cm and a surface water velocity between 5 and 10 cm s^{-1}. The flow-through channels were continuously fed the aerated groundwater from the same source that was used to fill the re-circulating channel at a volume ranging between 1500 and 1900 l min^{-1}. In each of the flumes, the gravel bed was manipulated to create five 1 m long 'riffle bars' that were approximately 10 cm higher than the rest of the gravel bed.

Stock buckets were placed at the head of the channel and specified mixtures of clay, salmon, or salmon + clay were added to the bucket at the beginning of the exposure period. Water from the header tank entered stock buckets (72-litre Rubbermaid container) that had a 200 cm^2 grid of sixteen 0.6 cm holes in the front and rear central portion of the container (Figure 10). The upstream grid was not screened but the downstream grid was screened with 200 μm Nitex to prevent large particles from leaving the stock bucket.

3.2.2 Stock Solution Preparation

Stock solutions for treatment conditions within the flow-through and re-circulating flumes consisted of Takla sediment (2004 only), laboratory grade kaolin clay (2005-2008) and SOM that was derived from rotting Pink salmon (*O. gorbuscha*). Takla sediment was added to the flume at 5 mg l^{-1} while kaolin clay was added to the flumes to bring them to a

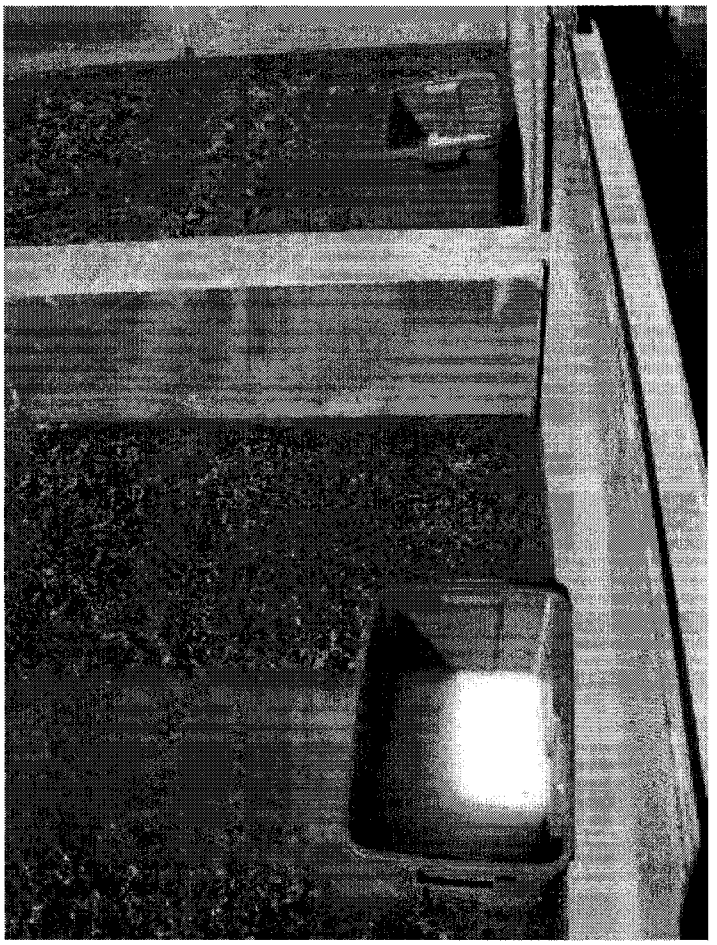

Figure 10: View of stock buckets in the flow-through channels. The clay treatment bucket is in the forefront and the salmon-clay mixture near the top of the photo. Note: water from the header tank passes through the turbulence baffle, enters the stock bucket on the right side of the photo, and then exits the stock bucket on the left as shown by clouds of clay (bottom) and salmon + clay (top).

final concentration of 0.5 or 5 mg l^{-1} (Chapter 2, Table 2). Prior to their introduction to the re-circulating channel, the Takla sediment and kaolin clay solution were disaggregated using an ultrasonic probe to minimize clay aggregates (Misonix Inc, Sonicator, Ultrasonic Processor XL 2020, 10 minute exposure at amplitude setting 4, ~ 220 watts). The kaolin clay size distribution had a mode of 2 μm, which is similar in magnitude to the lacustrine sediment of the Takla Lake area that had a mode of 8 μm. SOM preparation followed the procedure identified in section 2.2.2.

The study period was the same as in Chapter 2 (*s.* 2.2.2) extending from mid-August to early September and overlapping with the early salmon run in the Quesnel River. Each of the four flow–through flumes were exposed to a baseline period when no treatment mixtures were added to the water column. Following this, one of the four individual, treatments (clay, salmon, salmon + clay or control) was applied to each of the flow-through channels simultaneously so that exposure periods were equivalent between flumes. In contrast, clay and SOM were added to the re-circulating flume in sequence to assess the effect of each material on floc formation. Specifically, they followed the sequence of a baseline, clay, salmon, salmon + clay, and a post-treatment recovery sample for 2005, 2006 and 2007. In 2004, sequential treatments included control, clay, and salmon + clay while in 2008 only baseline and salmon + clay treatments were implemented (Table 4).

3.2.3 Sampling Program

To assess the influence of each treatment condition on floc sedimentation and interception by the gravel bed, samples were collected using infiltration bags and gravel buckets (Table 5).

Samples were removed in accordance to a sequence provided by a random number generator. Prior to sample removal, overlying water depth and velocity data was collected to identify if there were substantial differences in hydrologic conditions between collected samples. Once collected, the fine sediments captured by the gravel beds were assessed for carbon and nitrogen ratios, fine sediment mass, absolute particle size distribution (APSD), effective particle size distribution (EPSD), settling velocity, and bacterial enumeration.

3.2.3.1 Infiltration Bags

Infiltration bags were chosen for this study because they provide a measure of the amount of sediment moving vertically and horizontally through a streambed (Lisle and Eads, 1991). They were constructed of a waterproof fabric bag 20 cm in diameter and 35 cm long that was attached with a hose clamp or large zip-tie to a brightly coloured steel ring that was also 20 cm in diameter. The fabric bag was collapsed into the ring and placed in a hole excavated to a depth of 25-30 cm in the gravel-bed. This was covered with washed reference gravel that had been excavated from the holes. Reference gravel was washed and sieved to remove particles less than 2 mm (Figure 11). Infiltration bags were placed in the shallow riffle bars a minimum of 30 cm from the sidewalls of the flume to prevent edge effects due to surface drag. They were installed in 30 cm deep holes that had a 30 cm diameter. Infiltration bags were installed in a downstream direction and removed in an upstream direction, to minimize contamination. During bag recovery, retrieval ropes attached to the buried iron ring were located on the streambed and the sample bag was lifted through the gravel column capturing reference gravel and its trapped sediments. Once the ring was lifted above the

Table 5: Gravel bed sampling techniques used and analyses completed for each project year.

Year	Sampling Equipment	Analyses
2004	Infiltration Bags	TSS, C:N, APSD
2005	Infiltration Bags	TSS, C:N
2006	Infiltration Bags and Gravel Buckets	TSS, C:N, APSD,EPSD, Bacterial Enumeration
2007	Infiltration Bags and Gravel Buckets	TSS, C:N, APSD,EPSD, Bacterial Enumeration
2008	Infiltration Bags and Gravel Buckets	C:N, Nitrogen

TSS-Total Suspended Sediment ~ Fine Sediment Mass, C:N-Carbon to Nitrogen ratio, APSD- Absolute Particle Size Distribution, EPSD-Effective Particle Size Distribution, Nitrogen: Total Nitrogen, Ammonia, and Dissolved Organic Nitrogen

Figure 11: Infiltration bag deployed in a flow-through flume in 2007. Pull ropes extend from the flume's gravel surface and float in the water column for ease of location during their removal. Inset photo shows an infiltration bag lying horizontally on the ground prior to its installation in the channel – note the iron ring and the flexible waterproof bag (distance between ropes is ~20 cm).

streambed surface, it was covered with a lid to prevent loss of captured sediments during retrieval through the water column. The sample was then transferred to a clean 20-liter sample bucket. Infiltration bag sample numbers varied over the sampling program depending upon the number of treatments and flumes used (Table 6).

3.2.3.2 Gravel Buckets

Gravel buckets measure deposition onto and infiltration into the streambed from surface sediments. Accordingly, they generally capture less sediment than infiltration bags because they do not sample sediment moving horizontally through the gravel bed (Rex, 2002). Gravel buckets were placed in lower elevation flume bed areas located in front of the riffle bars where infiltration bags were placed. They consisted of a 4-liter hard-walled plastic bucket filled with surface gravels from the flume that were pre-screened and washed with channel water to remove particles < 2 mm. Sealed buckets were buried to their rim and lids were removed in a downstream direction once disturbed sediment had settled (Rex, 2002). Buckets were retrieved in an upstream direction after removing the infiltration bags. Lids were placed on the bucket opening to prevent sampling the overlying water column and then the buckets were removed. Gravel bucket sample numbers varied over the sampling program depending upon the number of treatments and flumes used (Table 7).

3.2.4 Sediment Sample Preparation

Once gravel-bed samples were removed from the flume via infiltration bags or gravel buckets, they were re-screened and washed using a 2 mm screen and distilled water into a

Table 6: Total number of infiltration bags used in the re-circulating and each flow-through channels during sample program years 2004-2008.

Year	Re-circulating Channel	Flow-Through Channels
2004	21	
2005		15
2006	10	10 per channel
2007	15	15 per channel
2008	15	

Table 7 Total number of gravel bucket samples collected in re-circulating and flow-through channels during sample program years 2005-2007.

Year	Re-circulating Channel	Flow-Through Channels
2005		15
2006	10	10 per channel
2007	15	15 per channel

Rubbermaid container. Water volume in the container was measured after which representative sub-samples were collected for biochemical oxygen demand (BOD), APSD/EPSD, carbon to nitrogen ratios, bacterial enumeration, and total suspended solids analyses.

3.2.4.1 Total Suspended Solids Analysis

To identify if there was an increase in the mass of fine sediment stored in the gravel bed over the course of the study, total suspended solids samples were collected from infiltration bags. Total suspended solids concentrations were determined using standard techniques (APHA, 1998). Specifically, known volumes of intergravel water were filtered with pre-ashed and pre-weighed glass fibre filters (Whatman GF/F 47 mm) that were desiccated and dried at 105 °C for 24 hours to remove water content, and then ashed at 500 °C for 3 hours to remove organic content. Total solids mass was converted to total mass for each infiltration bag sample. Total mass per bag was compared between treatments each year using a one-way ANOVA for re-circulating channel samples and a two-way ANOVA for flow-through channels (baseline/exposure and treatment as factors) followed by post-hoc comparison using Tukey's HSD (Sokal and Rohlf, 1995).

3.2.4.2 Particle Size Analysis

The 2004 particle size samples were analyzed using a Coulter Counter electro-resistance particle counting technique. The 2006-2008 samples were analyzed by LISST-ST to allow samples to be analyzed in their aqueous condition. The LISST-ST provides the opportunity to measure both inorganic and flocculated particles (EPSD) of a sample followed by physical and chemical dispersion and then re-measurement of the sample for inorganic

particles as well as the dispersed organic and inorganic floc component sizes (APSD). In contrast, the Coulter method only measures the inorganic fraction (APSD) from combusted and filtered samples. Samples were collected and prepared for Coulter analysis in 2005 but were not analyzed.

Coulter samples were filtered on pre-washed and weighed 0.8 μm millipore filters that were then dried, burned, and analyzed for APSD (T. G. Milligan, Bedford Institute of Oceanography using the technique of Milligan and Kranck (1991). The grain size distribution provided ranged from 0.75 μm to 675 μm over 53 bin sizes.

LISST-ST particle size analysis for EPSD samples required dilution of field samples in a 1:3 ratio with distilled water (Williams, 2006). Once EPSD was measured, the sample was re-bottled and treated with the chemical dispersant Calgon (sodium hexametaphosphate and sodium carbonate anhydrous), followed by physical dispersion (ultrasonic probe or bath) and then re-measured to determine the absolute particle size (Williams, 2006). Detailed procedures for LISST analysis including full sampling file descriptions are provided in Appendix 1.

Coulter samples from the 2004 sampling program were compared using the Kolmogorov-Smirnov test (Siegel, 1956), which compared averaged inorganic particle size distributions for each treatment period (n = 3, baseline and Takla sediment, n = 13 salmon + Takla sediment). The LISST-ST, APSD, and EPSD data for 2006 and 2007 data were compared using a two-way ANOVA of treatment on d_{16} and d_{84} (n = 10 and 15 infiltration

bag samples in 2006 and 2007 respectively). The d_{16} and d_{84} are the particle size at the 16th and 84th percentile of the grain size distribution. They are commonly used indicators describing the range of the grain size distribution (Knighton, 1998).

3.2.4.3 Particle Settling Velocities

Particle settling studies require a 12-hour sampling period and were completed overnight. Due to sample time requirements only one sample could be measured daily. Once collected, the settling study sample was diluted to a 1:3 ratio with distilled water bringing the final sample volume to 550 ml. The sample was then added to the settling column of the LISST-ST (Figure 12), which collected particle size information at 32 intervals based on a logarithmic time scale. Sample frequency starts at milliseconds to capture the fastest settling particles and ends at hourly intervals to measure the finest particles with the slowest settling times. In 2006, eight particle size bin ranges were used, namely 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. In 2007, a 32-bin size range was used that also extended from 0-460 μm. The settling column was equipped with baffles to minimize turbulence within the tube that might alter settling rates (Figure 12). Particle size cumulative frequency distribution figures were generated by averaging data gathered from the 32 samples collected during the sampling period.

3.2.4.4 Carbon to Nitrogen Ratios

Carbon to nitrogen (C:N) ratios were determined by elemental analysis of filtered sediment samples collected on pre-ashed (550 °C) glass fibre filters. The C:N ratio was determined using the Dumas principle of complete and instantaneous oxidation of the sample

Figure 12: LISST-ST showing the 30 cm settling column and baffles (identified by water drops) prior to sample addition.

by flash combustion using a FISON NA-1500 Elemental Analyzer (Milan, Italy) (Chikaraishi *et al.*, 2005). The C:N ratio is used here because it is an effective indicator of MDN enrichment, it decreases when suspended and bedload sediments contain nitrogen rich salmon organic matter (McConnachie and Petticrew, 2006; Rex and Petticrew, 2006). Samples were compared across treatments in the re-circulating channel using a one-way ANOVA for treatment and a two-way ANOVA for the flow-through channels (baseline/exposure and treatment as factors).

3.2.4.5 Bacterial Samples

Bacteria sample collection, sample preparation, lab analysis, and statistical analysis procedures used here are the same as described in Chapter 2 (*s.* 2.2.3).

3.3 Results

3.3.1 Re-circulating Channel

The single re-circulating channel was used in 2004, and 2006-2008 (Table 4). Recall that in 2004 Takla sediment was used rather than laboratory grade kaolin clay and the 2008 samples consisted of only a baseline and salmon + clay treatments.

3.3.1.1 Total Suspended Solids

The re-circulating channel had significantly more TSS in the salmon + clay samples than the salmon samples in 2006 ($F_{4,7} = 15.5$, $p < 0.05$; average of 13.7 mg and 8.4 mg, respectively). In 2007, the baseline samples had significantly higher TSS than the salmon, salmon + clay, and recovery samples ($F_{4,10} = 3.15$, $p < 0.05$; average of 4.0 mg, 2.3 mg, 2.1 mg, and 2.0 mg respectively). The 2007 clay samples were similar to both the baseline and

the SOM based samples. There were no significant differences between treatment conditions in 2004.

3.3.1.2 Particle Size: Coulter Analysis

Coulter technique results for fine sediments in 2004 indicate a shift in the particle size distribution of infiltration bag samples toward larger silt fraction between 5 - 40 μm in the infiltration bags following the addition of salmon + Takla sediment (Figure 13).

The baseline samples of fine sediment had a d_{50} of 2.6 μm, while the Takla-sediment only samples had a d_{50} of 8.0 μm, and the salmon + Takla sediment samples had a d_{50} of 14.0 μm. Similarly, the proportion of sediment greater than 10 μm captured in the gravel bed increased over the treatment periods as only 2.4% of the initial streambed sediment exceeded 10 μm, followed by 37.3% of the sediment-only samples, and 57.5% in the salmon + Takla sediment samples (Figure 13, K-S test, $p < 0.05$). Flocs and inorganic Takla sediment aggregates increased the delivery of larger silt fractions to the flume's gravel bed.

3.3.1.3 Particle Size: LISST-ST Technique

LISST-ST particle size results did not show an increase in the APSD of gravel-stored fine sediment samples collected from the re-circulating channel in 2006 and 2007 but they did show a significant change in EPSD, which includes flocs. Infiltration bag samples from 2006 ($F_{2, 8} = 9.1$, $p < 0.05$) and 2007 ($F_{3, 16} = 39.1$, $p < 0.05$, Figure 14) show a significant increase in the d_{16} and d_{84} following the addition of salmon + clay. Gravel bucket data from 2006 also show an increase in EPSD following the addition of SOM ($F_{2, 12} = 15.9$, $p < 0.05$,

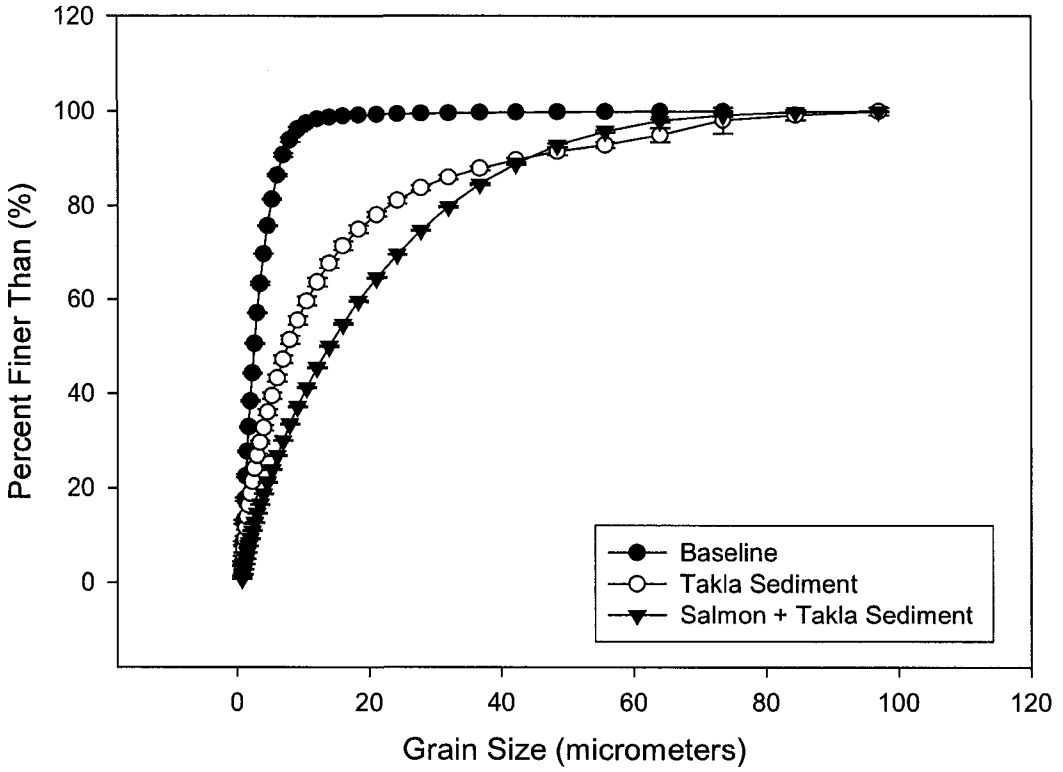

Figure 13: Mean percent composition and standard error (n=3) for inorganic fine-grained sediment (ASPD) captured in infiltration bags in the re-circulating channel in 2004 as determined by Coulter Counter technique.

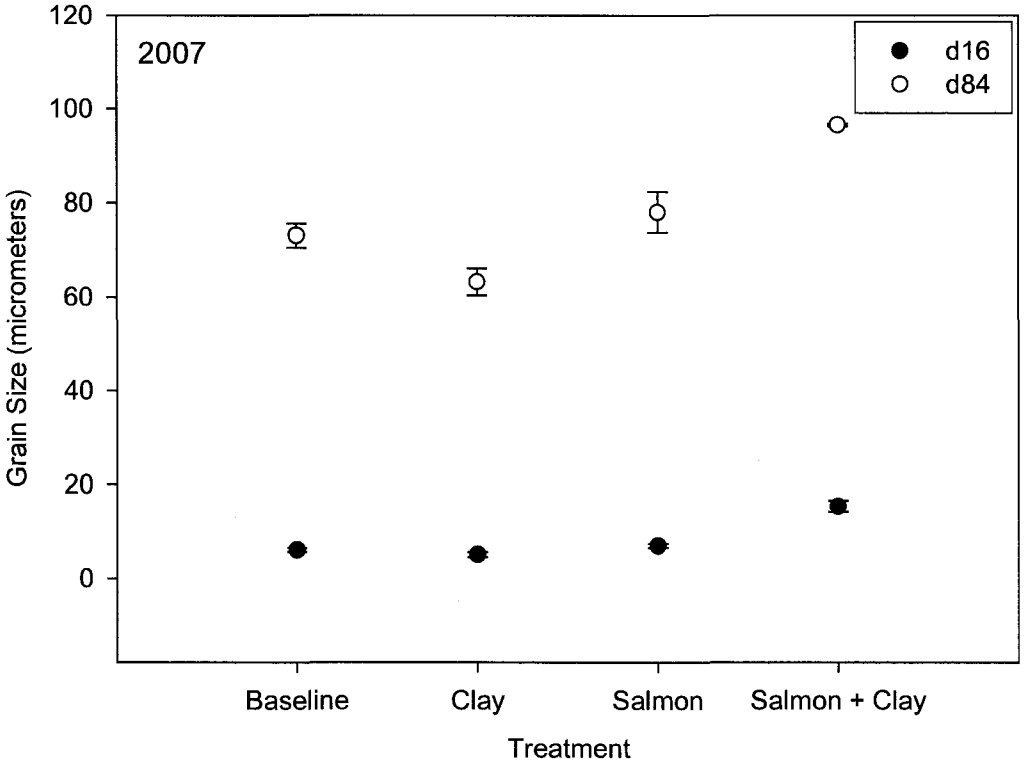

Figure 14: Average infiltration bag grain size for d_{16} and d_{84} in 2007 (triplicate analysis of 3 samples per treatment, error bars are one standard error).

Figure 15).

3.3.1.4 Particle Settling

Particle settling distributions of gravel-stored fine sediment collected from infiltration bags in the re-circulating channel show a higher proportion of large particles in the salmon + clay samples in both 2006 and 2007 but the trend is not significant (K-S test, Figure 16). Settling velocities calculated using Stokes Law estimated settling velocities for the salmon + clay particles to range between 4 and 8 mm s^{-1} for the 2006 and 2007 samples, which are comparable to those observed in O'Ne-eil Creek (Petticrew, 2005). Note that the particle size range as observed by values of d_{84} in gravel-stored sediments in 2006 and 2007 (50 – 90 μm) is less than observed in the water column (280 – 400 μm)(Figure 17).

3.3.1.5 Carbon to Nitrogen Ratio

The C:N ratios of sediment collected by the infiltration bags show the general trend of having the highest nitrogen content (i.e. lowest C:N) following the salmon + clay samples (Table 8). Although there was some variability in the level of enrichment of the salmon and recovery samples between 2006 and 2007 (Figure 18), the salmon + clay samples had the lowest carbon to nitrogen ratio each year (Table 8).

The gravel bucket C:N ratios exhibit different trends than the infiltration bag findings. In 2006, samples were generally similar except for salmon, which had a higher C:N ratio than the clay and recovery samples (Table 9, Figure 19). In 2007, the clay samples had a significantly higher C:N ratio than recovery, salmon, and salmon + clay samples (Table 9,

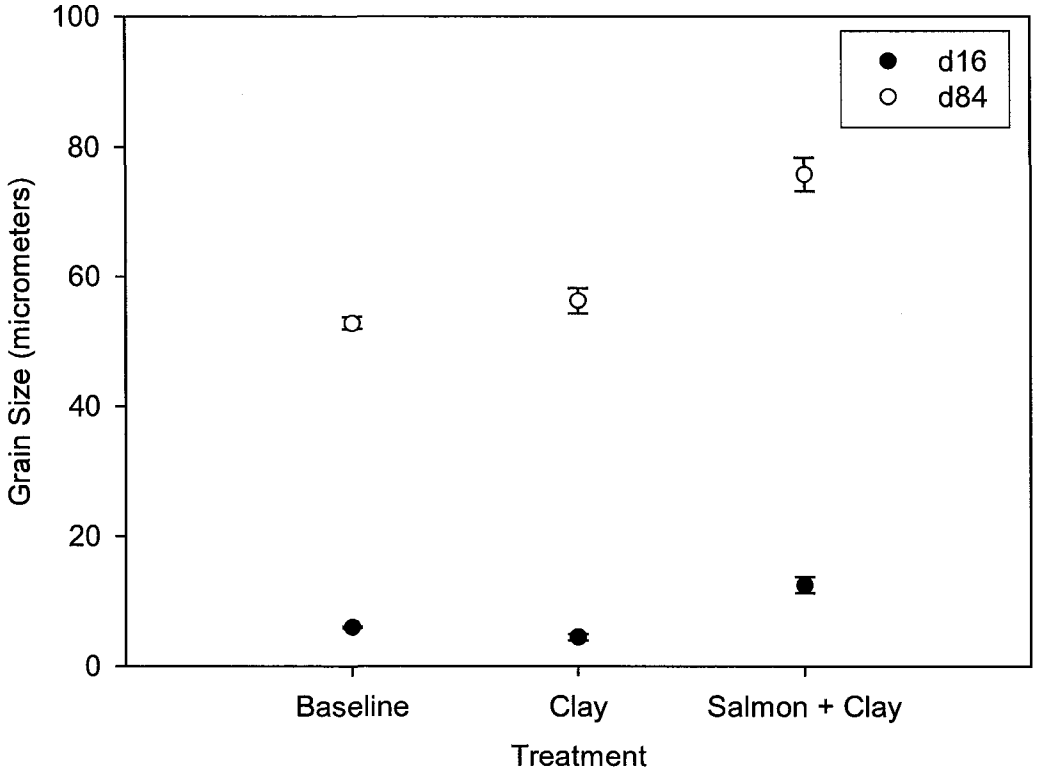

Figure 15: Average gravel bucket grain size estimates for d_{16} and d_{84}. Gravel-bed fine sediment distribution is shown for 2006 (triplicate analysis of 2 samples per treatment, error bars are one standard error of the mean).

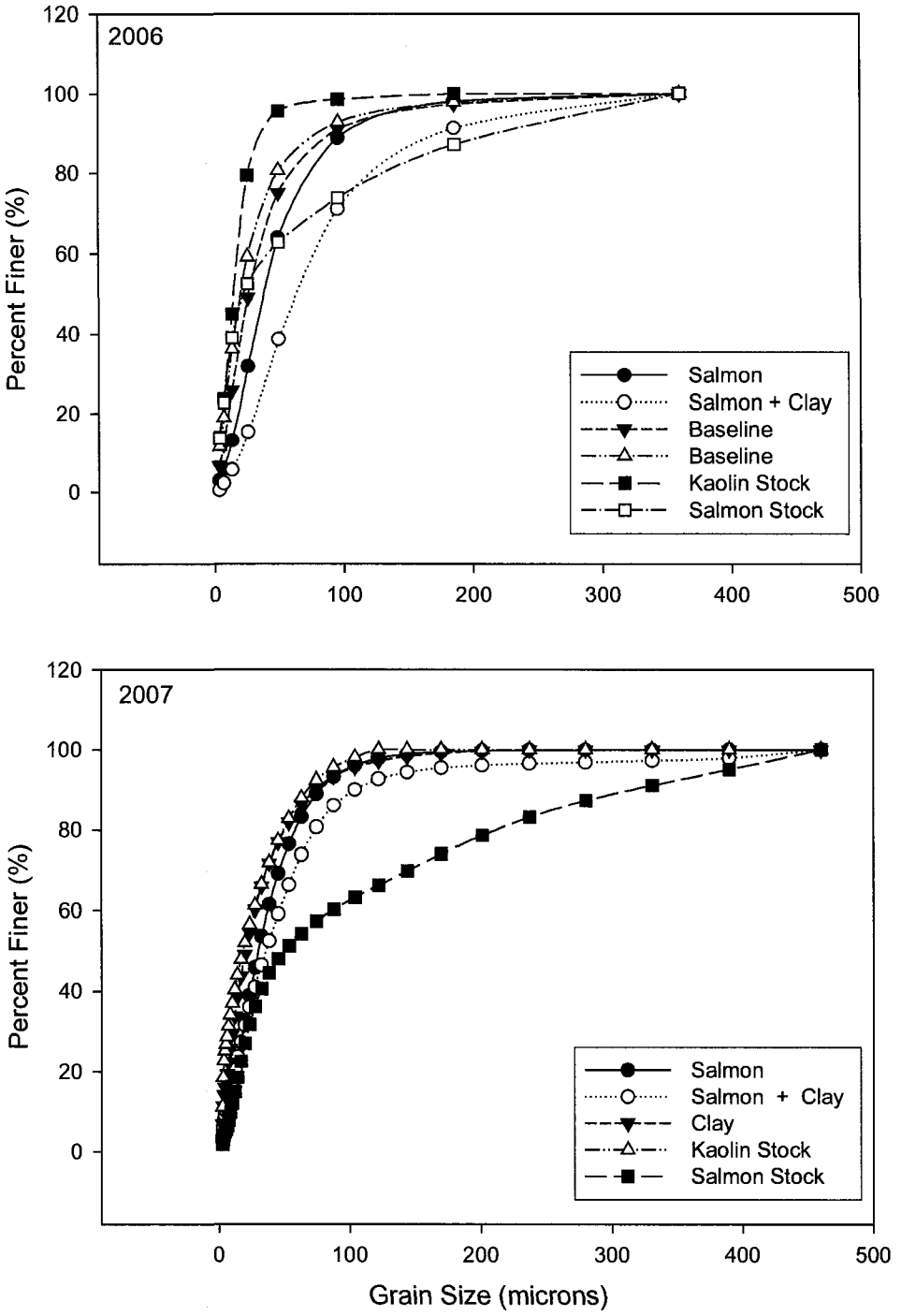

Figure 16: Cumulative grain size distribution curves for particle settling studies of re-circulating channel samples and stock solutions in 2006 and 2007. The 2006 curve is generated from 8 bin sizes while the 2007 curve comes from 32 bin grain size measurements. Clay stock solutions were disaggregated by sonication before delivery to the channel but salmon solutions were not sonicated, in order to prevent damage to bacteria.

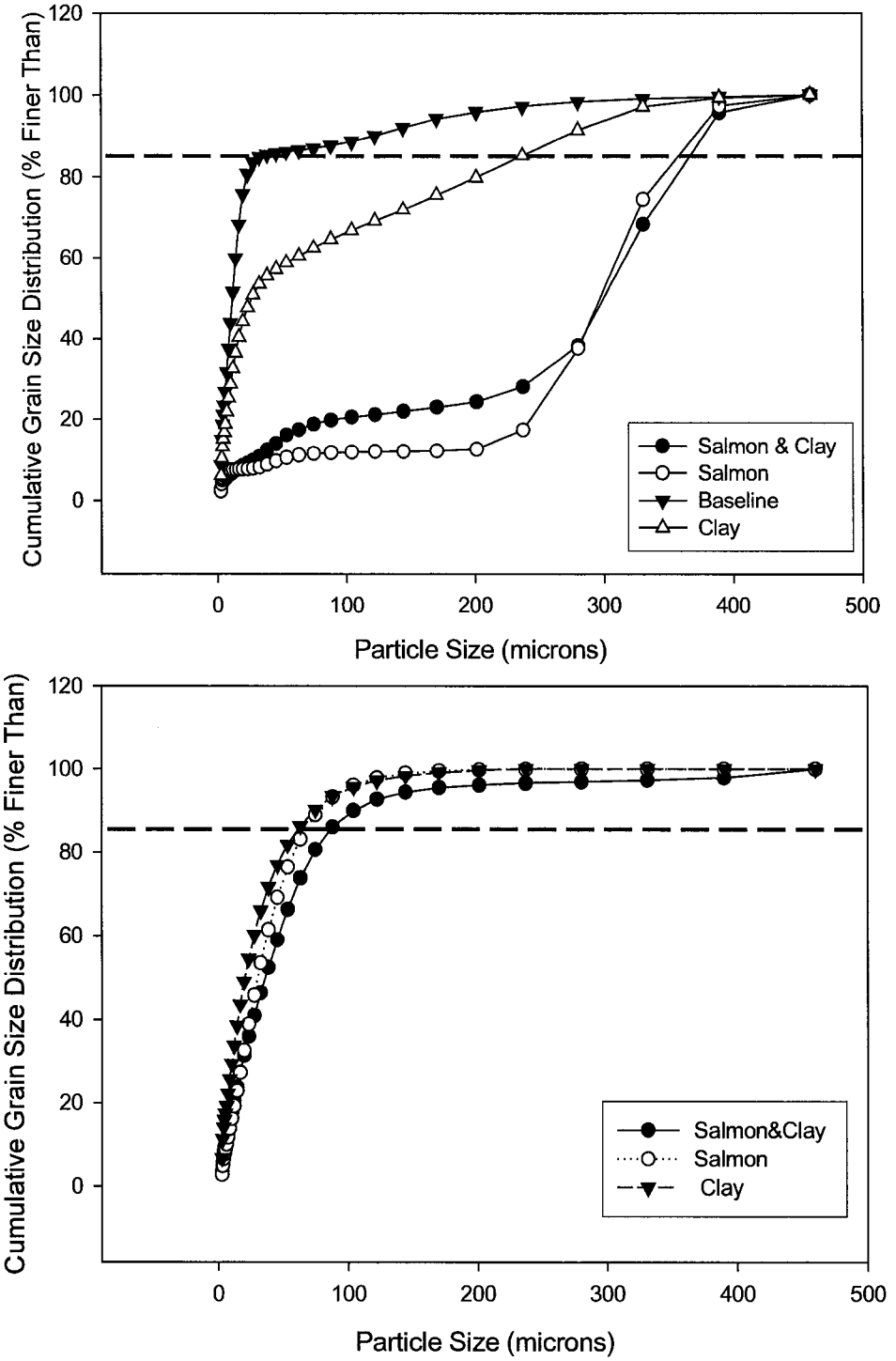

Figure 17: Suspended sediment (top) and gravel-bed sediment (bottom) cumulative grain size distributions for the re-circulating channel in 2007. Dashed line represents the approximate d_{84} particle size.

Table 8: Summary ANOVA findings for infiltration bag samples that were analyzed for carbon to nitrogen ratios in 2004 and 2006-2008. Significant differences between treatments are bolded and similar group treatments are linked by the symbol (~).

Year	Findings
2004	$F_{3, 12} = 32.3, p < 0.01$ **Salmon + clay < Baseline ~ Clay**
2006	$F_{4, 9} = 7.2, p < 0.01$ **Salmon + clay < Baseline** Salmon + clay ~ Recovery ~ Salmon ~ Clay Baseline ~ Recovery ~ Salmon ~ Clay
2007	$F_{4, 5} = 19.7\ p < 0.01$ **Salmon + clay < Clay ~ Recovery ~ Salmon ~ Baseline**
2008	$F_{1, 12} = 13.6, p < 0.05$ **Salmon + clay < Baseline**

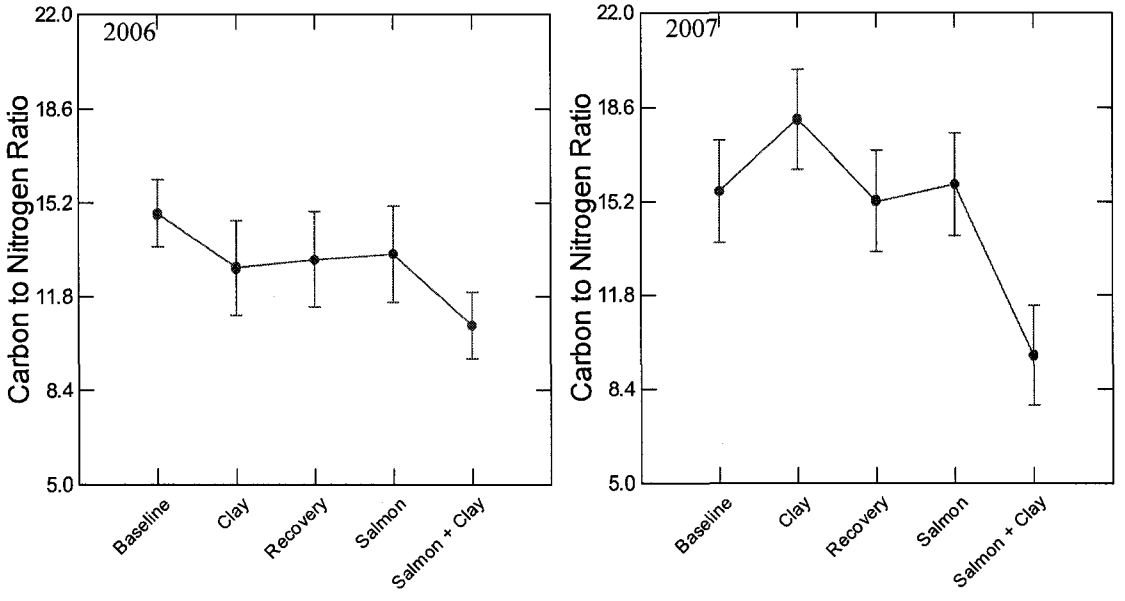

Figure 18: Least squares estimates of carbon to nitrogen ratios for infiltration bag samples collected from the re-circulating channel in 2006 and 2007 to show that salmon + clay is significantly lower than baseline in 2006 and lower than all other treatments in 2007. Sample size is n = 2 in 2006 and n = 3 in 2007, with error bars representing least squares error.

Table 9: Summary ANOVA findings for gravel bucket sediments analyzed for carbon to nitrogen ratios in 2006 and 2007. Significant differences between treatments are bolded.

Year	Findings
2006	$F_{4,7} = 6.1, p < 0.05$ **Salmon + clay, Clay, and Recovery < Salmon**
2007	$F_{4,10} = 9.1, p < 0.05$ **Recovery, Salmon, and Salmon + clay < Clay**

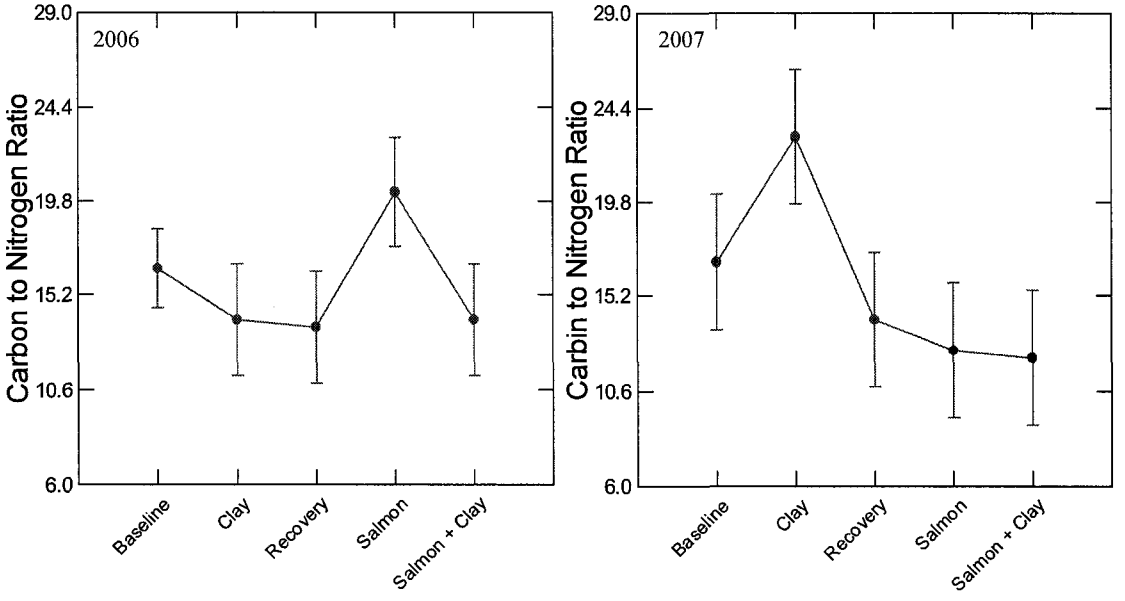

Figure 19: Least squares estimates of carbon to nitrogen ratios for gravel bucket samples collected from the re-circulating channel in 2006 and 2007 to show that salmon differs from all other treatments in 2006 while clay is significantly higher than all other treatments in 2007. Sample size is n = 2 in 2006 and n = 3 in 2007 with error bars representing least squares error.

Figure 19).

3.3.1.6 Bacterial Enumeration

As discussed in Chapter 2 (*s.* 2.3.3), there was a significant increase in bacterial counts following the addition of salmon (2006) and salmon + clay (2006-2007) (Chapter 2, Figures 7 and 8). The salmon + clay and recovery samples generally had the highest concentration of bacteria followed by salmon, clay and control samples. In addition, bacteria were predominantly observed in their attached form (Table 10).

3.3.2 Flow-through Channels

3.3.2.1 TSS

Total suspended solids (TSS) samples collected in the flow-through channels did not significantly differ between baseline and treatment periods or across channel conditions (i.e. control to salmon + clay) in 2006 or 2007. Further, the 2005 samples did not significantly differ across the sequential treatment periods.

3.3.2.2 Particle Size and Settling

LISST-ST analysis did not indicate a treatment difference in the APSD or EPSD of infiltration bag samples collected from the flow-through flumes in 2006 or 2007 (Figure 20). The gravel-stored particle size settling distribution curves show a trend of increasing grain size with the introduction of SOM-plus-clay but the difference is not significant (K-S test, Figure 21). Settling velocities for a 96 μm particle are similar to those observed in the re-circulating flume, showing an increase in settling velocity from clay, to salmon, and salmon + clay (Table 11).

Table 10: Summary ANOVA findings for infiltration bag bacterial enumeration data for 2006 and 2007. Significant differences between treatments are bolded and similar group treatments are linked by the symbol (~).

Year	Findings
2006	Treatment: $F_{4, 10} = 15.9, p < 0.01$ **Baseline ~ Clay < Salmon + clay ~ Recovery** **Baseline ~ Clay < Recovery ~ Salmon** Condition: $F_{1, 10} = 197.5, p < 0.01$ **Unattached < Attached** Treatment* Condition: $F_{4, 10} = 14.9, p < 0.01$ **All Treatment Unattached < All Treatment Attached**
2007	Treatment: $F_{4, 23} = 12.1, p < 0.01$ **Salmon ~ Clay ~ Baseline < Salmon + clay ~ Recovery** Condition: $F_{1, 23} = 17.2, p < 0.01$ **Unattached < Attached** Treatment* Condition: $F_{4, 23} = 4.6, p < 0.01$ **All Treatment Unattached < All Treatment Attached**

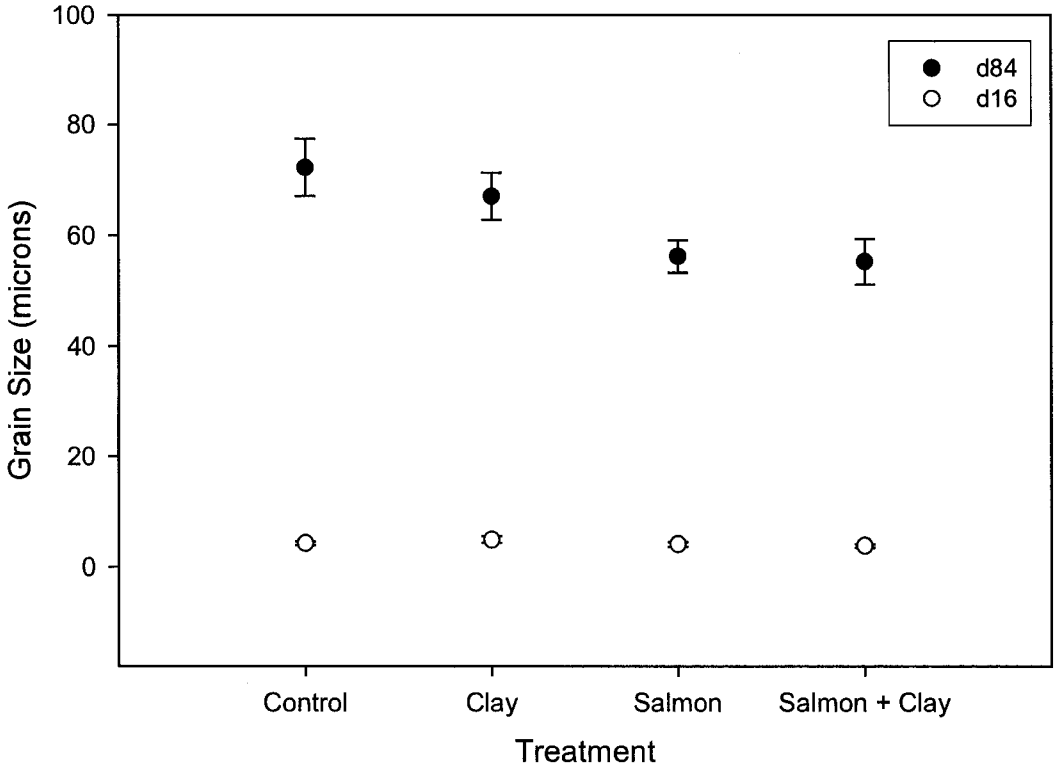

Figure 20: Average d_{16} and d_{84} grain size for flow-through flume's infiltration bag samples collected in 2007 (n = 3 per treatment, error bars represent one standard error of the mean).

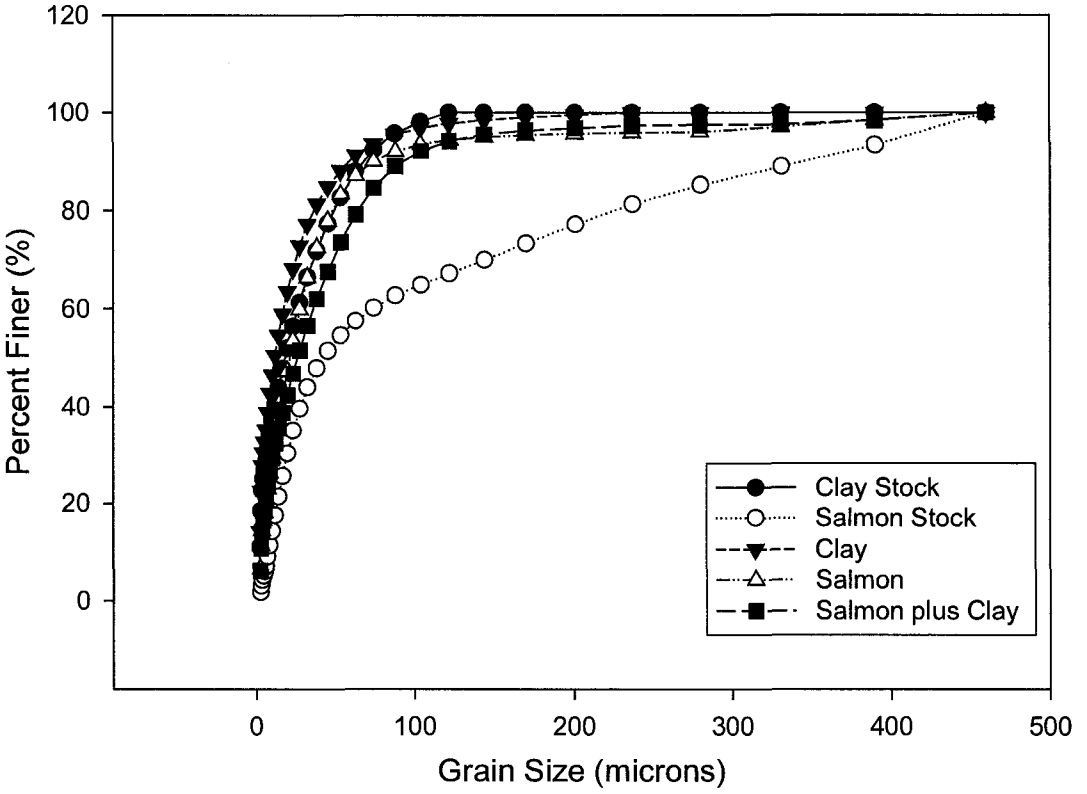

Figure 21: Settling distribution curves for infiltration bag sediment samples collected from flow-through channels in 2007 as well as for stock solutions added to the channels. Clay stock solutions were disaggregated by sonication before LISST-ST analysis but the salmon was not sonicated because it might damage SOM attached bacteria.

Table 11: Proportion of gravel-stored sediments less than 96 microns from flow-through channel infiltration bag samples and their calculated settling velocity (mm s^{-1}).

	2006		2007	
Sample	Proportion <96 μm	Settling Velocity (cm s^{-1})	Proportion * <96 μm	Settling Velocity (cm s^{-1})
Clay	Not measured	Not measured	~94	0.08
Salmon	89	0.43	~94	0.21
Salmon + clay	71	0.52	~87	0.83

*Settling bin sizes differed in 2007, becoming more resolute, the average of the two bin sizes encompassing 96 μm were included namely 88 μm and 104 μm .

3.3.2.3 Carbon to Nitrogen Ratios

Flow-through channel data do not exhibit the same trend as consistently as the re-circulating channel. Infiltration bag samples do not indicate a treatment effect for any of the years sampled but there was a significant exposure effect (i.e. baseline versus exposure to stock solutions) in 2006 and 2007. Carbon to nitrogen ratios generally decreased after the addition of stock solutions but they did not consistently decrease for any specific treatment type (i.e. control vs. salmon + clay) (Table 12). Gravel buckets also did not have a significant treatment effect in 2005 and 2006 but in 2007 their samples show nitrogen enrichment of salmon + clay and clay samples compared to the control and salmon samples (Table 13).

3.3.2.4 Bacterial Enumeration

Gravel-stored bacterial counts were significantly higher in the salmon + clay treatment than all other treatments in 2006 (Table 14, Figure 22). The 2006 samples also had higher levels of attached bacteria than unattached. The 2007 samples did not identify a significant difference between treatments (Table 14, Figure 23). Despite the absence of a treatment effect, there was a significant difference between bacterial condition with the attached bacterial numbers exceeding the unattached counts and an interaction effect between condition and exposure with the treatment attached bacterial numbers exceeding the attached baseline estimates. The highest counts were observed as attached bacteria from the salmon-only treatment. Although, there was no significant interaction effect for treatment/condition/exposure it is worth noting that the attached salmon estimate is five-fold higher than the control and clay samples and is close to twice the estimate from the salmon + clay samples.

Table 12: Summary ANOVA findings for gravel bags collected from the flow-through channels for carbon to nitrogen ratios from 2005-2007. Significant differences between treatments are bolded and similar treatment groups are joined by the symbol (~).

Year	Results
2005	No significant difference
2006	Treatment Effect – No significant difference Exposure Effect – $F_{1, 18} = 11.6, p < 0.05$ **Treatment < Baseline** Interaction – No Significant Difference
2007	Treatment Effect – No significant difference Exposure Effect – $F_{1,40} = 4.5, p < 0.05$ **Treatment < Baseline** Interaction – No significant difference

Table 13: Summary ANOVA results for gravel bucket samples collected from the flow-through channel for carbon to nitrogen ratios from 2005-2007. Significant differences between treatments are bolded and similar treatment groups are joined by the symbol (~).

Year	Results
2005	No significant difference
2006	No significant difference
2007	Treatment $F_{3, 38} = 6.3, p < 0.01$ **Salmon + clay < Control ~ Salmon** **Clay ~ Salmon + clay** **Clay ~ Control ~ Salmon** Exposure – No significant difference Interaction – No significant difference

Table 14: Summary ANOVA findings for infiltration bag bacterial enumeration data for 2006 and 2007 from the flow-through channels. Significant differences between treatments are bolded and similar group treatments are linked by the symbol (~).

Year	Findings
2006	Treatment: $F_{3, 8} = 8.9$, $p < 0.01$ **Salmon ~Control ~ Clay < Salmon + clay** Condition: $F_{1, 8} = 42.5$, $p < 0.01$ **Unattached < Attached** Treatment* Condition: $F_{3, 8} = 9.7$, $p < 0.01$ **All Treatment unattached < All Treatment Attached**
2007	Treatment: No significant difference Condition: $F_{1, 75} = 14.6$, $p < 0.01$ **Unattached < Attached** Condition* Exposure: $F_{1, 17} = 4.00$, $p < 0.05$ **Attached Baseline < Attached Treatment**

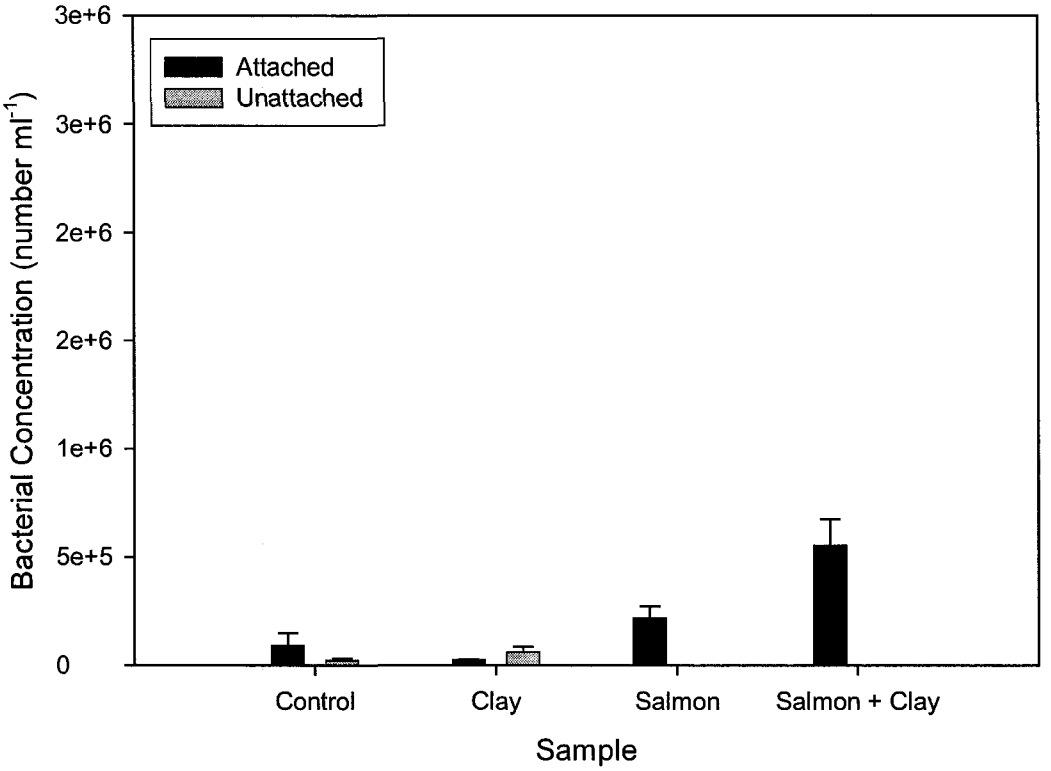

Figure 22: Average attached and unattached bacterial concentrations from gravel bag samples collected from the flow-through channels in 2006. Error bars represent one standard error of the mean (n = 3).

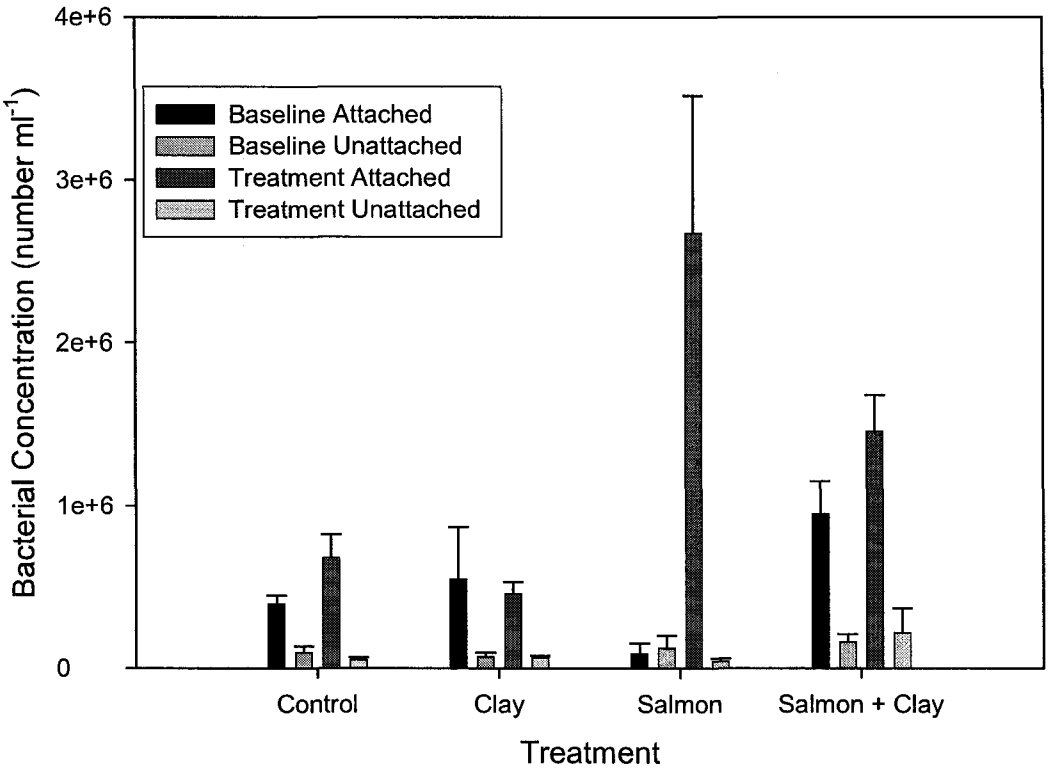

Figure 23: Average attached and unattached bacterial concentrations from gravel bag samples collected from the flow-through channels in 2007. Error bars represent one standard error of the mean ($n_{baseline} = 3$, $n_{treatment} = 9$).

3.4 Discussion

The second stage of the salmon-floc feedback loop, floc sedimentation and storage was verified by the flume studies completed between 2004 and 2007 (2008 focused on C:N only). These flume studies identified floc sedimentation and storage by documenting i) a coarsening of the particle size distribution of fine sediments in the flume bed (APSD in 2004 and EPSD in 2006-07), ii) an increase in the nitrogen content of flume bed sediment in the re-circulating flume and iii) a corresponding increase in bacterial levels in the re-circulating channel. Although there was some annual variability in the scale of response, there were significant responses for all parameters within the re-circulating channel and for some parameters within the flow-through channels after the addition of salmon and salmon + clay.

The re-circulating channel exhibited the strongest response to the addition of salmon and salmon + clay. In 2004, the largest increase in absolute particle size distribution (APSD) was observed for the salmon + Takla sediment. After the addition of salmon and salmon + clay to the re-circulating channel in 2006 and 2007, the particle size distribution of fine sediments in the gravel bed shifted toward larger particles. There was an increase in the EPSD of fine sediments in the gravel in 2006 and 2007 indicating the presence of SOM-based flocs, but the APSD did not have a corresponding increase because the sediment added was kaolin clay. The Takla sediment has a larger modal size than kaolin clay, which explains the 2004 coarsening of the distribution. The settling velocities observed for aggregated fine sediments collected from the gravel bed of the re-circulating channel following the salmon + clay exposure ranged between 5 and 8 mm s^{-1}, which are similar to those observed in O'Ne-eil Creek (Petticrew, 2005). The measured settling velocity of kaolin clay was 0.8 mm s^{-1}

while salmon-only samples from the water column were between 2 and 4 mm s^{-1}. The increase in settling rate after the addition of salmon + clay indicates flocculation along with a corresponding change in particle size and density (i.e. settling rate).

The C:N of re-circulating channel sediments consistently decreased from a baseline condition of more than 15:1 to approximately 10:1 following the salmon + clay exposure (2006-2008), which is the same decrease observed in suspended sediments from O'Ne-eil Creek during the spawning period (McConnachie and Petticrew, 2006). C:N ratios complicate data interpretation because ratios may change due to factors other than the addition of SOM alone. Other factors include respiration by benthic bacteria and natural variability in captured sediment. Although these and other factors may alter the C:N of captured sediments, I suggest the shift in C:N observed in the re-circulating channel is due to the addition of SOM because no other type of organic material was added to the channel during the treatment period. Further, the addition of low C:N ratio SOM (between 3:1 and 5:1 in McConnachie, 2003 and Unger *et al.,* 2003) to the gravel bed following floc sedimentation and storage should lower the C:N ratio from baseline conditions (~ 15:1).

The C:N ratio data from the re-circulating channel in 2006 identifies the baseline as significantly higher than the salmon + clay, and the 2007 and 2008 data identify the SOM-based samples as having lower ratios than non-SOM samples. However, the re-circulating channel bacterial counts were consistently highest following the salmon + clay addition. Further, these bacteria were primarily in their attached form indicating the delivery of SOM and bacteria flocs to the gravel bed. This combination of observations indicates that SOM

settled onto, or was intercepted by, the gravel bed and that bacteria were associated with the formation of these SOM-based flocs. Bacterial involvement, particularly the critical role EPS performs in forming and providing structure to biofilms and other non-salmon-based floc types is well documented (Battin *et al.*, 2003; Droppo, 2004; Wotton, 2004; Wotton, 2007).

Flocs settling onto the gravel bed of the re-circulating channel were limited in quantity but they were organically rich. The simulated riffle areas captured and retained water column-based flocs as surface water passed through the gravel bed elevated areas. The settling studies identified that the range of settling velocities observed for fine sediment from the gravel is lower than surface water velocities (4 to 8 mm s^{-1} compared to 5-10 cm s^{-1}). This indicates that direct settling may play a smaller role in the delivery of flocs to the gravel bed in the flume than bed-elevations. Therefore, the construction of the artificial riffles generating bed elevation differences may have enhanced advective flow as has been observed in other lab-based flume studies (Tonina and Buffington, 2007) and field based experiments that identified increased particle capture in shallower riffle areas with high velocities (Petticrew *et al.*, 2007).

Field investigation of Pacific salmon spawning areas have shown that streambed sorting and coarsening during redd construction reduces streambed mobility and lessens scour potential (Montgomery *et al.*, 1996). Redd design also facilitates more hydrologic exchange between the water column and the intergravel environment known as the hyporheic zone where surface and groundwater mix (Marion *et al.*, 2002; Tonina and Buffington, 2007). The simulated riffles used in the flumes do not have hyporheic zones, but their

increased elevation and clean intergravel pores likely enhances hydrologic exchange with surface water, increasing the probability of capturing large organic flocs within intergravel pores. Although these flume systems are artificial environments, their flume-bed surface conditions have some characteristics of natural systems. The riffles were constructed from clean gravel, of a size preferred by spawning salmon, similar to that of the redd banks used by Zimmerman and Lapointe (2005) in Atlantic salmon streams. They used redd banks to investigate intergravel flow velocities and fine sediment retention in Atlantic salmon redds. Similar to Zimmerman and Lapointe, (2005) the simulated riffles used here were effective models of natural redds as far as their ability to intercept fine sediments/ flocs.

Numerous studies on fine sediment entrainment and salmon spawning success have demonstrated that redds and surrounding stable gravel bed areas are capable of intercepting and retaining fine sediments (Waters, 1995). These studies have shown that increased fine sediment content in the streambed lowers surface water and dissolved oxygen exchange, and in the most severe cases prevents surface mixing due to the formation of sediment caps on salmon redds (Anderson *et al.,* 1996; Waters, 1995). Increased sediment delivery to salmon redds can lower reproductive success when the sediment load is high due to natural or anthropogenic causes. Although the capacity for streambeds in the areas of redds to intercept and store fine sediment may seem counterproductive, the ecological benefits are realized by the capacity for these modified gravels to facilitate floc and MDN storage. The capture and incorporation of nutrient rich fine sediment flocs in salmon redds provides a positive feedback opportunity as long as the organic matter and inorganic loading does not exceed the threshold where surface water exchange is reduced or metabolism of gravel-stored organic

matter depresses oxygen levels around developing eggs to lethal limits. Feedback may be direct such as in the case of MDN spurring benthic productivity that in turn supports resident salmon juveniles. Alternatively, it may be less direct such as MDN storage in the hyporheic zone of the streambed and riparian area benefiting overall ecosystem function and salmon production (O'Keefe and Edwards, 2002).

The change from the use of Takla sediments in 2004 to kaolin clay in the following years provided an interesting observation. In the presence of the Takla sediments' broader grain size distribution, the receiving gravel bed APSD shifted as silts collected on and in the bed (Figure 13). The 2004 data only represent the inorganic grain sizes (APSD) because the organics have been removed before analysis. Following the salmon + Takla sediment treatment the gravel-stored inorganic sediment distribution coarsened even further. In this latter treatment, if the inorganic sediment were settling or trapped via the same processes as occurred in the sediment-only treatment the same particle size distribution should have resulted, as they were added to the water column in the same proportion. It is important to note that it is not the change in added mass of sediments represented in these data but the proportional change in size classes. The coarsening of the bed between ~5-40 μm shows that a greater proportion of these silt-sized particles are being sequestered from the water column by SOM and delivered to the gravels.

Kaolin clay is comprised of smaller-sized particles and when it was used as the inorganic substrate for potential floc formation (2005-2008) it was observed that floc recruitment to the streambed changed the EPSD of gravel-bed materials without shifting the

APSD. APSD measurements would include the kaolin, with a modal size of ~2 μm. Therefore any selective size sequestering of the inorganic matter would be difficult to distinguish with LISST-ST analysis as the APSD are at the lower end of its detection limit. Organic matter-based flocs are larger than their individual inorganic components so alterations in the gravel bed's EPSD are easier to observe and they will remain significantly higher than the APSD until the organic content is mineralized.

Effective particle size was lower for gravel-stored sediments (d_{84} ~ 80 - 100 μm) than it was for water column based-flocs (d_{84} ~ 350 μm) because water column flocs are typically less dense than those found on the streambed (Leppard and Droppo, 2005). Bacterial EPS, which helps form the floc, swells upon hydration and therefore water column flocs exhibit great porosity, but when these settle and/or are forced into smaller gravel spaces they can be expected to break and/or compress resulting in smaller structures.

The flow-through channels did not show a consistent or comparable response to the re-circulating channel. Flow-through channels did not show a significant difference in gravel bed particle size distributions for EPSD or APSD. Carbon to nitrogen ratios did not show significant differences between the four treatments despite a significant decrease in each individual treatment response when compared to its baseline period. The bacterial response was similar to the re-circulating channel trend in 2006 with the salmon + clay exposure having more bacteria than the other channels. However, in 2007, the bacteria data did not identify any significant difference between treatments despite the large number of attached bacteria for the salmon sample, but it did show that gravel-stored bacteria were primarily in

their attached form.

The differences observed between the flow-through and re-circulating channels can be explained by their design and these differences should be considered during future use of these systems to model natural stream conditions and processes. The flow-through channels do not reflect stream conditions observed in O'Ne-eil Creek as effectively as the re-circulating channel. The treatment conditions applied in this study were based on field observations from O'Ne-eil Creek (McConnachie and Petticrew, 2006; Petticrew and Rex, 2006) with salmon tissue quantities based on an areal estimate of post-spawning salmon biomass per square meter of gravel bed and suspended sediment levels corresponding to active and post-spawn periods. The re-circulating channel can maintain these spatial estimates more effectively than the flow-through channels because the residence time of the material added to the channels is longer. Based upon pump volumes, the flow-through channel has a water parcel residence time of approximately ten minutes for surface water. The reach containing the artificial riffles only includes 60% of the flume's total bed length, meaning that flocs must form and settle within this length as the water parcel and its flocs are flushed out of the system. In the flow-through channels the LISST-ST was positioned 10-12 cm off the flume bed surface and it detected that flocs do form in the water column by mid-length in these flumes. Unfortunately the other parameters indicate that they did not settle in sufficient quantity to consistently detect differences between treatment types. Floc settling in flow-through channels may be enhanced by lowering water velocities or extending the channel and sample area. In contrast, the re-circulating system does not lose the clay and SOM added to the water column and so, on an areal basis, better approximates natural

conditions. This is supported by the fact that significant responses to treatments were observed in the 2006 data which represent the lower range of post-spawning clay concentrations observed in natural systems. Wipfli *et al.* (1998) also identified that flow-through channels were less effective in determining a response observed in natural channels, because the concentration of MDN was maintained at higher levels. As mentioned a similar influence would exist for the re-circulating channel where treatment solutions added were retained.

TSS levels in the re-circulating channel did not show a consistent trend between 2006 and 2007. In 2006, the salmon + clay sample had higher TSS than the salmon-only samples which is expected because the salmon-only samples did not have 9 g of kaolin added to the mixture. The 2007 samples in contrast indicated that the baseline sample had the highest TSS level even though a larger mass of kaolin was added compared to 2006. In each of the years the mass of fine sediment collected per infiltration bag or bucket is not large and is quite variable as the total amounts of added clay were not high relative to the area of the gravel bed (1.5 to 0.15 g m^{-2} for active and post-spawning respectively). However, it is clear from other metrics which reflect the delivery of SOM to the gravels (C:N ratio and bacterial counts) in 2006 and 2007 that the suspended clay acts as a vector, via flocculation to deliver significantly increased amounts of SOM to the gravels even though gravimentrically it is difficult to distinguish.

Chapter 4: Floc Storage and MDN Release

4.1 Background

Once flocs settle onto and/or infiltrate the streambed they can be metabolized by benthic biota on the streambed or in the hyporheic zone. Benthic food webs are central to stream nutrient cycling processes because organic matter such as flocs are continuously provided through surface and subsurface water exchange (Mulholland and DeAngelis, 2000; Poole *et al.*, 2008). For example, Claeson *et al.* (2006) identified that MDN contributions from Pacific salmon were mostly removed from the water column within 10 m of the carcass and were not measurable 250 m downstream. The flume-based studies presented here cannot model the complex surface and subsurface water exchange processes of natural streams because the flumes are closed systems, but they do provide insight on gravel bed salmon-floc delivery, nitrogen sequestration, and the microbial respiration response to MDN introduced into the water column.

4.1.2 Floc Metabolism and MDN Release

Pacific salmon spawning density influences bacterial response in natal streams with high spawner numbers having higher streambed bacterial levels than years with lower spawning salmon numbers (Richey *et al.*, 1975). Bernhardt and Likens (2002) identified that dissolved organic carbon (DOC) enrichment of the streambed stimulated bacterial growth, which in turn caused increased nitrogen requirements and microbial respiration. SOM will stimulate bacterial growth in the gravel-bed because the nitrogen released as decay products from salmon carcasses, provides the nitrogen they require. For example, Cak *et al.* (2008)

identified a 30-350 % increase in ammonium (NH_4^+) during the salmon spawning period in three southeastern Alaskan streams near Juneau while Pinay *et al.* (2008) identified peak microbial use of nitrate (NO_3^-) within 1 hr of it entering the hyporheic zone of a south-central Alaskan stream. Nitrogen forms, particularly ammonium are rapidly removed from the water column where they are oxidized to NO_3^- (O'Keefe and Edwards, 2002). Further they are preferentially absorbed by phytoplankton as a nutrient source (Dortch, 1990). Much of the work on MDN in salmon streams has been performed in the water column of spawning streams. As a result, MDN delivery and retention processes within the streambed and its hyporheic zone is an area of growing interest particularly so for determining how gravel bed conditions such as permeability and hydrologic exchange influence intergravel storage (Triska *et al.*, 1993; Pinay *et al.*, 2008).

The objective of this portion of the study was to investigate the occurrence of stage three of the salmon-floc feedback loop (floc dissociation and MDN release), occurring in the flumes. To determine if floc dissociation and MDN release occurred (research question three, Chapter 1), I investigated microbial respiration and nitrogen response to SOM and clay mixtures by measuring biochemical oxygen demand (BOD, Smith, 1998) of gravel-bed sediments (2004-2008), intergravel dissolved oxygen (2004-2008), and nitrogen content and forms in the water column and intergravel area (2008). Nitrogen was selected over phosphorus because it is present in higher concentrations in salmon tissue (Schindler *et al.*, 2003). If stage three of the salmon-floc feedback loop occurs, there should be nitrogen sequestration from the water column to the streambed along with a microbial respiration response that can be quantified through measurements of BOD and intergravel oxygen

concentrations.

4.2 Methods

The microbial respiration and nitrogen-sampling program used both the re-circulating and flow-through flumes, as well as the same stock solutions described in Chapter 3 (*s. 3.2.1*, Table 4). Intergravel dissolved oxygen probes (Point Four Systems Inc., Oxyguard Stationary Probe) were installed in different flumes through 2004 and 2008 depending upon the availability of equipment and configuration of channels (Table 15). The dissolved oxygen probes were placed into a 10 cm diameter down-pipe with openings at 25-35 cm depth to allow intergravel flow to pass by the probe's membrane. To ensure dissolved oxygen readings were not negatively biased due to consumption of oxygen during measurement, the probe was equipped with a wiper that continuously moved water over the membrane during the 1-minute oxygen measurement period (Figure 24). A burst sample of 100 measurements was collected at the end of each 15-minute interval and the mean of these 100 samples was recorded as the 15-minute value using a Starlogger (Unidata Systems Inc).

Dissolved oxygen traces were first viewed graphically to identify trends following the addition of clay and salmon stock solutions. If a trend was observed, a two-factor ANOVA of daily mean oxygen levels was used to assess differences between treatment (i.e. salmon + clay vs. baseline) and condition (i.e. baseline vs. treatment) in the flow-through system while a one-way ANOVA was used for the re-circulating channel using treatment as the main factor (Manly, 2000).

Table 15: Intergravel dissolved oxygen probe placements and BOD sample allocation.

Year	Intergravel Dissolved Oxygen Probes	BOD Samples
2004	Re-circulating Channel	Re-circulating Channel
2005	Flow-Through Channel	Flow-Through Channel
2006	Flow-Through Channels	Re-circulating and Flow-Through Channels
2007	Flow-Through Channels	Re-circulating and Flow-Through Channels
2008	Re-Circulating Channel	Re-Circulating Channel

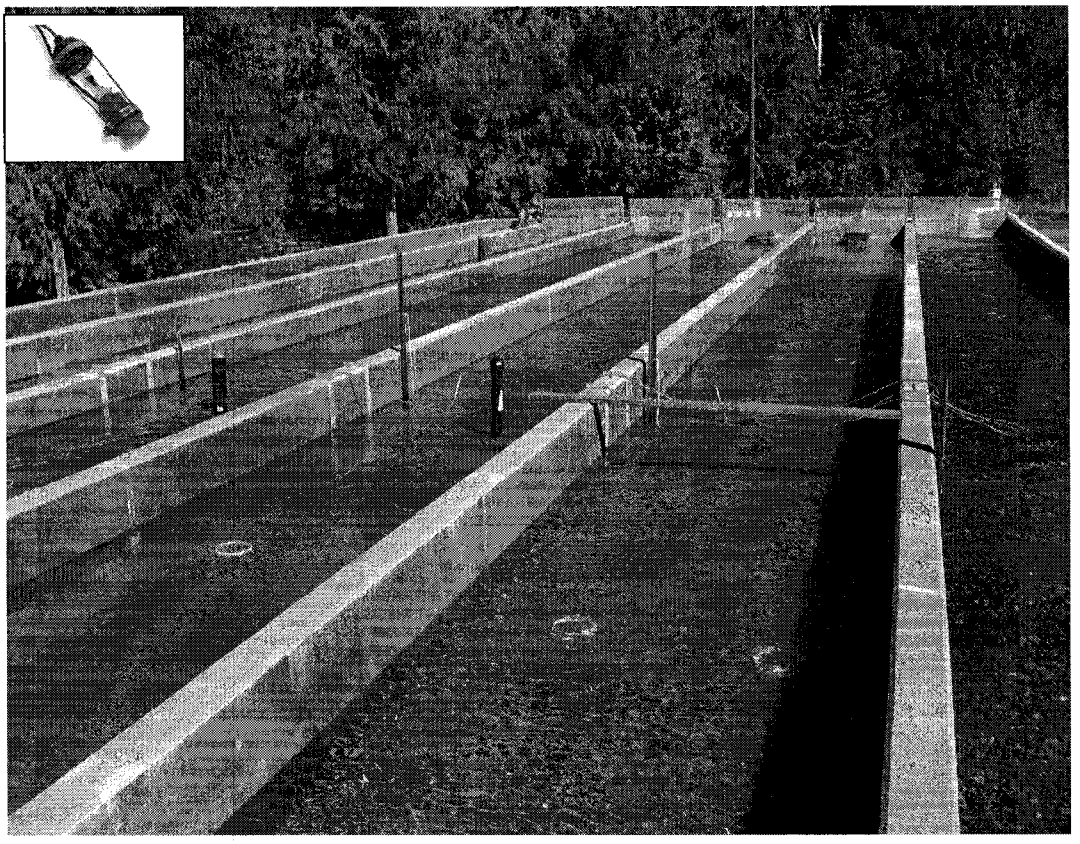

Figure 24: Upstream view of the flow-through channels showing the dissolved oxygen probe and down-pipes in the flumes along with the dissolved oxygen probe in the inset photo.

Biochemical oxygen demand samples were collected after wet sieving the infiltration bag samples with a 2 mm screen to remove gravel (n = 2, 3 per treatment depending on the year, Chapter 3). After screening the gravel, a 300 ml sub-sample of the wash water and its associated fine sediment was collected in an acid-washed borosilicate BOD bottle. Samples were incubated in the flumes at ambient flume temperatures and were measured on a daily basis for four to five days (APHA, 1998). Daily changes in dissolved oxygen and temperature in the bottles were measured using a Yellow Springs Instrument (YSI) 5010 BOD probe connected to a YSI 52 portable meter. The YSI probe measured dissolved oxygen concentration, percent saturation, and water temperature. BOD for each treatment was calculated as the oxygen consumed in the bottles over the monitoring period and it was analyzed in the same manner as the dissolved oxygen data, namely a one-way and two-way ANOVA for re-circulating and flow-through channels respectively with Tukey's HSD for post-hoc comparisons.

4.2.1 Nitrogen and Carbon to Nitrogen Ratio

Nitrogen samples were collected from the re-circulating channel in 2008 following a baseline period and then over a two-week period after the addition of salmon + clay. Aqueous nitrogen samples were collected from three locations including the i) water column ii) infiltration bags and, iii) gravel-bed using 25-cm deep piezometers. Mid-depth water column samples were collected during the baseline period (n = 1) as well as 1, 2, 4, 6, 7, and 14 days (n = 2) after the addition of salmon + clay to the water column. Infiltration bag samples (n = 3) were collected during the baseline period as well as 2, 4, 7, and 14 days after the addition of salmon + clay to the channel. Piezometer samples (n = 3) were collected during the

baseline period as well as 1, 2, 3, 5, 7, and 14 days after addition of the salmon + clay. Piezometers were screened with 200 μm Nitex to prevent sampling large particles from the streambed.

All three types of aqueous nitrogen samples were collected in sterile Nalgene ™ wide-mouth bottles. Once collected, samples were kept on ice (~4 °C) and shipped to a commercial laboratory for spectrophotometric analysis in accordance with APHA standards of total nitrogen (TN), dissolved organic nitrogen (DON), Kjeldahl nitrogen (KN), Nitrate + Nitrite ($NO_3^- + NO_2^-$) and Ammonium (NH_4^+) within 72-96 hours. For brevity DON, TN, and NH_4^+ are discussed. Each sample set was shipped with duplicate samples and a travel blank. In addition, batch quality assurance and control (QA/QC) data including spike recovery samples for the commercial laboratory's equipment are provided in Appendix 2. No sample data were excluded based on QA/QC limits. Samples were to be excluded if spiked samples exceeded lab QA/QC limits or blank samples were three times larger than the detection limit. A two-way ANOVA using nitrogen form and date was used to compare samples.

Sediment bound nitrogen levels were determined by elemental analysis of fine sediment collected from infiltration bags. A 220 ml sample was collected from infiltration bag samples. It was centrifuged to concentrate the fine sediments into a plug that was then oven dried at 60 °C. Carbon to nitrogen ratios were determined using the Dumas principle of complete and instantaneous oxidation of the sediment plug by flash combustion using a FISON NA-1500 Elemental Analyzer (Milan, Italy) (Chikaraishi *et al.*, 2005). To assess the C:N response to a single salmon + clay treatment over a 14-day period a repeated-measures

ANOVA was applied (Manly, 2001).

4.3 Results

A significant BOD response was observed for those samples that were observed to have salmon-based flocs, as identified in Chapter three. Carbon to nitrogen ratios decreased in gravel-bed sediments after the addition of salmon + clay, indicating nitrogen enrichment in the re-circulating channel in 2004, 2006, 2007, and 2008. In the 2008 study, aqueous nitrogen concentrations in the water column and gravel bed peaked within the first 1-4 days after the addition of salmon + clay and gradually decreased over the remaining 10-11 day period. Intergravel dissolved oxygen levels did not show a signal, possibly due to low organic loading (i.e. lower range of field conditions) and sample size (i.e. one probe per channel).

4.3.1 Biochemical Oxygen Demand

The BOD response was consistent across years in the re-circulating channel. Salmon + clay samples consistently had the highest biological oxygen demand (Table 16, Figure 25). The flow-through channels did not show differences in 2006 and 2007. In 2005, the single flow-through channel had a significantly higher BOD for the salmon + clay samples than the other treatments (Table 16). An algal bloom (Figure 26) in the channel and BOD samples in 2008 compromised the results that year so they are not presented.

Table 16: ANOVA summary findings for BOD tests from 2004-2007. Significant differences between treatments are identified by a < while similar treatments are linked by the symbol (~)

Year	Re-Circulating	Flow-Through
2004	Treatment $F_{4, 10} = 55.6, p < 0.05$ **Baseline ~ Clay < Salmon + Clay**	
2005		Treatment $F_{5, 12} = 4.8, p < 0.05$ **Control ~ Clay ~ Salmon < Salmon + Clay**
2006	Treatment $F_{3, 8} = 4.5, p < 0.05$ **Baseline ~ Clay ~ Salmon < Salmon + Clay**	No significant differences
2007	Treatment $F_{3, 5} = 46.7, p < 0.05$ **Baseline ~ Clay < Salmon ~ Salmon + Clay**	No significant differences
2008	Algal bloom compromised BOD challenge	

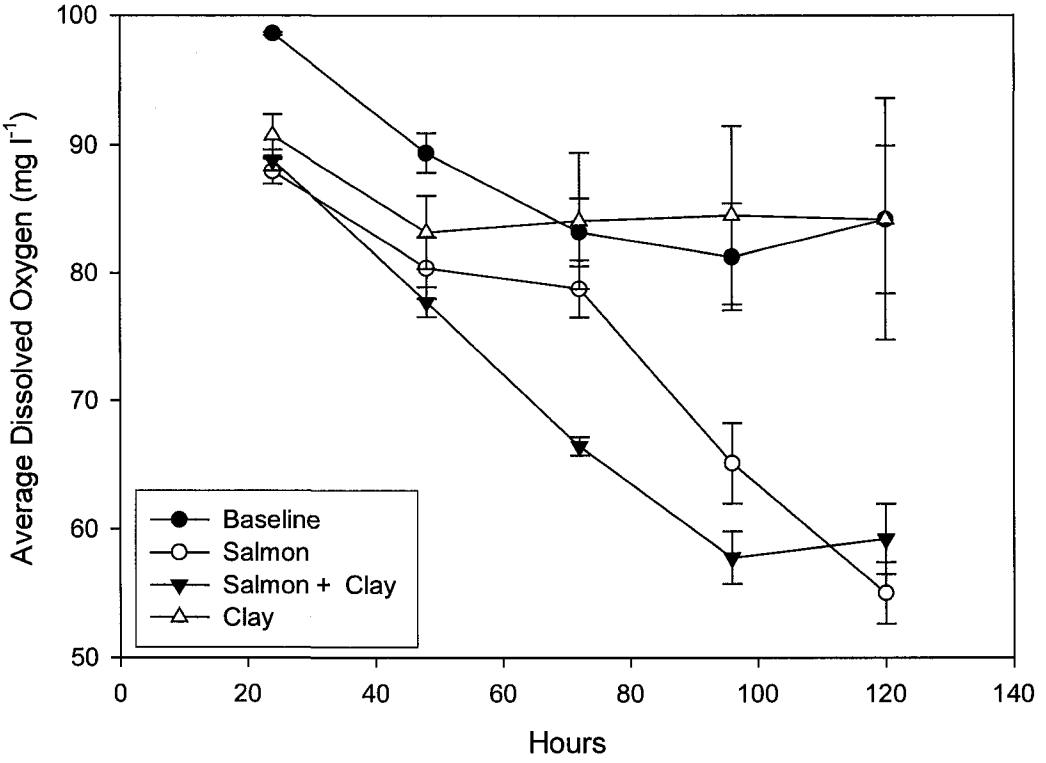

Figure 25: Dissolved oxygen levels for infiltration bag samples collected from the re-circulating channel in 2007 (n = 2, error bars represent one standard error of the mean).

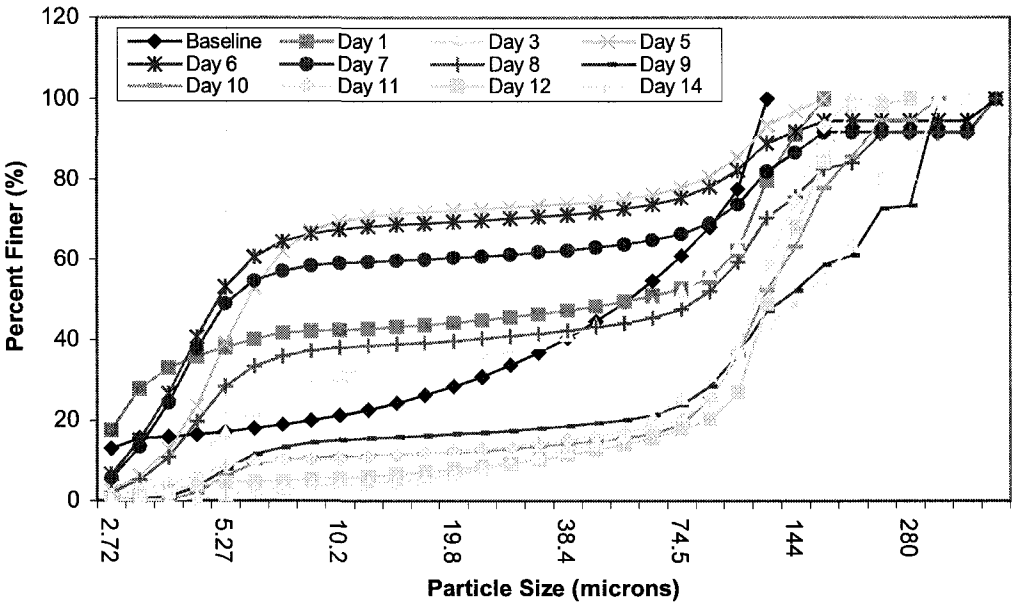

Figure 26: Average LISST-ST in-situ trace for suspended sediments in the re-circulating channel in 2008. Note the algae bloom identified by the peak in grain size distribution for particles between 5 and 10 microns from day 5 to day 7.

4.3.2 Intergravel Dissolved Oxygen

There was no intergravel oxygen response to treatment conditions. Subtle changes were observed at the same time as temperature shifts in both the re-circulating and flow-through channels each year but there was no apparent response to treatment mixtures of SOM and clay. Due to the observed lack of trend, no further analysis was completed.

4.3.3 Nitrogen

Surface water concentrations increased from baseline conditions after the addition of salmon + clay for ammonia and total nitrogen (approximately 15-fold and 4-fold, respectively) (Figure 27). There was a significant difference between the concentrations of different nitrogen forms ($F_{2, 21} = 80.3$, $p < 0.01$) with total nitrogen levels exceeding ammonia, which was higher than dissolved organic nitrogen. Over the 14-day sampling period the concentration of total nitrogen varied significantly with the baseline and day 14 samples being similar to each other and significantly lower than the remaining days in between ($F_{4, 21} = 25.5$, $p < 0.01$). There was also a significant interaction effect ($F_{8, 21} = 5.1$, $p < 0.01$), which identified the highest levels of total nitrogen and ammonia on day 1.

Infiltration bag samples generally had 10 – 50% higher concentrations of total nitrogen and ammonia compared to the water column while the concentrations of dissolved organic nitrogen were 10-fold higher. Infiltration bag total nitrogen levels were significantly higher on days 2 and 4 than during baseline, day 7 and day 14 (Figure 28, $F_{4, 21} = 11.3$, $p < 0.01$). Both dissolved organic nitrogen and ammonia had lower concentrations when compared to total nitrogen ($F_{2, 21} = 35.0$, $p < 0.01$) as would be expected given that total

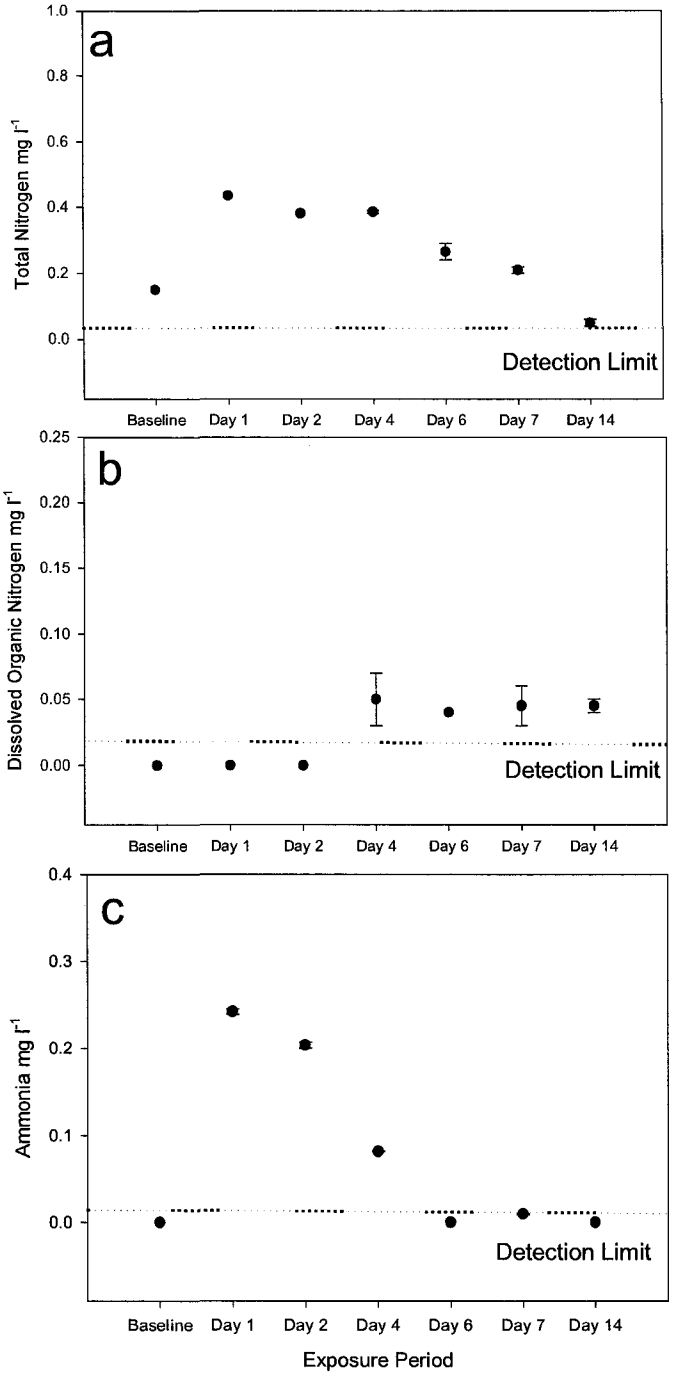

Figure 27: Average nitrogen data for water column samples collected from the re-circulating channel in 2008 (n = 1 baseline, n = 3 post-treatment). Error bars represent one standard error of the mean.

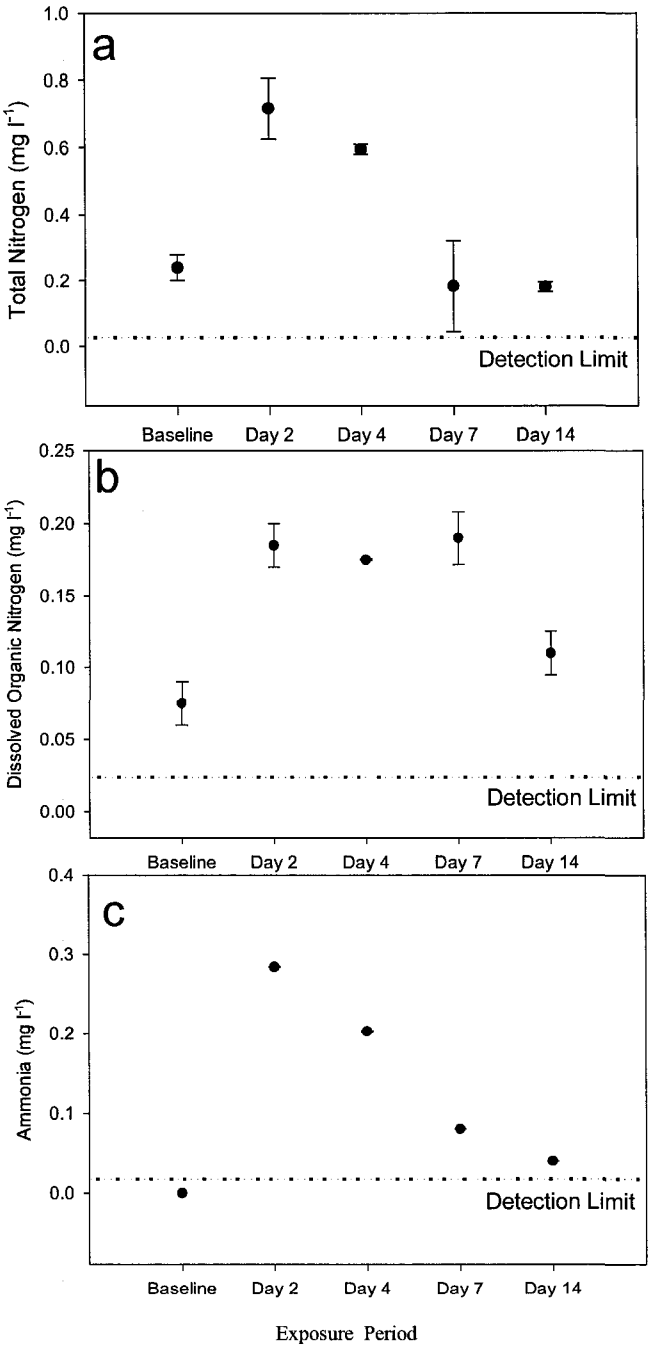

Figure 28: Average nitrogen data for infiltration bags collected from the re-circulating channel in 2008 (n = 3). Error bars represent one standard error of the mean.

nitrogen is the sum of these measured forms and inorganic nitrogen. On days 2 and 4, higher concentrations of ammonia and total nitrogen were observed than during the preliminary baseline period and one week after the addition of salmon plus clay (days 7 and 14) ($F_{8,21} = 4.75, p < 0.01$).

Piezometer data exhibited a similar trend to infiltration bag ammonia and total nitrogen levels but at a considerably lower concentration and with higher variability among sample replicates (Figure 29). As a result, there is no statistically significant difference between treatment periods. Despite the absence of statistically significant effects, the trend observed for these two forms of nitrogen in the piezometer samples follows that of the gravel bags. There is an increase between days 1 and 3 after the addition of SOM plus clay, which reduces over the remainder of 14-day exposure period. Dissolved organic nitrogen in the piezometers does not reflect the same pattern as the infiltration bags but is similar to that of the surface water samples in that the response is delayed (day 7) and of low magnitude.

Carbon to nitrogen ratios of gravel-stored fines in the re-circulating channel in 2008 significantly decreased after the addition of salmon + clay (Figure 30, $F_{4,8} = 4.8, p < 0.05$). This gravel bed enrichment extended up to two weeks and potentially longer, as indicated by a C:N lower than the baseline condition at the end of the 14-day monitoring period. This suggests that the sediment-bound nitrogen delivered to the bed via flocculation is retained and acts as a nutrient source for longer-term release of MDN to the stream.

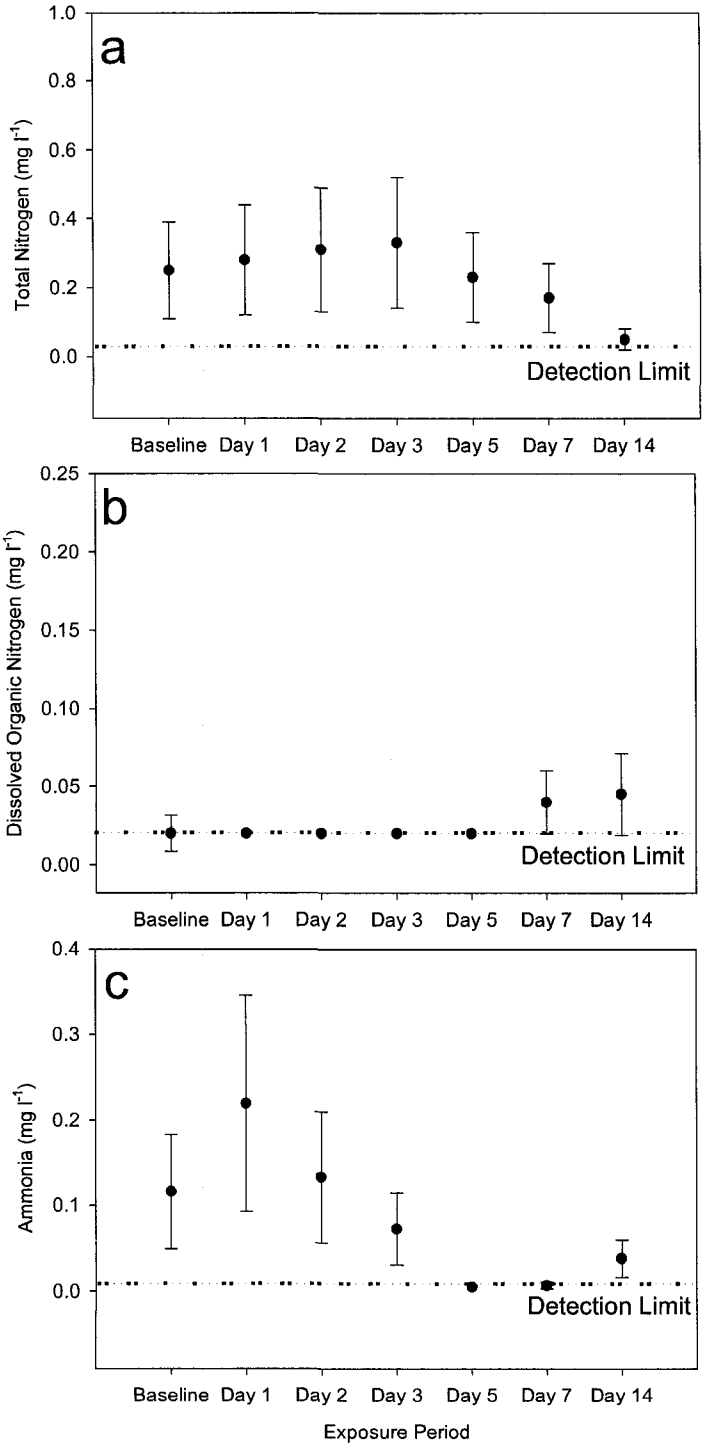

Figure 29: Average nitrogen levels from interstitial water as collected by piezometers in the re-circulating channel in 2008 (n = 3). Error bars represent one standard error of the mean.

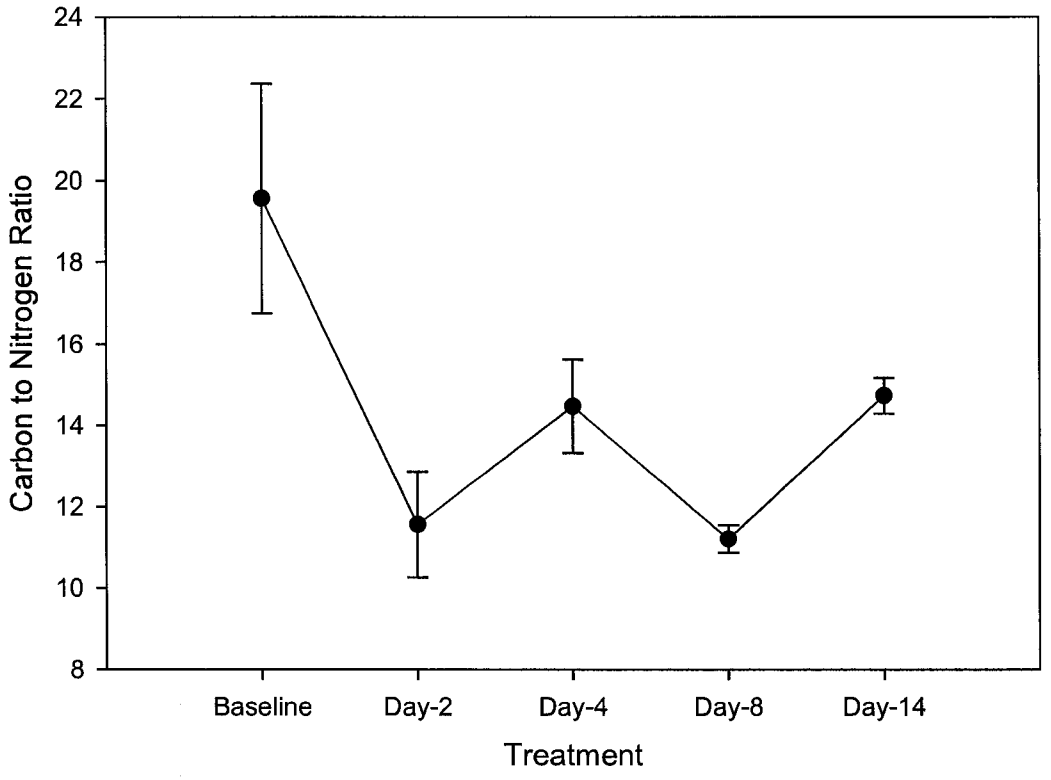

Figure 30: Average carbon to nitrogen ratio for infiltration bag samples collected from the re-circulating channel during a baseline period and then over 14 days following exposure to salmon + clay (n = 3, error bars represent one standard error of the mean).

4.4 Discussion

The third stage of the salmon-floc feedback loop (Figure 1), floc dissociation and MDN release was verified by the BOD and nitrogen studies. The intergravel oxygen study did not identify significant trends, which may be a function of the closed system (i.e. lack of hyporheic and riparian zones) and/or sample size (i.e. one probe per channel). Although not observed here in the experimental flumes, a dissolved oxygen response was observed during the field studies that led to this controlled study. After the 2002 spawning event in O'Ne-eil Creek, intergravel dissolved oxygen levels decreased by 10 to 20% of the pre-spawn levels and did not return to pre-spawn conditions for two-weeks after salmon die-off (Petticrew and Rex, 2006). BOD was not measured for the O'Ne-eil Creek gravel-stored sediments but it appears to be a good indictor for the range of additions and conditions (re-circulating and flow-through) tested in these flumes as it isolates the samples to allow comparisons over a set time period. Despite the absence of an intergravel dissolved oxygen response in the flumes, BOD findings demonstrate that addition of salmon-based flocs increased the oxygen demand of re-circulating channel sediments. The flow-through system did not show a BOD response every year, likely the result of low floc recruitment to the flume bed within those systems as was discussed in Chapter 3. The significant increase in BOD of the re-circulating flume gravel-bed samples following the addition of salmon + clay, indicates that flocs that settle onto/into the re-circulating channel bed have sufficient organic quality to consistently elevate the oxygen demand. The increased BOD is a result of significantly higher attached bacterial numbers that were actively respiring during digestion of SOM-floc based organic matter after the addition of SOM (Chapters 2 and 3). Smith (1998) identified that there is a significant relationship between the abundance of viable bacteria and respiration measured by BOD.

Nitrogen levels increased significantly after the addition of salmon + clay to the re-circulating channel and were observed to follow a pulse-like pattern in the water column and intergravel water. The pulse lasted approximately four to seven days. Total nitrogen and ammonium decreased more quickly than dissolved organic nitrogen did in the infiltration bag samples. Infiltration bag samples generally had 10 – 50% higher concentrations of total nitrogen and ammonia compared to the water column while the gravel bag concentrations of dissolved organic nitrogen were 10-fold higher.

The difference between water column and intergravel dissolved organic nitrogen concentrations can be explained by an algal bloom that appeared within a couple of days after the addition of salmon + clay. The dissolved organic nitrogen was readily taken up by algal cells, which helped to intensify the bloom. However, the intergravel region, which is limited by light, did not experience a bloom so DON concentrations were higher. After the bloom receded, as identified by increased water clarity and loss of its green hue as well as LISST-ST particle size data, dissolved organic nitrogen was measurable above the detection limit in the water column. The decline of this bloom and recruitment of algal contained MDN to the streambed represents a delayed delivery several days after the addition of SOM and clay. By day 14, all aqueous nitrogen levels returned to baseline conditions but the sediment-bound nitrogen levels did not. Although there was some variability in the C:N ratio response after the addition of salmon + clay, levels were still significantly lower than baseline conditions after 14 days. This observation supports the assumption proposed in Chapter 3 that the decrease of C:N ratios in fine sediment stored in the gravel bed is due to

the capture of low C:N ratio SOM-flocs. In the absence of nitrogen mass information for sediments, aqueous nitrogen can be used as a proxy. The addition of SOM + clay significantly increased the mass of aqueous nitrogen in the channel and its gravel bed. These findings indicate that the delivery of flocs to the streambed increased gravel bed nitrogen concentrations. Further, it also indicates that the delivery of flocs to the gravel bed can enhance nutrient retention period if MDN are sediment bound. These findings are similar to Cak *et al.*, (2008) who found that ammonia levels quickly responded to the addition of SOM in Alaskan streams and that ammonia was rapidly removed from solution because of its adherence to clay particles, identified here as a floc component. Together, these observations indicate that the formation of SOM-based flocs during the salmon die-off can deliver and enhance intergravel retention of sediment bound MDN. Once MDN is delivered to the streambed it is available to benthic food webs and hyporheic storage as was observed by O'Keefe and Edwards (2002).

Chapter 5: Salmon-Floc Feedback Loop: From Flumes to Streams

Pacific salmon play an important role in the ecology of their natal streams by providing SOM and MDN that maintains the productivity of these aquatic systems. Although natal streams require these MDN inputs to remain productive (Scheurell *et al.*, 2005), there has been limited research on in-stream MDN delivery and retention processes. This study addressed the lack of research on MDN delivery by proposing and verifying the salmon-floc feedback loop (Figure 1). The salmon-floc feedback loop presented here supports ecology-based studies on MDN in salmon streams that have shown i) the loss of the MDN signal within 10 m of salmon carcasses (Claeson *et al.*, 2006), ii) MDN delivery to hyporheic zones (O'Keefe and Edwards, 2002), and iii) post-spawning increases in- stream productivity (e.g. Naiman *et al.*, 2002; Chaloner *et al.*, 2004; Johnston *et al.*, 2004).

The salmon-floc feedback loop was studied in the controlled environment of experimental flumes using field-based salmon natal stream concentrations for SOM and sediment from O'Ne-eil Creek British Columbia. The flume-based experiments identified that the salmon-floc can deliver MDN to the streambed in sufficient quantity to decrease the carbon to nitrogen ratio of fine sediments in the flume bed for up to two weeks. Specifically, the studies described here determined that:

- SOM-based flocs formed in the presence of salmon decay products and inorganic particulate matter,
- SOM-based flocs settled or otherwise became entrained within gravel beds,
- SOM-based flocs enriched the streambed and those nutrients were available for entry into benthic foodwebs.

Flocs formed in the water column of the re-circulating flume in the presence of SOM and inorganic particulate matter. The size of flocs generated in the water column during the SOM exposures agrees with field observations of suspended sediment particle size distributions from O'Ne-eil Creek where particle diameters in excess of 400 μm were recorded (McConnachie and Petticrew, 2006). Floc formation in the flume occurred through a combination of physical, chemical, and biological processes. In this study, physical and chemical parameters were regulated to determine the influence of SOM on floc formation. The role of bacteria in floc formation was identified by the increase in attached bacteria following SOM addition. SOM supports surface-living bacteria that exude EPS, which has been well documented as important to the formation of non-SOM flocs (Droppo, 2004).

Once salmon-based flocs formed they were recruited to the simulated riffles in the re-circulating flume where they enriched the intergravel environment with MDN and attached bacteria. These attached bacteria along with existing intergravel microbes metabolized floc-based organic matter initiating the nutrient cycling process for MDN. Although these findings were generated from flume-based studies, I suggest the information presented here is applicable to Pacific salmon natal streams in the interior of British Columbia from which the field conditions were drawn.

Supporting information for the role of flocs as a MDN transport and delivery mechanism comes from the literature of other floc-based fields of study including contaminant transport, industrial waste management, and biogeochemical cycling in marine environments (Santschi et al., 2005; Geesey and Kloeke, 2005; Bhaskar and Bholse, 2005). Research in these fields

has shown that trace metals, pollutants, and nutrients are incorporated into flocs through attachment to the sediment floc components or microbial EPS (Santschi et al., 2005; Geesey and Kloeke, 2005). In the marine environment, flocs are prominent in the carbon, nitrogen and sulfur nutrient cycles (Bhaskar and Bholse, 2005). Due to their nutrient rich condition flocs are capable of supporting food webs in the mid-depths and benthos (Geesey and Kloeke, 2005). Similar processes can occur in freshwater systems because flocs comprise a significant portion of the suspended sediment load (Droppo et al., 1998). Further, MDN enriched flocs will deliver these nutrients and support benthic foodwebs similar to their marine counterparts.

Salmon play an important role in the ecology of their natal streams as demonstrated by investigations on the influence of redd creation on fine and coarse sediment movement (Kondolf, 2000; Hassan *et al.*, 2008), streambed stability (Montgomery *et al.*, 1996), and habitat maintenance (Poirier, 2004). The influence salmon have on the channel bed and the biota it supports is significant, prompting some researchers to refer to salmon as "ecosystem engineers" (Schindler *et al.*, 2003). The relationship between salmon decay, sediment, flocculation, and nutrient cycling is similarly complex to channel bed alteration but it occurs at both a microscopic (flocs and nutrients) and macroscopic scale (spawning and die-off). Floc formation and streambed delivery requires SOM from decaying salmon, inorganic sediments, bacterial populations, low-flow stream conditions, porous raised gravel bed areas, and hyporheic exchange to be an effective MDN vector. These are the typical conditions that occur in the die-off period following active spawning of salmon.

Redd construction enhances floc-bound MDN delivery to the hyporheic zone because

redds roughen the streambed surface and enhance down-welling surface-water exchange with groundwater (Bjornn and Reiser, 1991; Tonina and Buffington, 2005). Oxygen-rich surface water passes downward through salmon redds where it mixes with groundwater (Bjornn and Reiser, 1991) and deposits salmon-based flocs. In addition to increasing surface water exchange, higher bed elevation profiles increase the storage time of down-welling surface water within the hyporheic zone (Marion *et al.*, 2002), increasing the opportunity for MDN dispersal and storage within the hyporheic zone. Increased surface areas associated with redd structures may further enhance particle settling because Ren and Packman (2002) identified that particle recruitment in a gravel bed is due to a combination of particle settling and advective flow across the streambed interface.

Down-welling surface water that contains suspended flocs can lose them as water passes through the streambed gravel matrix. Flocs passing through streambed can be captured and metabolized by streambed biota or they can become trapped where the interstitial pore size is smaller than the floc. Minshall *et al.* (2000) identified that fine particulate organic matter retention time and associated nutrient cycling was greatly influenced by hyporheic filtration and biotic retention. Similarly, extensive research on inorganic sediment effects on Pacific salmon spawning success has documented that the deposition and retention of fine inorganic sediments can negatively influence salmon embryo survival because trapped fine sediments can block interstitial pores and reduce intergravel oxygen exchange for extended periods of time (McNeil and Ahnell, 1969; Chapman, 1988; Waters, 1995; Anderson *et al.*, 1999).

The capture and incorporation of nutrient rich fine sediment flocs in the streambed,

including salmon redds, provides a positive feedback opportunity that benefits spawning areas as well as downstream reaches in the watershed possibly increasing productivity and thereby benefiting natal salmon populations over the long-term. Stream fertilization programs attempt to emulate the natural salmon-floc feedback loop by contributing nutrients (drip fertilization or salmon-carcass analogues) to streams to enhance stream productivity, including fish stocks (Kohler *et al.*, 2008). The salmon-floc feedback loop may help to enhance the effectiveness of these programs by defining how nutrients can be delivered to streambeds through flocculation.

Floc structure will also influence MDN storage potential. Specifically, gravel-stored floc structure has been observed to change during the spawning cycle, with post-spawning flocs having an organic film-like covering (Petticrew and Arocena, 2003). This film contributes to floc strength and likely enhances its retention within gravel-bed interstices. The re-circulating flume experiment also documented this by identifying increased EPSD of gravel-stored flocs following the salmon + clay exposure in 2006 and 2007. These larger flocs will not be re-suspended from the streambed by interstitial flow unless the floc compacts to a size where it can pass through restricting pores or streambed scour redistributes the gravel surrounding the floc (Rehg *et al.*, 2005).

The salmon-floc loop also indicates that there is quite likely a threshold where the benefits of MDN additions can be outweighed by habitat alteration due to excess sedimentation of flocs. Flocs can alter streambed conditions similar to their inorganic components by blocking intergravel flow and oxygen exchange. In addition, the metabolic

activities of SOM attached microbes and microbial populations within the streambed may further lower intergravel oxygen levels. The reduction of oxygen levels can negatively influence salmon spawning success if they occur near eggs and alevins. Land-use activities that increase fine sediment levels during the low-flow salmon spawning period may increase floc formation and streambed delivery, which may alter watershed ecology by shifting the natural distributions patterns of MDN.

The active spawning simulation of 2007 resulted in more flocs > 200 μm forming in the water column when clay levels were higher than the post-spawning simulation. This observation indicates that large quantities of flocs may form if natural background levels of fine sediments within natal streams are increased due to land-use activities. The consequence of increased floc formation and delivery are unknown but may include excess enrichment of streambeds in spawning areas and consequently a lowering of MDN exported to downstream environments, which may include nursery lakes. To minimize the disruption of MDN delivery and distribution processes, activities increasing the contribution of fine sediments to salmon-bearing streams should be minimized particularly during and following spawning activities. Further, field studies should be initiated to identify fine sediment thresholds where the mass balance relationship between MDN retention and export changes in relation to land-use activities such as urban development, agriculture, and forestry which generally add sediment to streams and hydroelectric facilities or other sediment retention activities that remove fine sediment from downstream areas.

Wotton (2007) emphasized that the elucidation of floc-based nutrient delivery processes is essential to improve our basic scientific understanding of aquatic ecology as well as to

effectively plan, manage, and conserve our watersheds. This study addressed this need within Pacific salmon watersheds by verifying the salmon-floc feedback loop as a viable nutrient delivery process. Further, the findings presented here in the context of cited literature suggests exciting future research opportunities. For example, field-based investigations of floc retention and marine derived nutrient cycling processes within streambeds of productive Pacific salmon streams to discern the influence of various hydrologic regimes on nutrient storage and Pacific salmon spawning success would be useful from both a scientific and management perspective. In addition, salmon enhancement activities may benefit from the information provided by this study by mimicking processes that stimulate benthic foodwebs in fertilization programs. Nutrient cycling research in Pacific salmon streams will benefit from knowledge of the salmon-floc feedback loop because it identifies the mechanisms associated with nutrient delivery and retention in a watershed.

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Appendix 1

LISST –ST Technical Specifications

Parameters measured/derived:

size distribution
settling velocity
optical transmission
pressure
temperature

- Optical path length: 5 cm standard, 2.5 cm optional with Path Reduction Module (PRM),
- Optical transmission: 12 bit resolution
- Particle size range: 1.2 - 250 micron diameter (Type B), 2.5-500 micron for Type C.
- Resolution (for settling velocity): 8 size classes, log-spaced
- Data storage memory: 512K (6500 samples, or 75 settling experiments) expandable to 2 MB (26,000 samples or 300 settling experiments)
- Maximum sample speed: 4 size distributions per second (standard)
- Temperature-sensor range: -10 to 45$_o$C resolution: 0.01$_o$C
- Pressure-sensor range: 0 to 300 m of H_2O, resolution: 8 cm of H_2O
- Dimensions: 13 cm (5") dia x 81 cm (32")
- Weight in air: 12 Kg (25 lb)
- Weight in water: 4 Kg (8.5 lb)
- Depth rating: 300 m (standard)

Procedures for Particle Size Analysis using LISST-ST

Effective Particle Size Distribution (EPSD)

- Suspended sediment samples do not require dilution
- Bed (trap) sediment samples should be diluted at a ratio of 1 part wet sediment to 3 parts water, i.e. 16ml wet sediment : 48 ml water

1. Acquire background calibration (.zsc) using groundwater (GW is as clean as DI, and is run through flumes),
2. Load LAB TEST.lop sample routine from C:\Program Files\Sequoia\LISST100,
3. Mix sample bottles by gentle agitation: wash all sediment settled onto bottom of bottle into suspension, then turn bottle end over end 10 times,

4. For bed sediment samples, measure 16ml of sample into a 50ml centrifuge tube. Measure 48ml GW into a separate 50ml centrifuge tube. Pour sediment sample into a 500ml bottle, pour water into sediment centrifuge tube then into 500ml bottle to wash all sediment into suspension. Pour contents of 500ml bottle into LISST sample chamber. This dilutes and mixes sediment sample rigorously for analysis,
5. For suspended sediment samples, mix by turning bottle end over end 10 times and add directly into LISST sample chamber,
6. Add LISST PRM if necessary,
7. Use mechanical switch to initiate LISST analysis routine,
8. Retain sample in 50 ml centrifuge tube for absolute particle size analysis,
9. Rinse all apparatus.

Absolute Particle Size Distribution (APSD)

1. Acquire background calibration (.zsc) using groundwater,
2. Into 50ml centrifuge tubes, weigh 0.22g sodium hexametaphosphate and 0.4g sodium carbonate (anhydrous),
3. Mix sample bottles by gentle agitation: wash all sediment settled onto bottom of bottle into suspension, then turn bottle end over end 10 times,
4. Place centrifuge tube samples in ultra-sonic bath for 25 mins,
5. Meanwhile, load LAB TEST.lop sample routine from C:\ProgramFiles\Sequoia\LISST100 and add 15ml GW into LISST sample chamber,
6. Mix each tube by turning end over end 10 times, and add to LISST sample chamber,
7. Allow sample to degas for 2 minutes, while stirring,
8. Stir sample to ensure all particles are in suspension,
9. Add LISST PRM,
10. Use mechanical switch to initiate LISST analysis routine,
11. Rinse all apparatus

Procedures for Particle Settling Velocity Analysis using LISST

- Suspended sediment samples do not require dilution
- Bed (trap) sediment samples should be diluted at a ratio of 1 part wet sediment to 3 parts water:
  - With PRM: 57.5ml wet sediment : 172.5 ml water
  - Without PRM: 70 ml wet sediment : 210 ml water

1. Acquire background calibration (.zsc) using groundwater (GW is as clean as DI, and is run through flumes),

2. Fix PRM if necessary, and fix settling column,
3. Load Settling Experiment (SE) routine from LISST-SOP software,
4. Use look-up table in C:\Program Files\Sequoia\LISST Manuals\LISST ST Technical Info.xls to determine the experiment duration (number of scans),
5. Mix sample bottles by gentle agitation: wash all sediment settled onto bottom of bottle into suspension, then turn bottle end over end 10 times,
6. Dilute bed (trap) samples as appropriate using groundwater, and mix by turning bottle end over end 10 times,
7. For suspended sediment samples, dilution is not required, but mix by turning bottle end over end 10 times,
8. Add sample to the settling column, so that the column is filled to HALFWAY (15cm above laser). This level is marked on the column,
9. Start SE analysis routine via the LISST-SOP software,
10. Move the LISST so that the column is out of direct sunlight,
11. When analysis is complete, thoroughly rinse all apparatus,
12. When analysing data, the reported settling velocity values should be doubled to account for halfway settling distance.

Appendix 2

Table: Water column sample nitrogen data from the re-circulating flume in 2008, no data were excluded to QA/QC issues.

Maxxam ID	L11960	L11969	L11970	L11977	L11978	L18408	L18409
Sampling Date	2008-08-09	2008-08-10	2008-08-10	2008-08-11	2008-08-11	2008-08-13	2008-08-13
COC Number	F118527	F118527	F118527	F118528	F118528	F118439	F118439
	SURFACE WATER	**SURFACE WATER A**	**SURFACE WATER B**	**SURFACE WATER A**	**SURFACE WATER B**	**SURFACE WATER A**	**SURFACE WATER B**
Nutrients							
Dissolved Organic Nitrogen (N)	<0.02	<0.02	<0.02	<0.02	<0.02	0.07	0.03
Total Total Kjeldahl Nitrogen (Calc)	<0.02	0.26	0.27	0.20	0.20	0.17	0.18
Ammonia (N)	<0.005	0.239	0.245	0.207	0.200	0.081	0.082
Nitrate plus Nitrite (N)	0.17	0.17	0.16	0.18	0.17	0.22	0.22
Dissolved Nitrogen (N)	0.15	0.39	0.37	0.34	0.25	0.36	0.33
Total Nitrogen (N)	0.14	0.43	0.44	0.38	0.38	0.38	0.39

Maxxam ID	L18413	L18414	L20877	L20878	L31881	L31882
Sampling Date	2008-08-15	2008-08-15	2008-08-16	2008-08-16	2008-08-23	2008-08-23
COC Number	F118439	F118440	8208443	8208443	F118864	F118864
	SURFACE WATER A	**SURFACE WATER B**	**SURFACE H2O A**	**SURFACE H2O B**	**SURFACE WATER A**	**SURFACE WATER B**
Nutrients						
Dissolved Organic Nitrogen (N)	0.04	<0.02	0.06	0.03	0.05	0.04
Total Total Kjeldahl Nitrogen (Calc)	0.08	0.03	0.09	0.06	0.06	0.04
Ammonia (N)	<0.005	<0.005	0.009	0.010	<0.005	<0.005
Nitrate plus Nitrite (N)	0.22	0.21	0.13	0.13	<0.02	<0.02
Dissolved Nitrogen (N)	0.26	0.22	0.20	0.17	0.05	0.04
Total Nitrogen (N)	0.29	0.24	0.22	0.20	0.06	0.04

Table: Piezometer sample nitrogen data from the re-circulating flume in 2008, no data were excluded to QA/QC issues.

Maxxam ID		L11957	L11958	L11959	L11961	L11967	L11968
Sampling Date		09/08/2008	09/08/2008	09/08/2008	10/08/2008	10/08/2008	10/08/2008
COC Number		F118527	F118527	F118527	F118527	F118527	F118527
	Units	**PIEZOMETER 7**	**PIEZOMETER 9**	**PIEZOMETER 15**	**PIEZOMETER 5**	**PIEZOMETER 8**	**PIEZOMETER 9**
Nutrients							
Dissolved Organic Nitrogen (N)	mg/L	0.02	<0.02	0.02	<0.02	<0.02	<0.02
Total Total Kjeldahl Nitrogen (Calc)	mg/L	0.03	0.22	<0.02	0.26	0.20	<0.02
Ammonia (N)	mg/L	<0.005	0.225	0.007	0.227	0.212	<0.005
Nitrate plus Nitrite (N)	mg/L	0.14	0.17	0.15	0.16	0.17	0.14
Dissolved Nitrogen (N)	mg/L	0.16	0.39	0.19	0.39	0.34	0.10
Total Nitrogen (N)	mg/L	0.16	0.39	0.17	0.42	0.36	0.16

Maxxam ID		L11971	L11972	L11973	L18405	L18406	L18407
Sampling Date		11/08/2008	11/08/2008	11/08/2008	13/08/2008	13/08/2008	13/08/2008
COC Number		F118528	F118528	F118528	F118439	F118439	F118439
	Units	**PIEZOMETER 2**	**PIEZOMETER 12**	**PIEZOMETER 14**	**PIEZO #3**	**PIEZO #8**	**PIEZO #6**
Nutrients							
Dissolved Organic Nitrogen (N)	mg/L	0.03	<0.02	<0.02	0.04	0.04	0.04
Total Total Kjeldahl Nitrogen (Calc)	mg/L	0.14	0.20	0.06	0.15	0.18	0.21
Ammonia (N)	mg/L	0.109	0.206	0.085	0.056	0.079	0.083
Nitrate plus Nitrite (N)	mg/L	0.20	0.18	0.31	0.23	0.22	0.22
Dissolved Nitrogen (N)	mg/L	0.34	0.30	0.28	0.32	0.33	0.34
Total Nitrogen (N)	mg/L	0.34	0.37	0.37	0.38	0.40	0.43

		L18410	L18411	L18412	L18410	L18411	L18412	L31878
Sampling Date		15/08/2008	15/08/2008	15/08/2008	15/08/2008	15/08/2008	15/08/2008	23/08/2008
COC Number		F118439	F118439	F118439	F118439	F118439	F118439	F118864
	Units	**PIEZO #1**	**PIEZO #2**	**PIEZO #3**	**PIEZO #1**	**PIEZO #2**	**PIEZO #3**	**PIEZOMETER #5**
Nutrients								
Dissolved Organic Nitrogen (N)	mg/L	0.02	<0.02	<0.02	0.02	<0.02	<0.02	0.03
Total Total Kjeldahl Nitrogen (Calc)	mg/L	0.09	0.10	0.11	0.09	0.10	0.11	0.04
Ammonia (N)	mg/L	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Nitrate plus Nitrite (N)	mg/L	0.22	0.22	0.21	0.22	0.22	0.21	<0.02
Dissolved Nitrogen (N)	mg/L	0.24	0.21	0.23	0.24	0.21	0.23	0.03
Total Nitrogen (N)	mg/L	0.31	0.31	0.32	0.31	0.31	0.32	0.04

Table: Infiltration bag sample nitrogen data from the re-circulating flume in 2008, no data were excluded to QA/QC issues.

Maxxam ID		L11954	L11955	L11956	L11974	L11975	L11976	L18402
Sampling Date		09/08/2008	09/08/2008	09/08/2008	11/08/2008	11/08/2008	11/08/2008	13/08/2008
COC Number		F118527	F118527	F118527	F118528	F118528	F118528	F118439
	Units	**BAG 7**	**BAG 9**	**BAG 15**	**BAG 2**	**BAG 12**	**BAG 14**	**BAG #3**
Nutrients								
Dissolved Organic Nitrogen (N)	mg/L	0.06	0.09	0.19	0.30	0.17	0.20	0.23
Total Total Kjeldahl Nitrogen (Calc)	mg/L	0.05	0.12	0.21	0.73	0.63	0.45	0.38
Ammonia (N)	mg/L	<0.005	<0.005	<0.005	0.350	0.297	0.270	0.169
Nitrate plus Nitrite (N)	mg/L	0.16	0.15	0.09	0.18	0.18	0.18	0.22
Dissolved Nitrogen (N)	mg/L	0.22	0.24	0.28	0.83	0.65	0.64	0.61
Total Nitrogen (N)	mg/L	0.20	0.28	0.30	0.91	0.80	0.63	0.59

Maxxam ID		L20874	L20875	L20876	L31875	L31876	L31877
Sampling Date		16/08/2008	16/08/2008	16/08/2008	23/08/2008	23/08/2008	23/08/2008
COC Number		8208443	8208443	8208443	F118864	F118864	F118864
	Units	**BAG #1**	**BAG #4**	**BAG #10**	**BAG #5**	**BAG #11**	**BAG #13**
Nutrients							
Dissolved Organic Nitrogen (N)	mg/L	0.23	0.18	0.17	0.13	0.08	0.12
Total Total Kjeldahl Nitrogen (Calc)	mg/L	0.32	0.27	0.28	0.16	0.17	0.15
Ammonia (N)	mg/L	0.088	0.07	0.068	0.026	0.061	0.027
Nitrate plus Nitrite (N)	mg/L	0.14	0.13	0.13	<0.02	<0.02	0.06
Dissolved Nitrogen (N)	mg/L	0.45	0.38	0.37	0.15	0.14	0.20
Total Nitrogen (N)	mg/L	0.46	0.4	0.41	0.16	0.17	0.21

Table: Blank sample nitrogen data in 2008, no samples were excluded based on these results.

Maxxam ID			L18415	L31883	L20879	L11980
Sampling Date			2008-08-15	2008-08-23	2008-08-16	2008-08-11
COC Number			F118440	F118864	8208443	F118528
	Units	**RDL**	**DI TRAVEL BLANK**	**DI TRAVEL BLANK**	**DI TRAVEL BLANK**	**DI TRAVEL BLANK**
Nutrients						
Dissolved Organic Nitrogen (N)	mg/L	0.02	<0.02	<0.02	<0.02	<0.02
Total Total Kjeldahl Nitrogen (Calc)	mg/L	0.02	<0.02	0.05	<0.02	<0.02
Ammonia (N)	mg/L	0.005	<0.005	<0.005	<0.005	<0.005
Nitrate plus Nitrite (N)	mg/L	0.02	<0.02	<0.02	<0.02	<0.02
Dissolved Nitrogen (N)	mg/L	0.02	<0.02	<0.02	<0.02	<0.02
Total Nitrogen (N)	mg/L	0.02	<0.02	0.05	<0.02	<0.02

RDL = Reportable Detection Limit

Table: Spiked sample recovery for 2008, no data were excluded based on these results.

QA/QC Batch Num	Init	QC Type	Parameter	Date Analyzed yyyy/mm/dd	Value	Recovery	Units	QC Limits
2522178	TS1	MATRIX SPIKE	Total Nitrogen (N)	2008-08-20		103	%	80 - 120
		SPIKE	Total Nitrogen (N)	2008-08-20		98	%	80 - 120
		BLANK	Total Nitrogen (N)	2008-08-20	<0.02		mg/L	
		RPD	Total Nitrogen (N)	2008-08-20	NC		%	25
2522897	IC4	MATRIX SPIKE	Nitrate plus Nitrite (N)	2008-08-21		84	%	80 - 120
		SPIKE	Nitrate plus Nitrite (N)	2008-08-21		100	%	80 - 120
		BLANK	Nitrate plus Nitrite (N)	2008-08-21	<0.02		mg/L	
		RPD	Nitrate plus Nitrite (N)	2008-08-21	NC		%	25
2522974	BB3	MATRIX SPIKE	Ammonia (N)	2008-08-21		88	%	80 - 120
		SPIKE	Ammonia (N)	2008-08-21		95	%	80 - 120
		BLANK	Ammonia (N)	2008-08-21	<0.005		mg/L	
		RPD	Ammonia (N)	2008-08-21	NC		%	25
2523585	TS1	MATRIX SPIKE	Dissolved Nitrogen (N)	2008-08-21		98	%	80 - 120
		SPIKE	Dissolved Nitrogen (N)	2008-08-20		96	%	80 - 120
		BLANK	Dissolved Nitrogen (N)	2008-08-20	<0.02		mg/L	
		RPD	Dissolved Nitrogen (N)	2008-08-20	0.4		%	25
2517264	BB3	MATRIX SPIKE	Ammonia (N)	2008-08-19		98	%	80 - 120
		SPIKE	Ammonia (N)	2008-08-19		85	%	80 - 120
		BLANK	Ammonia (N)	2008-08-19	<0.005		mg/L	
		RPD	Ammonia (N)	2008-08-19	1.4		%	25
2517586	TS1	MATRIX SPIKE	Total Nitrogen (N)	2008-08-20		85	%	80 - 120
		SPIKE	Total Nitrogen (N)	2008-08-20		104	%	80 - 120
		BLANK	Total Nitrogen (N)	2008-08-20	0.02	RDL=0.02	mg/L	
		RPD	Total Nitrogen (N)	2008-08-20	1.5		%	25
		RPD	Total Nitrogen (N)	2008-08-20	NC		%	25
2518054	IC4	MATRIX SPIKE	Nitrate plus Nitrite (N)	2008-08-19		96	%	80 - 120
		SPIKE	Nitrate plus Nitrite (N)	2008-08-19		98	%	80 - 120
		BLANK	Nitrate plus Nitrite (N)	2008-08-19	<0.02		mg/L	
		RPD	Nitrate plus Nitrite (N)	2008-08-19	0.5		%	25
2523585	TS1	MATRIX SPIKE	Dissolved Nitrogen (N)	2008-08-21		98	%	80 - 120
		SPIKE	Dissolved Nitrogen (N)	2008-08-20		96	%	80 - 120
		BLANK	Dissolved Nitrogen (N)	2008-08-20	<0.02		mg/L	
		RPD	Dissolved Nitrogen (N)	2008-08-20	NC		%	25

QA/QC Batch Num	Init	QC Type	Date Analyzed Parameter	yyyy/mm/dd	Value	Recovery	Units	QC Limits
2506874	SC2	MATRIX SPIKE	Nitrate plus Nitrite (N)	2008-08-14		99	%	80 - 120
		SPIKE	Nitrate plus Nitrite (N)	2008-08-14		102	%	80 - 120
		BLANK	Nitrate plus Nitrite (N)	2008-08-14	<0.02		mg/L	
		RPD	Nitrate plus Nitrite (N)	2008-08-14	0.6		%	25
2509513	BB3	MATRIX SPIKE	Ammonia (N)	2008-08-15		98	%	80 - 120
		SPIKE	Ammonia (N)	2008-07-03		98	%	80 - 120
		BLANK	Ammonia (N)	2008-07-03	<0.005		mg/L	
		RPD	Ammonia (N)	2008-07-03	6.5		%	25
2513877	TS1	MATRIX SPIKE	Total Nitrogen (N)	2008-08-18		96	%	80 - 120
		SPIKE	Total Nitrogen (N)	2008-08-20		105	%	80 - 120
		BLANK	Total Nitrogen (N)	2008-08-20	<0.02		mg/L	
		RPD	Total Nitrogen (N)	2008-08-18	0.9		%	25
2513925	TS1	MATRIX SPIKE	Dissolved Nitrogen (N)	2008-08-18		94	%	80 - 120
		SPIKE	Dissolved Nitrogen (N)	2008-08-20		95	%	80 - 120
		BLANK	Dissolved Nitrogen (N)	2008-08-20	<0.02		mg/L	
		RPD	Dissolved Nitrogen (N)	2008-08-20	3		%	25
2537431	IC4	MATRIX SPIKE	Ammonia (N)	2008-08-28		95	%	80 - 120
		SPIKE	Ammonia (N)	2008-08-28		92	%	80 - 120
		BLANK	Ammonia (N)	2008-08-28	<0.005		mg/L	
		RPD	Ammonia (N)	2008-08-28	NC		%	25
2538711	SC2	MATRIX SPIKE	Nitrate plus Nitrite (N)	2008-08-28		100	%	80 - 120
		SPIKE	Nitrate plus Nitrite (N)	2008-08-28		108	%	80 - 120
		BLANK	Nitrate plus Nitrite (N)	2008-08-28	<0.02		mg/L	
		RPD	Nitrate plus Nitrite (N)	2008-08-28	NC		%	25
2539768	TS1	MATRIX SPIKE	Total Nitrogen (N)	2008-08-28		80	%	80 - 120
		SPIKE	Total Nitrogen (N)	2008-08-20		95	%	80 - 120
		BLANK	Total Nitrogen (N)	2008-08-20	<0.02		mg/L	
		RPD	Total Nitrogen (N)	2008-08-20	NC		%	25
2539973	TS1	MATRIX SPIKE	Dissolved Nitrogen (N)	2008-08-28		94	%	80 - 120
		SPIKE	Dissolved Nitrogen (N)	2008-08-20		102	%	80 - 120
		BLANK	Dissolved Nitrogen (N)	2008-08-20	<0.02		mg/L	
		RPD	Dissolved Nitrogen (N)	2008-08-20	NC		%	25

QA/QC Batch Num	Init	QC Type	Date Analyzed Parameter	yyyy/mm/dd	Value	Recovery	Units	QC Limits
2506874	SC2	MATRIX SPIKE	Nitrate plus Nitrite (N)	2008-08-14		99	%	80 - 120
		SPIKE	Nitrate plus Nitrite (N)	2008-08-14		102	%	80 - 120
		BLANK	Nitrate plus Nitrite (N)	2008-08-14	<0.02		mg/L	
		RPD	Nitrate plus Nitrite (N)	2008-08-14	0.6		%	25
2509513	BB3	MATRIX SPIKE	Ammonia (N)	2008-08-15		98	%	80 - 120
		SPIKE	Ammonia (N)	2008-08-15		98	%	80 - 120
		BLANK	Ammonia (N)	2008-08-15	<0.005		mg/L	
		RPD	Ammonia (N)	2008-08-15	6.5		%	25
2513877	TS1	MATRIX SPIKE	Total Nitrogen (N)	2008-08-18		96	%	80 - 120
		SPIKE	Total Nitrogen (N)	2008-08-18		105	%	80 - 120
		BLANK	Total Nitrogen (N)	2008-08-18	<0.02		mg/L	
		RPD	Total Nitrogen (N)	2008-08-18	0.9		%	25
2513925	TS1	MATRIX SPIKE	Dissolved Nitrogen (N)	2008-08-18		94	%	80 - 120
		SPIKE	Dissolved Nitrogen (N)	2008-08-18		95	%	80 - 120
		BLANK	Dissolved Nitrogen (N)	2008-08-18	<0.02		mg/L	
		RPD	Dissolved Nitrogen (N)	2008-08-18	3		%	25
2522178	TS1	MATRIX SPIKE	Total Nitrogen (N)	2008-08-20		103	%	80 - 120
		SPIKE	Total Nitrogen (N)	2008-08-20		98	%	80 - 120
		BLANK	Total Nitrogen (N)	2008-08-20	<0.02		mg/L	
		RPD	Total Nitrogen (N)	2008-08-20	NC		%	25
2522897	IC4	MATRIX SPIKE	Nitrate plus Nitrite (N)	2008-08-21		84	%	80 - 120
		SPIKE	Nitrate plus Nitrite (N)	2008-08-21		100	%	80 - 120
		BLANK	Nitrate plus Nitrite (N)	2008-08-21	<0.02		mg/L	
		RPD	Nitrate plus Nitrite (N)	2008-08-21	NC		%	25
2522974	BB3	MATRIX SPIKE	Ammonia (N)	2008-08-21		88	%	80 - 120
		SPIKE	Ammonia (N)	2008-08-21		95	%	80 - 120
		BLANK	Ammonia (N)	2008-08-21	<0.005		mg/L	
		RPD	Ammonia (N)	2008-08-21	NC		%	25
2523585	TS1	MATRIX SPIKE	Dissolved Nitrogen (N)	2008-08-21		98	%	80 - 120
		SPIKE	Dissolved Nitrogen (N)	2008-08-21		96	%	80 - 120
		BLANK	Dissolved Nitrogen (N)	2008-08-21	<0.02		mg/L	
		RPD	Dissolved Nitrogen (N)	2008-08-21	0.4		%	25

QA/QC Batch Num	Init	QC Type	Parameter	Date Analyzed yyyy/mm/dd	Value	Recovery	Units	QC Limits
2517264	BB3	MATRIX SPIKE	Ammonia (N)	2008-08-19		98	%	80 - 120
		SPIKE	Ammonia (N)	2008-08-19		85	%	80 - 120
		BLANK	Ammonia (N)	2008-08-19	<0.005		mg/L	
		RPD	Ammonia (N)	2008-08-19	1.4		%	25
2517586	TS1	MATRIX SPIKE	Total Nitrogen (N)	2008-08-19		85	%	80 - 120
		SPIKE	Total Nitrogen (N)	2008-08-19		104	%	80 - 120
		BLANK	Total Nitrogen (N)	2008-08-19	0.02	RDL=0.02	mg/L	
		RPD	Total Nitrogen (N)	2008-08-19	1.5		%	25
		RPD	Total Nitrogen (N)	2008-08-19	NC		%	25
2518054	IC4	MATRIX SPIKE	Nitrate plus Nitrite (N)	2008-08-19		96	%	80 - 120
		SPIKE	Nitrate plus Nitrite (N)	2008-08-19		98	%	80 - 120
		BLANK	Nitrate plus Nitrite (N)	2008-08-19	<0.02		mg/L	
		RPD	Nitrate plus Nitrite (N)	2008-08-19	0.5		%	25
2523585	TS1	MATRIX SPIKE	Dissolved Nitrogen (N)	2008-08-21		98	%	80 - 120
		SPIKE	Dissolved Nitrogen (N)	2008-08-21		96	%	80 - 120
		BLANK	Dissolved Nitrogen (N)	2008-08-21	<0.02		mg/L	
		RPD	Dissolved Nitrogen (N)	2008-08-21	NC		%	25
2537431	IC4	MATRIX SPIKE	Ammonia (N)	2008-08-28		95	%	80 - 120
		SPIKE	Ammonia (N)	2008-08-28		92	%	80 - 120
		BLANK	Ammonia (N)	2008-08-28	<0.005		mg/L	
		RPD	Ammonia (N)	2008-08-28	NC		%	25
2538711	SC2	MATRIX SPIKE	Nitrate plus Nitrite (N)	2008-08-28		100	%	80 - 120
		SPIKE	Nitrate plus Nitrite (N)	2008-08-28		108	%	80 - 120
		BLANK	Nitrate plus Nitrite (N)	2008-08-28	<0.02		mg/L	
		RPD	Nitrate plus Nitrite (N)	2008-08-28	NC		%	25
2539768	TS1	MATRIX SPIKE	Total Nitrogen (N)	2008-08-28		80	%	80 - 120
		SPIKE	Total Nitrogen (N)	2008-08-28		95	%	80 - 120
		BLANK	Total Nitrogen (N)	2008-08-28	<0.02		mg/L	
		RPD	Total Nitrogen (N)	2008-08-28	NC		%	25
2539973	TS1	MATRIX SPIKE	Dissolved Nitrogen (N)	2008-08-28		94	%	80 - 120
		SPIKE	Dissolved Nitrogen (N)	2008-08-28		102	%	80 - 120
		BLANK	Dissolved Nitrogen (N)	2008-08-28	<0.02		mg/L	
		RPD	Dissolved Nitrogen (N)	2008-08-28	NC		%	25

NC = Non-calculable

RPD = Relative Percent Difference