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Biofilm responses to salmon carcass analog addition in central Idaho streams

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BIOFILM RESPONSES TO SALMON CARCASS ANALOG ADDITION IN

CENTRAL IDAHO STREAMS

By:

Jonathan D. Ebel

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

(Biological Sciences)

MICHIGAN TECHNOLOGICAL UNIVERSITY

2012

This thesis, "Biofilm Responses to Salmon Carcass Analog Addition in Central Idaho Streams," is hereby approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE IN BIOLOGICAL SCIENCES.

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The migration of juvenile salmon from their natal streams to the ocean constitutes an enormous challenge; they must swim through hundreds of miles of slack water, escape the jaws of a plethora of beasts trying to eat them, and survive eight vertical drops through spinning hydroelectric turbines. It's amazing that any of the young salmon ever make it to the ocean. Each salmon must have a friend like Beth.

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Abstract

Pacific salmon populations have declined due to human activity in the Pacific Northwest, resulting in decreased delivery of marine-derived nutrients to streams. Managers use artificial nutrient additions to increase juvenile salmon growth and survival and assume that added nutrients stimulate biofilm production, which propagates up the food web to juvenile salmon. We assessed biofilm responses (standing crop, nutrient limitation, and metabolism) to experimental additions of salmon carcass analog in tributaries of the Salmon River, Idaho in 2010 and 2011. Biofilm standing crop and nutrient limitation did not respond to analog, but primary productivity and respiration increased in the subset of streams where they were measured. Discrepancies between biofilm productivity and standing crop may occur if standing crop is constrained by physical and biological factors. Thus, conclusions about biofilm response to analog should not be based on standing crop alone and mitigation research may benefit from nutrient budgets of entire watersheds.

1. INTRODUCTION

The movement of organisms, nutrients, and energy that connect ecosystems (i.e., resource subsidies) can substantially influence the biological and physiochemical dynamics of the receiving habitats (Polis et al. 1997). Stream ecologists have demonstrated that inputs of terrestrial organic matter (e.g., leaves) and prey (e.g., invertebrates) can dominate ecosystem energy budgets (Fisher and Likens 1973; Webster and Meyer 1997), control nutrient cycling rates (Valett et al. 2008), and alter intra- and inter-trophic level relationships (Nakano et al. 1999; Moore et al. 2004). More recently, ecologists have recognized the importance of interactions between the quantity and quality of resource subsidies to food webs and ecosystem processes (Marcarelli et al. 2011). Subsidies can enter streams as living or dead biomass. Nutrients and carbon are released as dissolved or particulate organic matter through a combination of interacting physical, chemical, and biological forces that drive decomposition rates (Suberkropp et al. 2010; Fenoglio et al. 2010). Dissolved nutrients and fine particulates are then available for capture by biofilms, which are corsortia of algal, bacterial, and fungal species that co-inhabit the surface of cobbles, sediments, wood, and plants in the stream channel. Biofilms are responsible for a significant proportion of primary production, carbon metabolism and nutrient cycling in streams.

Pacific salmon (*Oncorhynchus* spp.) are widely recognized as an important resource subsidy that regulates the ecological dynamics of the rivers in which they spawn. Salmon transport carbon and nutrients from marine ecosystems to their natal freshwater streams. The carbon and nutrients are released as labile compounds through

excretion, post-spawn carcass decomposition, and the direct consumption of tissue by other organisms (Chaloner et al. 2002). In regions with large salmon runs, organic material from dead salmon propogates through trophic levels of both aquatic and terrestrial communities (Kline et al. 1990; Bilby et al. 1996; Helfield and Naiman 2001; Hicks et al. 2005). In aquatic systems, salmon are also hypothesized to stimulate food webs via bottom up processes by alleviating algal nutrient limitation and increasing primary productivity (Juday et al. 1932; Nelson and Edmondson 1955). In turn, higher primary productivity increases invertebrate consumer production. Subsequently, higher invertebrate abundances should translate into increased growth and survival of juvenile salmon. In support of this hypothesis, studies have shown that spawning salmon and carcasses increase stream water nutrient concentrations (Mitchell and Lamberti 2005; Chaloner et al. 2007), eliminate biofilm nutrient limitation (Ruegg et al. 2011; Tiegs et al. 2011), and can increase biofilm and macroinvertebrate biomass (Wipfli et al. 1998; Wipfli et al. 1999).

In addition to bottom-up food-web stimulation, redd or nest digging behavior of spawning salmon is a disturbance mechanism, which may counteract the stimulatory effects of increased nutrient supply by dislodging benthic biofilms and macroinvertebrates (Peterson and Foote 2000; Moore and Schindler 2008; Tiegs et al. 2009; Holtgrieve and Schindler 2011). Generalized conclusions about the role of salmon as a consistent resource subsidy remain elusive (Janetski et al. 2009). Most likely, the diversity of physical and behavioral characteristics among and within salmon species across their historic ranges (i.e., body size, spawner density, timing of spawn) interacts with spatial and temporal variability in physical habitat characteristics to determine how

ecosystem processes are influenced by spawning salmon (Ruegg et al. 2012). The effect of salmon on ecosystems and the salmon themselves are highly diverse (Schindler et al. 2010) and it is clear that stream ecosystems are as much a product of their salmon as the salmon are a product of their natal streams.

Over the past century, human activity has negatively affected Pacific salmon populations throughout much of their historic range with the steepest declines occurring in the contiguous United States (Nehlsen et al. 1991). Salmon populations in the Columbia River Basin are 5-7% of historical levels because of hydroelectric dams, hatcheries, habitat destruction, and over-harvest (Lichatowich 1999; Gresh et al. 2000; Thomas et al. 2003). In many watersheds, the decline or extirpation of Pacific salmon has consequences for entire stream food webs. Streams with depressed salmon populations risk further salmon decline in a negative feedback pattern where nutrient limitation of algae and microbes limits juvenile salmon growth rates, in turn, decreasing their survival during and after out-migration (Larkin and Slaney 1997). Traditionally, salmon restoration initiatives have focused on hatchery supplementation and the maintenance, restoration, or creation of physical habitat for salmon spawning and rearing. Recently, researchers and managers have shifted their focus to the importance of spawning and dead salmon to production within stream food webs (Kiffney and Roni 2007; Wipfli and Baxter 2010), which ultimately has consequences for juvenile salmon and trout survival.

The use of artificial nutrient enrichment to bolster salmon populations was first tested in the 1950's as a way to stabilize commercial harvests. Inorganic fertilizer additions to sockeye salmon rearing lakes resulted in higher primary production and phytoplankton biomass, but failed to stimulate production in higher trophic levels

(Nelson and Edmondson 1955). Whole-lake enrichments for stock enhancement were discontinued, but the practice was introduced as a tool for salmon conservation in the 1970's in British Columbia, 1980's in Alaska, and mid 1990's in central Idaho (Hyatt et al. 2004). These later studies found that nitrogen and phosphorus additions translated into greater sockeye smolt weights and egg to smolt survival rates. Artificial nutrient enrichment of streams began in earnest in the mid-1990's, and tend to use organic rather than inorganic nutrients (Compton et al. 2006). Currently, salmon nutrient additions to streams are popular with state and tribal fisheries agencies in the Pacific Northwest as a salmon conservation and restoration tool commonly referred to as salmon nutrient mitigation (e.g., ODFW 2009; Kohler et al. 2012). Yet, the way nutrient subsidies enter the stream food web may limit the success of mitigation efforts.

Salmon nutrient mitigation research and application is dominated by the bottom-up hypothesis and has focused on biofilms as the primary vector by which salmon nutrients are transferred into the food web. The bottom-up hypothesis assumes that algae are nutrient limited, and that algae have the capacity to respond to salmon nutrient addition. Primary producers are often limited by nitrogen or phosphorus or co-limited by both N and P (Harpole et al. 2011). Stream biofilm autotrophs are no exception (Tank and Dodds 2003; Sanderson et al. 2009), and nutrient limited algae tend to respond positively to nutrient additions. Secondary production by heterotrophic microbes may also be an important pathway for salmon nutrients into the foodweb; however, nutrient mitigation research has largely ignored responses of biofilm heterotrophs. Biofilm heterotrophs can be limited by algal carbon (e.g. Haack et al. 1988; Scott et al. 2008) and respond positively to additions of labile dissolved organic matter (Cooney and Simon

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2009; Van Horn et al. 2011). Additionally, inorganic nutrient enrichment can increase food quality for detritus-feeding macroinvertebrates by decreasing the C:N ratio of fungi and bacteria (Cross et al. 2006). Yet, salmon nutrient enrichment does not consistently yield positive responses in either autotrophic or heterotrophic microbial standing crop (Janetski et al. 2009). The lack of consistent biofilm standing crop responses is likely due to spatial and temporal variability in physical and biological factors that constrain biomass accrual such as light (Hill et al. 1995), grazing (Rosemond 1993), and interactions between disturbance and biofilm development (Stoodley et al. 1999; Romani and Sabater 2001; Romani et al. 2004; Augspurger and Kusel 2010).

The objective of our study was to assess changes in biofilm nutrient limitation, standing crop, and metabolism following the addition of salmon carcass analog to tributaries of the Salmon River, Idaho. Salmon carcass analog is made of dried, pasteurized, and pelletized marine fishmeal (Pearsons et al. 2007) and may be a viable source of marine-derived carbon and nutrients to streams with depressed or extirpated salmon populations (Wipfli et al. 2004; Kohler et al. 2012). To date, most salmon-food web studies have used biofilm standing crop (i.e., chlorophyll *a* and AFDM) or stable isotopes to determine the incorporation of salmon nutrients into biofilms including previous research in tributaries of the Salmon River (Kohler et al. 2008; Kohler and Taki 2010). We measured biofilm standing crop, biofilm nutrient limitation, benthic metabolism, and whole-stream metabolism in upstream and downstream segments of streams that received different analog loads. Metabolism is a measure of the primary production and respiration, which is dominated by autotrophic and heterotrophic microbial metabolic activity and integrates the processes governing organic matter

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dynamics and nutrient cycling (Tank et al. 2010). We predicted that (i) biofilm algal and heterotroph standing crop would be higher in stream segments that received analog addition than in segments without analog; (ii) biofilms would be predominantly nitrogen limited in the absence of analog and analog addition would alleviate or change nutrient limitation; (iii) analog addition would increase autotrophic and heterotrophic metabolic activity.

2. METHODS

2.1. Study area and design:

From its headwaters in the Sawtooth and Salmon mountain ranges, the Salmon River flows north through the mountainous region of central Idaho before turning west to its confluence with the Snake River, a major tributary of the Columbia River. Peak stream discharge occurs during high elevation snow-melt between mid-June and early July and baseflow conditions typically persist from mid-July through the winter (Figure 2.1). Our study region is underlain by the Idaho Batholith geologic formation with Cretaceous granite and quartz diorite being the dominant rock types (Omernik 1987). Riparian vegetation is dominated by willow (*Salix* sp.) whereas upland areas consist mostly of lodgepole pine (*Pinus contorum*). As a result of watershed and regional characteristics, the study streams receive little geologic or vegetative nutrient inputs, and have low rates of atmospheric nitrogen deposition (242 kg N km^{-2} year $^{-1}$; National Atmospheric Deposition Program 2010, site ID03). Biofilms in nearby streams are nitrogen limited or co-limited by nitrogen and phosphorous (Marcarelli and Wurtsbaugh 2007; Sanderson et al. 2009). Streams in the upper Salmon River region are relatively undisturbed by human land-use practices other than scattered mines and dispersed livestock grazing. Natural disturbances include mountain pine beetle infestations (Logan and Powell 2001) and stand-removing wildfires in upland lodgepole pine forests (Malison and Baxter 2010). Watersheds are largely under federal ownership, draining areas of the Sawtooth Wilderness, Sawtooth National Recreation Area and the Salmon-Challis National Forest.

To evaluate the effect of analog addition on biofilms, we compared adjacent upstream control and downstream treatment segments in six streams during 2010 and 2011 (Figure 2.3). Segments were approximately 3 km in length. Biofilm standing crop sampling followed a Before-After-Control-Impact (BACI) design and a Control-Impact design was used to assess changes in biofilm nutrient limitation and metabolism. Study streams were 3rd-4th order with baseflow discharges between 0.04 – 0.97 m^3 s^{-1} (Table 2.1). We separated the streams into two groups by geographic region: Elk Creek, Basin Creek and Cape Horn Creek near Stanley, ID and Panther Creek, Musgrove Creek and Moyer Creek north-west of Challis, ID (Figure 2.2). All of these streams likely supported healthy runs of Chinook salmon in the past, yet returning adults are rare in five of the study streams (Table 2.1). Cape Horn Creek, which drains into the Middle Fork of the Salmon River, is the only study stream with a sizeable run of Chinook salmon relative to the other study streams, albeit still small relative to historical levels (see Evermann 1896).

We experimentally added three different salmon carcass analog loads to treatment segments: No analog, 30 g / m^2 (low analog), or 150 g / m^2 (high analog) of bank-full channel area. Low analog density was based on target levels suggested by Wipfli et al. (2003) for comparison with earlier salmon carcass analog additions in the Pacific Northwest (see Kohler et al. 2012), whereas the high analog density was based on salmon escapement levels described by Bilby et al. (2001). The location and length of segments was decided based on the accessibility of the treatment segment and the proximity of the downstream edge of the study site to junctions with major tributaries. Analog addition

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occurred in the middle of August 2010 and 2011 to mimic the historical annual peak in

Chinook spawning.
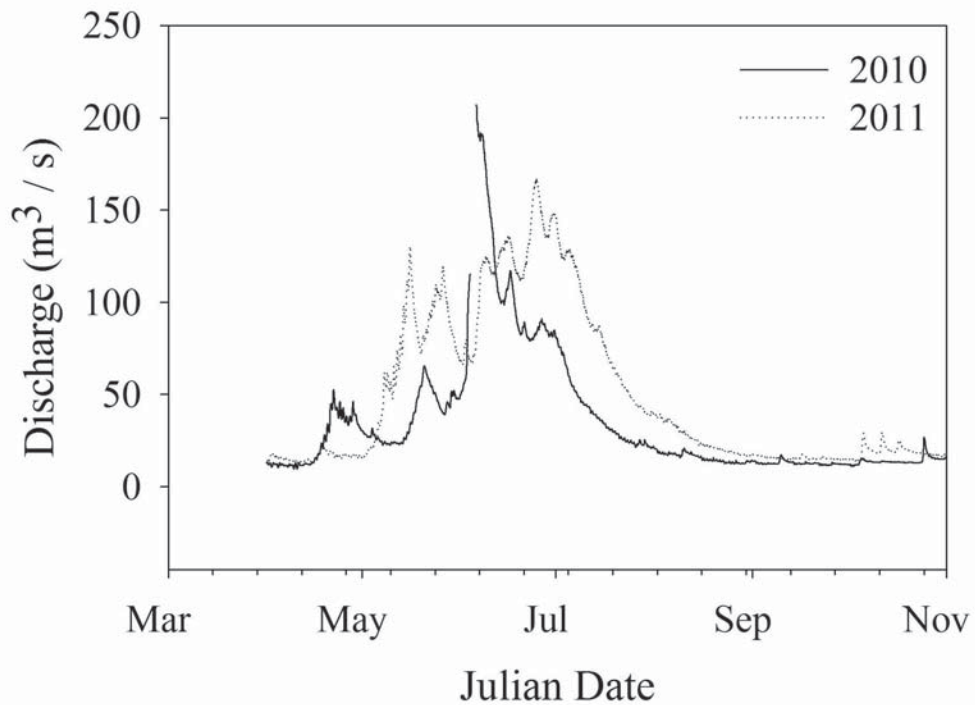
Figure 2.1 Maximum daily discharge of measured at the USGS discharge gauge on the mainstem Salmon River below Yankee Fork confluence (USGS #13296500) beginning prior to spring snowmelt and ending at the end of the study period during 2010 and 2011.
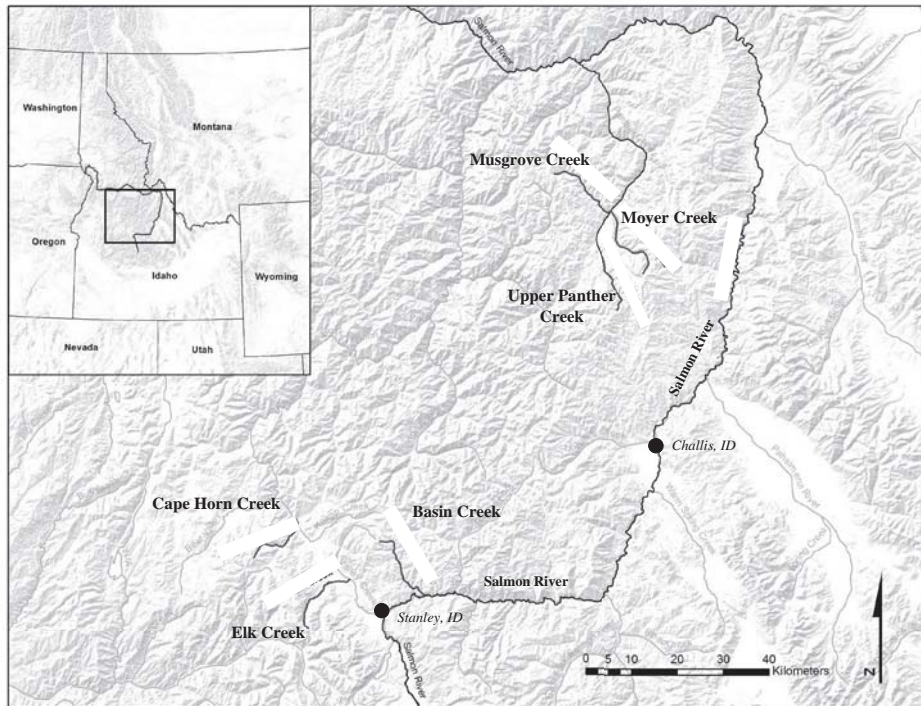
Figure 2.2 Location of study streams in central Idaho (inset, upper-right).
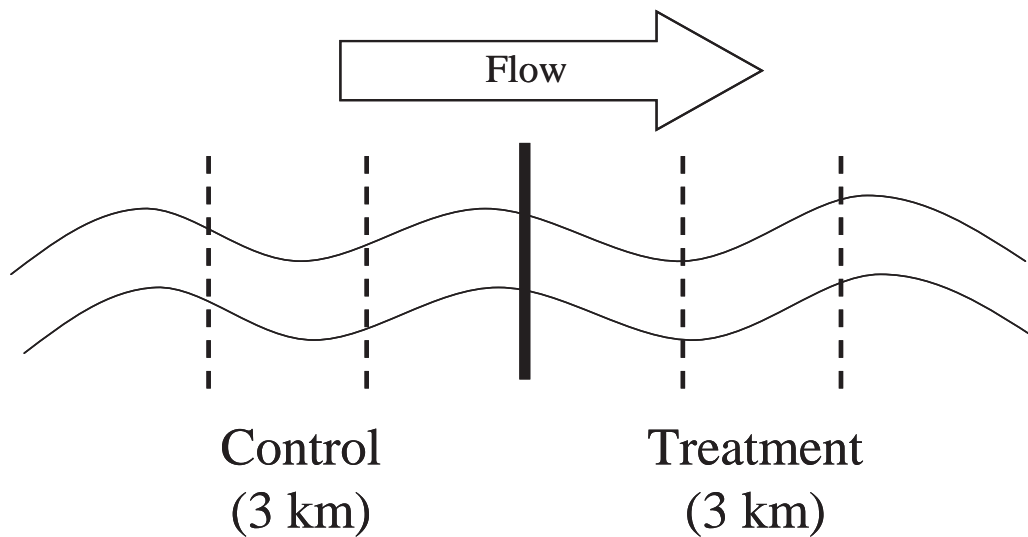
Figure 2.3 Schematic of study design. Upstream control and downstream treatment segments are separated by a solid black line. In 2010, study segments were further stratified into 1 km reaches as indicated by the dashed lines.

2.2. Physical characteristics

We measured stream channel and watershed characteristics in 2011. Stream depth and stream flow velocity was measured at 25 cm intervals along three transects in each segment with a calibrated wading rod and a Marsh McBirney Flo-Mate Model 2000 flow meter, respectively. Depth and flow velocity measurements across transects were used to calculate discharge. Temperature was measured at 15 minute intervals from July-September 2011 using HOBO temperature loggers at the midpoint of each study segment. We estimated canopy cover at all biofilm standing crop sample collection sites (see Section 2.3) with a spherical densitometer. Habitat measurements within each segment were averaged. Basin area draining to the downstream edge of each segment was determined with the USGS StreamStats web application. We calculated channel gradient of segments by dividing the difference in elevation between the upstream and downstream boundaries of each segment by the total length of the segment. Physical stream and watershed characteristics are shown in Table 2.1.

Table 2.1
Stream characteristics at study segments.

Stream	Analog Level	Segment	Basin Area (km²)	Elevation (m)	Gradient (m/km)	Canopy Cover (%)	Depth (cm)	Discharge (m³/s)	Temp (°C)	Chinook salmon redds
Elk Creek	None	Treatment	53	1989	9.9	9.2	19.8	0.71	11.3	3
		Control	51	2013	11.2	8.5	21.6	0.70	10.9	0
Musgrove Creek	None	Treatment	61	1676	23.7	36.4	16.7	0.37	9.8	5
		Control	57	1744	38.9	30.4	18.3	0.28	9.2	1
Basin Creek	Low	Treatment	136	1851	11.6	8.1	20.2	0.94	10.3	0
		Control	126	1885	11.1	10.1	21.3	0.74	9.6	0
Moyer Creek	Low	Treatment	107	1671	19.6	19.8	25.2	0.74	9.0	8
		Control	103	1729	23.8	22.8	23.5	0.70	9.1	6
Cape Horn Creek	High	Treatment	69	1994	7.0	8.1	23.8	0.97	9.1	23
		Control	63	2013	4.6	5.2	24.9	0.89	8.4	28
Panther Creek	High	Treatment	36	2052	25.6	71.4	12.0	0.18	7.5	0
		Control	26	2131	49.0	27.2	8.6	0.04	8.6	0

2.3. Biofilm Standing Crop

We measured biofilm standing crop on natural substrates as chlorophyll *a* and ash free dry mass (AFDM) two weeks prior and four weeks after analog addition in both 2010 and 2011. In 2010, we stratified our 3 km control and treatment segments into 1 km upper, middle, and lower reaches. We collected three rocks from each of two randomly selected riffles within the three reaches. In 2011, we removed the within-segment stratification and collected three rocks from nine randomly selected riffles along each study segment. Our sampling protocol for collecting biofilm substrates changed between years because our initial sampling design did not provide sufficient statistical power to detect subtle differences. The change in sampling design increased our power to detect differences in both chlorophyll *a* and AFDM (Table 2.2, Table 2.3).

Biofilms were scrubbed from rocks into approximately 400 mL of stream water and the resultant slurry was filtered through 0.7 μm glass-fiber filters. Filters were frozen for storage and transportation before being analyzed for chlorophyll *a* and AFDM according to standard methods (APHA 2005). To obtain an estimate of standing crop per m^2 of substrate, we determined rock surface area by tracing the planar area onto paper and weighing the cut-out (Bergey and Getty 2006). We analyzed the difference in standing crop between treatment and control segments (i.e., treatment minus control) using a two-way analysis of variance (ANOVA) with analog level (no analog, low analog, high analog) and sampling period (before, after) as fixed factors. We explored the whether physical characteristics of our study segments influenced biofilm standing crop using simple linear regressions. Chlorophyll *a* and AFDM values were log-transformed

before all analyses to meet statistical assumptions of residual normality. Percent canopy

cover was also log transformed to reduce skewness of the residuals caused by one

segment with substantially higher canopy cover than other 11 segments.

Table 2.2
Power analysis for chlorophyll *a* in summer 2010 and 2011, analyzed as the difference between treatment and control segments within streams. Two-tailed power tests were calculated with a confidence level of 5%. Ideal sample sizes were calculated using a confidence level of 5% and a beta error level of 50%. Calculations made at http://www.dssresearch.com/KnowledgeCenter/toolkitcalculators/samplesizecalculators.aspx

Year	Comparison	Analog (mean T-C ± SD	No Analog (mean T-C ± SD)	Analog vs Control (power; sample size)
2010	High v. No Analog	6.01 ± 12.02	3.53 ± 14.60	7.3%; 157
	Low v. No Analog	-5.65 ± 8.29	3.53 ± 14.60	19.2%; 9
2011	High v. No Analog	1.59 ± 4.21	5.62 ± 6.53	18.1%; 7
	Low v. No Analog	0.15 ± 2.04	5.62 ± 6.53	30.4%; 4

Table 2.3
Power analysis for AFDM in summer 2010 and 2011, analyzed as the difference between treatment and control segments within streams (mean T-C). Two-tailed power tests were calculated with a confidence level of 5%. Ideal sample sizes were calculated using a confidence level of 5% and a beta error level of 50%. Calculations made at http://www.dssresearch.com/KnowledgeCenter/toolkitcalculators/samplesizecalculators.aspx

Year	Comparison	Analog (mean T-C ± SD	No Analog (mean T-C ± SD)	Analog vs Control (power)
2010	High v. No Analog	61.75 ± 145.54	11.82 ± 41.72	11.9%; 25
	Low v. No Analog	-3.82 ± 67.39	11.82 ± 41.72	8.6%; 69
2011	High v. No Analog	0.27 ± 0.58	2.13 ± 2.31	29.4%; 4
	Low v. No Analog	0.97 ± 1.68	2.13 ± 2.31	14.2%; 16

2.4. Nutrient limitation

We deployed nutrient diffusing substrates (NDS) in each study segment 4 weeks prior to analog addition in 2010 and one week after analog addition during 2010 and 2011 to examine whether analog addition altered biofilm nutrient limitation. NDS consisted of 37 ml PolyCon vials filled with a nutrient amended agar and capped with a 24-mm diameter fritted glass disk. Nutrients diffused out of the agar and through the glass disk which provided a substrate for biofilm colonization. The rate of diffusion is not greatly affected by temperature, but is strongly dependent on the nutrient concentration in the agar and diffusion rates decrease log-linearly with time (Rugenski et al. 2008). NDS were amended with 0.8 mol nitrogen L^{-1} as $NaNO_3$, 0.05 mol phosphorus L^{-1} as KH_2PO_4, or both N + P. Nitrogen and phosphorus concentrations in substrates were designed to diffuse at a rate greater than algal uptake and, thus, remove the possibility of biofilm nutrient limitation for that amendment. NDS containing agar only (i.e., no added nutrient) served as our control. Six replicates of each amendment were placed in metal racks installed at the downstream edge of each segment. Following a 21 day deployment, fritted glass disks were wrapped in aluminum foil and frozen until analysis of chlorophyll *a* and AFDM by standard methods (APHA 2005).

We determined nutrient limitation of biofilms in individual study segments using a two-way ANOVA with N and P as fixed factors. To examine the effect of analog treatments on nutrient limitation, we used a three way ANOVA with amendment (N, P, N+P, No Nutrient), segment (treatment, control) and analog level (no analog, low analog, high analog) as factors. Chlorophyll *a* concentration and AFDM on NDS were log-

transformed before all analyses to meet the normality assumptions of a factorial ANOVA.

2.5. Metabolism

2.5.1. Whole-stream metabolism

We measured whole-stream metabolism with dissolved oxygen records from control and treatment segments of Basin Creek (low analog) and Cape Horn Creek (high analog) to assess whether analog addition increased microbial metabolic activity. Ecosystem metabolism can exhibit high day-to-day variability (Roberts et al. 2007), thus metabolism estimates based on short periods (i.e., 2-3 days) of continuous dissolved oxygen records may not accurately represent changes in metabolism due to analog addition. We estimated metabolism in control and treatment segments of only two streams in order maximize the length of continuous dissolved oxygen records to encompass day-to-day variability in metabolism and ensure that we were measuring a metabolism response to analog addition rather a response to changing weather patterns. Whole-stream metabolism was estimated from records of dissolved oxygen and temperature collected at 10 minute intervals between August 30th and September 26th, 2011 with YSI 6920 V2 water quality sondes equipped with YSI 6150+ ROX optical dissolved oxygen probes. Dissolved oxygen probes were calibrated before initial deployment and again 18 days after deployment using the saturated air technique. Daily metabolism was calculated for control and treatment segments using the single station open water method (Bott 2006). We used diel changes in oxygen concentration over a 24-

h period to estimate net ecosystem production (NEP as mg O_2 m^{-2} d^{-1}) according to the

equation

$$NEP(dt) = (C_t - C_{t-1})/dt - k(C_s - C_t) + A$$

Where: t = time, C_t = O_2 concentration at a single time step, C_s = saturation constant of O_2

in water, k = reaeration coefficient (i.e., O_2 exchange between water and the atmosphere),

and A = O_2 exchange between the stream and groundwater. Daily ecosystem respiration

(ER as mg O_2 m^{-2} d^{-1}) was estimated by averaging NEP over the night-time period, then

extrapolating this average rate over the entire 24 h period. Daily gross primary

productivity (GPP as mg O_2 m^{-2} d^{-1}) was calculated as the sum of NEP and ER. We

potentially underestimated GPP and overestimated ER because we did not account for O_2

exchange between the stream and groundwater (A) in our calculations (Hall and Tank

2005). We automated whole stream metabolism calculations with the RIVERMET tool

(Izagirre et al. 2007).

The use of diel oxygen curves for whole stream metabolism requires accurate

estimates of oxygen flux between the stream and the atmosphere (reaeration coefficient;

k). We could not robustly estimate reaeratation with direct gas evasion (Marzolf et al.

1994; Young and Huryn 1998) because of the high width to depth ratio of our study

streams. Therefore, we employed multiple different models to estimate reaeration. First,

we used the night-time regression method of Hornberger and Kelly (1975), which

regresses the night-time decrease in O_2 per time unit (dt^{-1}) against the oxygen saturation

deficit. The reaeration coefficient is estimated from the slope of the regression. Days with

non-significant night-time regressions were not included in the analysis. Because night-

time regression does not always provide robust estimates of k in streams less than 0.5 m

in depth (Young and Huryn 1999; Aristegi et al. 2009), we also used morphologic and

hydrologic characteristics of segments (i.e., slope, depth, discharge, water velocity) to

calculate k using fitted equations (E1-E6; Raymond et al. 2012), a fitted equation for

pool-riffle stream systems (E7; Melching and Flores 1999), and the energy dissipation

model (E8; Tsivoglou and Neal 1976) (Table 2.4). When solved, E1-6 produced a gas

transfer velocity normalized to a Schmidt number (Sc) of 600 (i.e., temperature-

dependent ratio of kinematic viscosity of water and the diffusion coefficient of a gas). We

converted predicted k_{600} to k_{20} for input to RIVERMET by first converting k_{600} to gas

transfer velocity for oxygen at 17.5° C ($k_{O2 \ at \ 17.5° \ C}$) followed by adjusting $k_{O2 \ at \ 17.5° \ C}$

Table 2.4
Empirical equations used to calculate the reaeration coefficient.

Code	Equation	Citation
E1	$k_{600} = (VS)^{0.89} \times D^{0.54} \times 5037$	Raymond et al. (2012)
E2	$k_{600} = 1162 \times S^{0.77} \times V^{0.85}$	Raymond et al. (2012)
E3	$k_{600} = (VS)^{0.76} \times 951.5$	Raymond et al. (2012)
E4	$k_{600} = (VS + 2841) + 2.02$	Raymond et al. (2012)
E5	$k_{600} = 929 \times (VS)^{0.75} \times Q^{0.011}$	Raymond et al. (2012)
E6	$k_{600} = 4725 \times (VS)^{0.86} \times Q^{-0.14} \times D^{0.66}$	Raymond et al. (2012)
E7	$k_{20} = 596 \times (VS)^{0.528} \times Q^{-0.136}$	Melching and Flores (1999)
E8	$k_{20} = k' * S * V$	Tsivoglou and Neal (1976)

k_{600} is the gas transfer velocity with a Schmidt number of 600, k_{20} is the reaeration
coefficient at 20°C. V = stream velocity in m s^{-1}; S = slope; Q = discharge in m^3 s^{-1};
D = depth in meters for E1-E7 & E8) ; k' = 1.53 x 10^4 s m^{-1} d^{-1} for stream flow > 560
L s^{-1}.

to $k_{O2 \ at \ 20° \ C}$ according to the known temperature dependency of Schmidt numbers for

oxygen ($Sc_{O2} = 1568 - 86.04 \ T + 2.142 \ T^2 - 0.0216 \ T^3$, where T = temperature in °C;

Raymond et al. 2012), and dividing $k_{O2 \ at \ 20° \ C}$ by mean segment depth (D, in meters) using

the following equation:

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$$k_{20} = [k_{600} / (600 / Sc_{O2})^{-0.5}] / D, \text{ units: } d^{-1}$$

We examined differences in whole-stream metabolism between segments by visually

comparing time-series plots of GPP, ER, and NEP.

2.5.2. Benthic Metabolism

In 2011, we also measured benthic metabolism in small light-dark re-circulating

chambers using short-term incubations in treatment and control segments of Cape Horn

Creek (high analog), Basin Creek (low analog), and Elk Creek (no analog) near Stanley,

Idaho. Chambers were constructed from 2-L polycarbonate containers with inlet and

outlet openings connected with Tygon tubing to submersible centrifugal bilge pumps.

Substrates were haphazardly collected from riffles and incubated in the chambers for 1-2

hrs in full sunlight. We used micro-Winkler titrations (APHA 2005) to measure dissolved

oxygen in chambers at the beginning and end of incubations. Clear chambers were used

to measure net community productivity (NCP) because changes in dissolved oxygen in

the presence of light can be attributed to algal photosynthesis and to respiration by both

autotrophs and heterotrophs. We use the term net community respiration to highlight the

differences between whole-stream and benthic metabolism; whole-stream metabolism

accounts for respiration by all organisms in the stream and the hyporheic zone whereas

chambers are used to measure the metabolic activity of only biofilms communities on

streambed rocks. Respiration was measured in dark chambers because changes in oxygen

can be attributed only to respiration when light is absent. At the end of each incubation

period, biofilm standing crop and rock surface area was measured as described in Section

2.3. NCP and R were determined from dissolved oxygen measurements in light and dark

chambers, respectively, using equations from Wetzel and Likens (2000):

$$NCP \ (mg \ C \ m^{-2} \ h^{-1}) = [(DO_{before} - DO_{after}) \ (V) \ (0.375)] \ / \ (t) \ (SA) \ (PQ)$$

$$R \ (mg \ C \ m^{-2} \ h^{-1}) = [(DO_{before} - DO_{after}) \ (V) \ (RQ) \ (0.375)] \ / \ (t) \ (SA)$$

Where t = time, DO_{before} and DO_{after} = dissolved oxygen concentrations before and after

incubation respectively, V = water volume in chamber (i.e., volume of chamber, tubing

and pump minus volume of stream substrate), SA = surface area of stream substrate, PQ

= photosynthetic quotient (i.e., the ratio of O_2 production to inorganic carbon fixation;

assumed to be 1), RQ = respiratory quotient (i.e., the efficiency of converting O_2 to

carbon through metabolism; assumed to be 1). The value 0.375 is a constant determined

by the molar ratio of carbon to O_2 (Wetzel and Likens 2000). GPP was calculated as the

sum of NCP + ER.

We assessed differences in NCP and R between control and treatment segments

using t-tests. Because we used multiple t-tests, we employed a bonferroni-correction to

our alpha value ($\alpha_{bonferroni} = 0.05/3 = 0.017$).

3. RESULTS

3.1. Biofilm Standing Crop

Our results show that biofilm and chlorophyll *a* and AFDM did not respond to analog addition under any analog level in 2010 (Figure 3.1) or 2011 (Figure 3.2). There were no significant differences in chlorophyll *a* or AFDM between control and treatment segments during before and after analog addition sampling periods under any analog level in either 2010 or 2011 (Table 3.1). The lack of difference between standing crop in treatment and control segments was consistent across sampling periods regardless of analog level.

Many of the physical characteristics we measured in our study were autocorrelated. Discharge was significantly correlated with depth ($p < 0.001$, $r^2 = 0.776$), velocity ($p = 0.009$, $r^2 = 0.515$), gradient ($p < 0.001$, $r^2 = 0.722$), and basin area ($p = 0.028$, $r^2 = 0.396$). Canopy cover was negatively correlated with discharge ($p = 0.002$, $r^2 = 0.639$), which is not surprising because higher discharge streams in our system tend to be wider than streams with lower discharges. Canopy cover differed significantly between the two geographic groups of streams ($t_5 = 3.41$, $p = 0.019$), as did discharge ($t_5 = 3.47$, $p = 0.013$). Despite the correlation between canopy cover and discharge, we regressed biofilm standing crop against both of these variables. Contrary to our expectations, chlorophyll *a* was positively associated with canopy cover (Figure 3.3a), and negatively associated with discharge (Figure 3.3b). AFDM was not correlated with canopy cover ($p = 0.304$, $r^2 = 0.105$) or discharge ($p = 0.485$, $r^2 = 0.050$).

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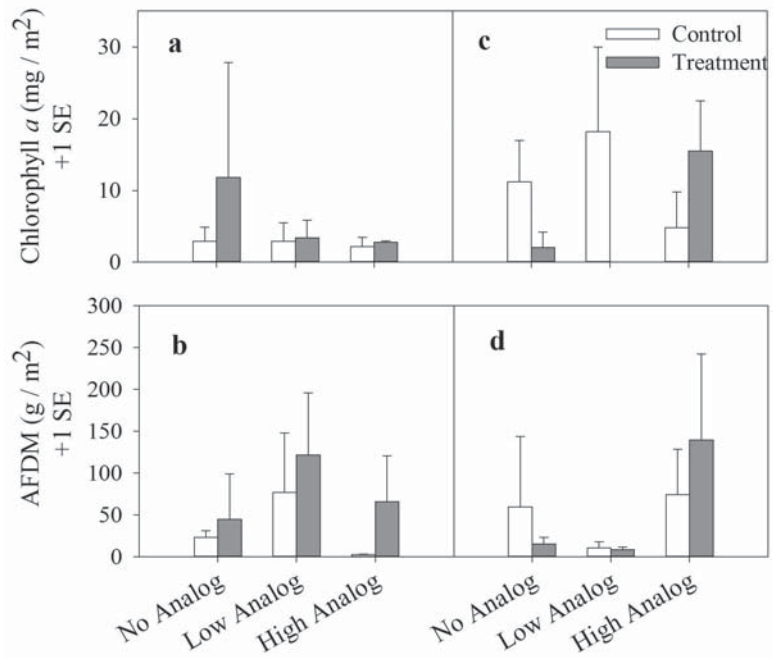
Figure 3.1 Chlorophyll *a* and AFDM on natural substrates collected before (*a, b*) and after (*c, d*) analog addition in 2010. n=2 for all error bars. Post-addition chlorophyll *a* samples from low analog treatment segments were lost during analysis.
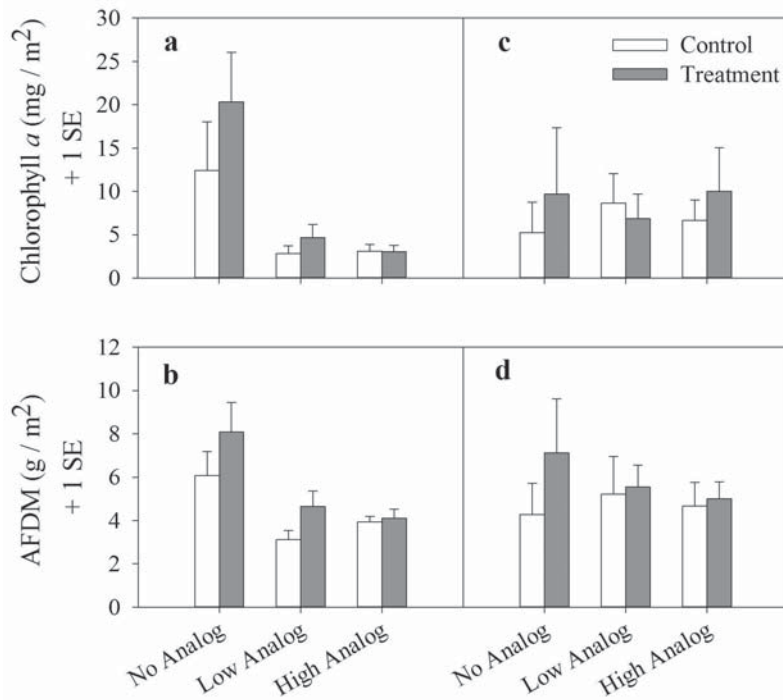
Figure 3.2 Chlorophyll *a* and AFDM on natural substrates collected before (*a, b*) and after (*c, d*) analog addition in 2011. n=2 for all error bars.

Table 3.1

Two-way ANOVA results for 2010 and 2011 Chlorophyll *a* and AFDM on natural substrates with sample period (levels = Before, After) and Analog Level (levels = High, Low, No Analog) as fixed factors.

	2010				2011			
	Chlorophyll a		AFDM		Chlorophyll a		AFDM	
	F	p	F	p	F	p	F	p
Level	$1.02_{2,6}$	0.415	$0.32_{2,6}$	0.735	$1.18_{2,6}$	0.370	$0.900_{2,6}$	0.456
BA	$0.48_{1,6}$	0.514	$0.15_{1,6}$	0.713	$0.06_{1,6}$	0.817	$0.010_{1,6}$	0.943
Level*BA	$1.40_{2,6}$	0.316	$0.10_{2,6}$	0.903	$0.51_{2,6}$	0.625	$0.240_{2,6}$	0.796
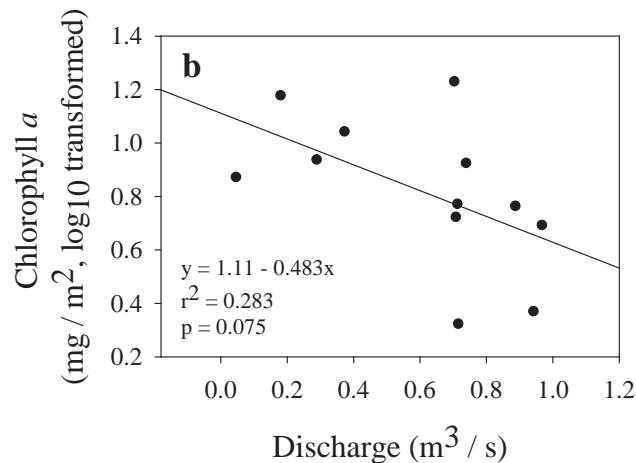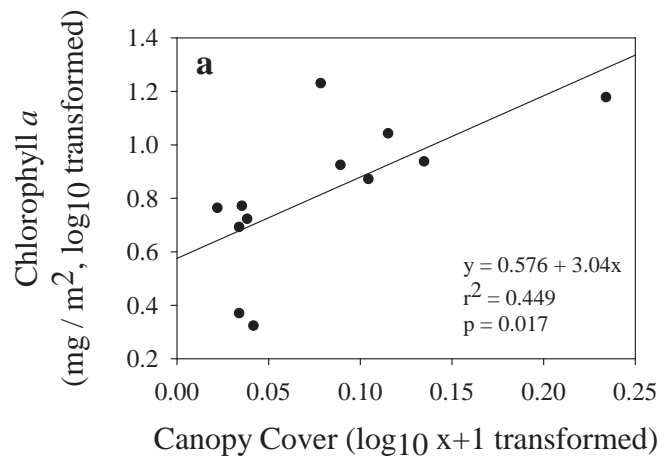
Figure 3.3. Relationship of chlorophyll *a* concentration on rocks collected after analog addition in 2011 with (*a*) canopy cover and (*b*) discharge (m^3 / s).

3.2. Nutrient Limitation

Biofilms in study streams were primarily N-limited and nutrient limitation did not change with analog addition (Level X Segment X Amendment; Table 3.1). Chlorophyll *a* and AFDM on nutrient diffusing substrates deployed in treatment segments before analog addition in July 2010 showed similar nutrient limitation patterns to substrates deployed in the same segment after the 2010 analog addition (Table 3.3). Chlorophyll *a* exhibited primary N-limitation in five of six treatment segments following analog addition during the post-analog period of both study years (Table 3.3), whereas primary N-limitation of chlorophyll *a* occurred in four and five control segments during the post-analog addition periods of 2010 and 2011, respectively (Table 3.3). After analog addition, N amendments in control and treatment segments stimulated AFDM accrual in two and three streams in 2010 and 2011, respectively (Table 3.4). Primary P-limitation was rare for biofilms in our streams, and occurred for AFDM in only one segment during July 2010.

Analog addition altered the amount of chlorophyll *a* on substrates, as indicated by a significant Level X Segment interaction (Table 3.2). Under the high analog addition, chlorophyll *a* was 2.2, 1.6 and 2 times higher on no nutrient, nitrogen, and N + P amended substrates in high analog segments than control segments in 2010. In the same high analog streams, NDS deployed in 2011 chlorophyll *a* was 3, 1.8, and 4 times higher on no nutrient, nitrogen and phosphorus amended substrates in treatment segments than control segments and only 1.1 times higher for N + P amendments (Figure 3.4). This pattern was not observed in AFDM (Figure 3.5).

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Table 3.2

Three-way ANOVA results on chlorophyll *a* concentrations on nutrient diffusing substrates deployed after analog addition in 2010 and 2011. Analog level (no analog, low, high), segment (treatment, control), amendment (no nutrient, N, P, N+P) were fixed factors. The response variable was log-transformed chlorophyll *a* (mg / m^2). F-ratios with degrees of freedom are shown and significant differences are indicated with a (*).

	2010		2011	
Factor	*F*	*p*	*F*	*p*
Level	6.77 $_{2, 245}$	*0.001	4.51 $_{2, 209}$	*0.012
Segment	8.08 $_{1, 245}$	*0.005	10.93 $_{1, 209}$	*0.001
Amendment	58.96 $_{3, 245}$	*< 0.001	36.18 $_{3, 209}$	*< 0.001
Level*Segment	8.11 $_{2, 245}$	*< 0.001	5.84 $_{2, 209}$	*0.003
Level*Amendment	3.51 $_{6, 245}$	*0.002	0.51 $_{6, 209}$	0.799
Segment*Amendment	1.29 $_{3, 245}$	0.277	1.35 $_{3, 209}$	0.260
Level*Segment*Amendment	1.51 $_{6, 245}$	0.174	0.78 $_{6, 209}$	0.583

Table 3.3

Results of chlorophyll *a* nutrient limitation from nutrient diffusing substrates deployed in upstream control and downstream treatment segments following analog addition in 2010 and 2011.

Time	Analog Level	Stream Segment	Treatment				Control			
			N	P	NxP	Limitation	N	P	NxP	Limitation
2010, before addition	High Analog	Cape Horn	<0.001*	0.069*	0.055*	Co	--	--	--	--
	Low Analog	Panther	0.009*	0.634	0.782	N+	--	--	--	--
		Basin	0.002*	0.538	0.504	N+	--	--	--	--
		Moyer	<0.001*	0.290	0.145	N+	--	--	--	--
	No Analog	Elk	<0.001*	0.701	0.063*	1° N +, 2° P+	--	--	--	--
		Musgrove	Lost	Lost	Lost	N/A	--	--	--	--
2010, after addition	High Analog	Cape Horn	0.003*	0.232	0.547	N+	0.110	0.112	0.838	None
		Panther	<0.001*	0.001*	0.463	N+, P-	0.552	0.312	0.677	None
	Low Analog	Basin	<0.001*	0.138	0.154	N+	<0.001*	0.138	0.154	N+
		Moyer	0.016*	<0.001*	0.357	Co	0.058*	0.413	0.928	N+
	No Analog	Elk	0.002*	0.084	0.005*	1° N+, 2° P+	0.001*	0.203	0.053*	1° N+, 2° P+
		Musgrove	0.001*	0.824	0.257	N+	<0.001*	0.129	0.042*	1° N+, 2° P+
2011, after addition	High Analog	Cape Horn	0.018*	0.168	0.001*	1° N+, 2° P+	0.001*	0.476	0.028*	1° N+, 2° P+
		Panther	0.526	0.470	0.076	None	0.918	0.400	0.353	None
	Low Analog	Basin	0.012*	0.797	0.313	N+	<0.001*	0.333	0.232	N+
		Moyer	0.012	0.647	0.755	N+	0.003	0.143	0.231	N+
	No Analog	Elk	0.003*	0.657	0.192	N+	<0.001*	0.711	0.013*	1° N+, 2° P+
		Musgrove	0.048*	0.634	0.405	N+	0.051*	0.334	0.485	N+

* $P < 0.05$ are indicated with an asterisk (*). (+) indicates that amendment had a stimulatory effect whereas (-) indicates suppression of chlorophyll a. (--) indicates that NDS were not deployed. A significant N-effect was interpreted as N-limitation, P effect as a P limitation. If both N and P, or NxP was significant, biofilms were deemed co-limited. If both N and NxP were significant, biofilms were primarily (1°) N-limited and secondarily (2°) P-limited (see Tank and Dodds 2003).

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Table 3.4

Results of AFDM nutrient limitation from nutrient diffusing substrates deployed in upstream control and downstream treatment segments following analog addition in 2010 and 2011.

Time	Analog Level	Stream	Segment	Treatment				Control			
				N	P	NxP	Limitation	N	P	NxP	Limitation
2010, before addition	High Analog	Cape Horn		0.019*	0.181	0.117	N+	--	--	--	--
		Panther		0.433	0.335	0.706	none	--	--	--	--
	Low Analog	Basin		<0.001*	0.353	0.793	N+	--	--	--	--
		Moyer		0.105	0.072	0.117	P+	--	--	--	--
	No Analog	Elk		0.028*	0.270	0.008*	1° N+, 2° P+	--	--	--	--
		Musgrove		Lost	Lost	Lost	N/A	--	--	--	--
2010, after addition	High Analog	Cape Horn		0.443	0.225	0.020*	Co	0.735	0.232	0.582	none
		Panther		0.005*	0.034	0.025*	Co	0.003*	0.325	0.011*	1° N+, 2° P+
	Low Analog	Basin		0.049*	0.009	0.091	Co	0.005	0.201	0.005*	1° N+, 2° P+
		Moyer		0.002*	0.841	0.780	N+	0.341	0.188	0.139	none
	No Analog	Elk		0.003*	0.103	0.121	N+	0.706	0.786	0.348	none
		Musgrove		0.362	0.065	0.756	none	0.188	0.095	0.040*	Co
2011, after addition	High Analog	Cape Horn		0.001*	0.294	0.096	N+	0.045*	0.247	0.15	N+
		Panther		0.184	0.946	0.946	none	0.017*	0.215	0.277	N+
	Low Analog	Basin		0.005*	0.698	0.001*	1° N+, 2° P+	<0.001*	0.049*	0.679	Co
		Moyer		0.034*	0.823	0.054*	1° N+, 2° P+	0.252	0.276	0.632	none
	No Analog	Elk		0.100	0.570	0.116	none	0.013*	0.693	0.256	N+
		Musgrove		0.639	0.985	0.160	none	0.204	0.147	0.781	none

* $P < 0.05$ are indicated with an asterisk (*). (+) indicates that amendment had a stimulatory effect whereas (–) indicates suppression of chlorophyll a. (--) indicates that NDS were not deployed. A significant N-effect was interpreted as N-limitation, P effect as a P limitation. If both N and P, or NxP was significant, biofilms were deemed co-limited. If both N and NxP were significant, biofilms were primarily (1°) N-limited and secondarily (2°) P-limited (see Tank and Dodds 2003).

Figure 3.4. Standing crop of chlorophyll *a* (+ 1 SE, n = 2) on nutrient diffusing substrates deployed for 21 days in 2010 (*a* , *c*, *e*) and 2011 (*b*, *d*, *f*) before analog addition (light gray, 2010 only) and after analog addition in control (white) and treatment (dark gray) segments. Treatment segments received either no analog (*a,b*), low analog (30 g / m^2; *c*, *d*), or high (150 g / m^2; *e*, *f*).

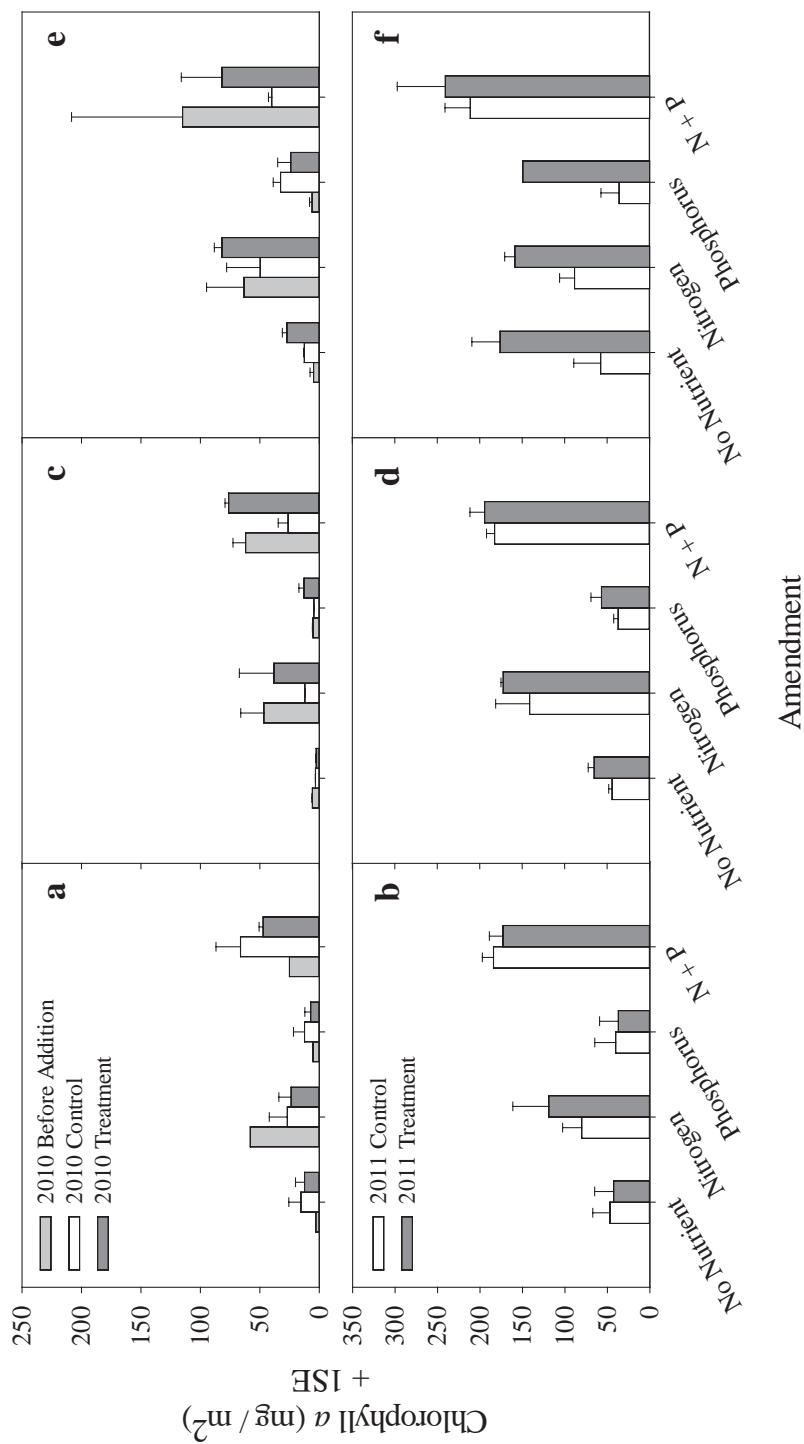Figure 3.5. Standing crop of AFDM (+ 1 SE, n = 2) on nutrient diffusing substrates deployed for 21 days in 2010 (*a*, *c*, *e*) and 2011 (*b*, *d*, *f*) before analog addition (light gray, 2010 only) and after analog addition in control (white) and treatment (dark gray) segments. Treatment segments received either no analog (*a*, *b*), low analog (30 g / m^2: *c*, *d*), or high (150 g / m^2: *e*, *f*)
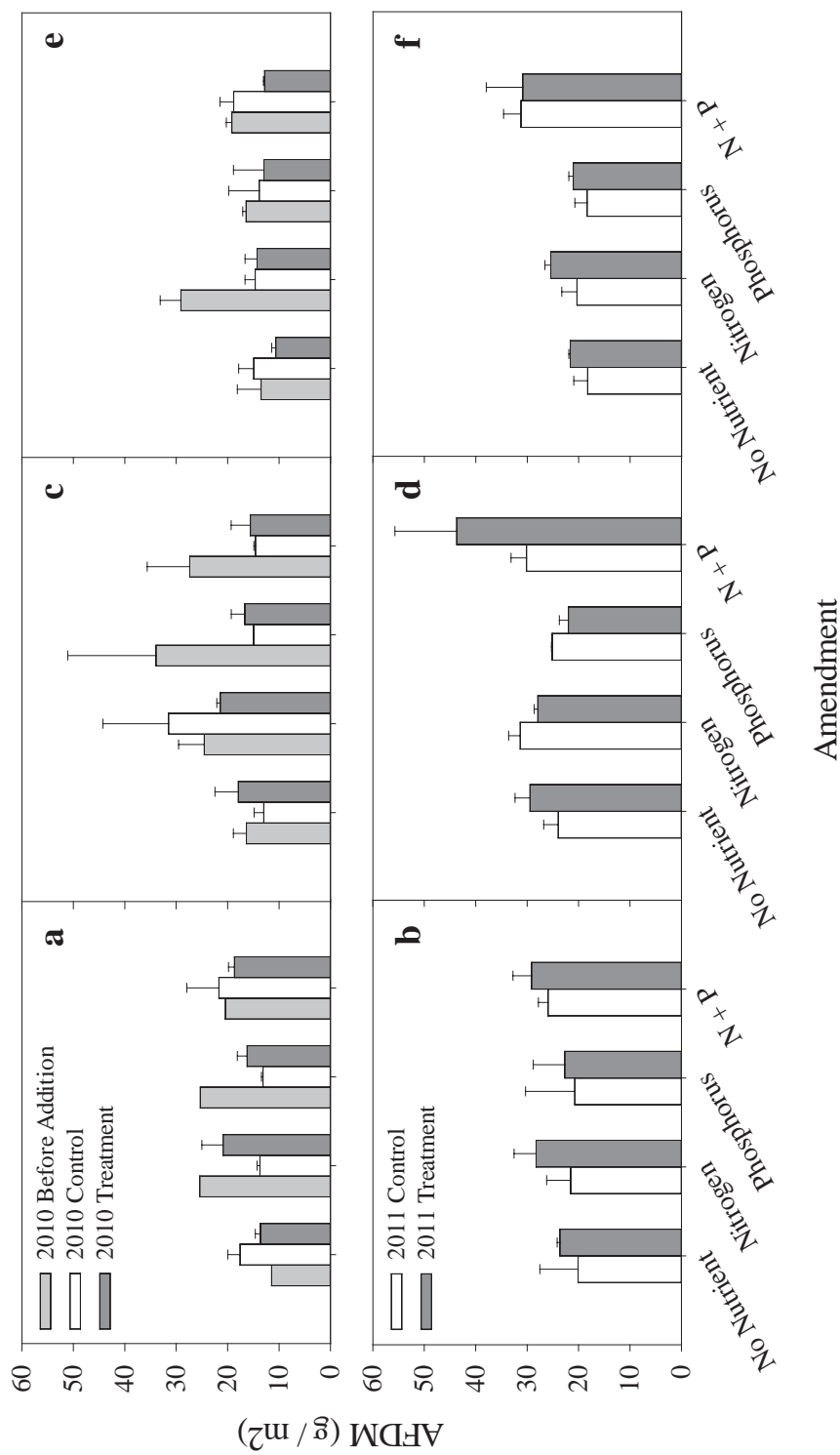
3.3. Metabolism

3.3.1. Whole-stream metabolism

Reaeration estimates from equations provided better metabolism estimates than the night-time regression method. Night time regression yielded k_{20} values two to three-fold lower than the next lowest k_{20} (E7), and GPP estimates using these k_{20} values were only positive in the high analog treatment segment. Consequently, we focused our attention on metabolism parameters estimated with equation-based estimates of k_{20} (Table 3.5).

Gross primary productivity and ecosystem respiration were higher in the high analog treatment segment versus the control, but were similar in low analog treatment and control segment (Table 3.6). Daily gross primary productivity in our streams ranged from 1.63 mg O_2 m^{-2} d^{-1} to 4.03 mg O_2 m^{-2} d^{-1}. Our high analog treatment had the highest GPP of the four segments. Mean daily GPP in the high analog treatment segment was 150% higher than the high analog control segment. Meanwhile, Mean daily GPP was approximately equal in the two segments of our low analog stream (Table 3.6). Ecosystem respiration exceeded GPP in all of our study segments throughout September 2011, but followed a similar pattern of differences between segments. Mean daily ER ranged from 15.55 to 38.38 mg O_2 m^{-2} d^{-1} across the four segments, and was 70% higher in the high analog treatment segment vs. the control segment (Table 3.6). Low analog control and treatment segments had higher ER than the high analog segments, but treatment differed from the control by only 16% in this stream. NEP was highly negative, ranging from -13.92 to -36.26 mg O_2 m^{-2} d^{-1}, with the lowest estimates coming from the

low analog segments. Finally, the ratio of production to respiration ranged from 0.05 in the low analog control segment to 0.17 in the high analog treatment segment.

Daily metabolism changed between the first nine (i.e., early September) and the last seven days of September (i.e., late September) (Figure 3.6 a-c). Sonde recalibration was concurrent with the change in metabolism and a sonde malfunction in the high analog treatment segment around September 10th caused nine day gap in metabolism estimates for this segment. This sonde was fixed, calibrated and redeployed at the same time as the sondes in the other segments. Daily GPP declined by 20-30% in high analog segments, with the greater decrease occurring in treatment segment. Low analog segments showed a steeper decline in GPP than high analog segments, with late September GPP being 41% and 85% lower in the treatment and control segments, respectively. Daily GPP appeared to converge around September 19th, 2011 at 1.5 mgO_2 $m^{-2} d^{-1}$ in high analog control, low analog treatment and low analog control segments, whereas the high analog treatment segment fell to between 3.03 to 3.37 $mgO_2 m^{-2} d^{-1}$. Daily ER decreased between early and late September in the high analog treatment segment by 24% and increased in the high analog control, low analog treatment, and low analog control segments by 11%, 5%, and 20% respectively.

Reaeration coefficients (k_{20}) of control and treatment segments of Cape Horn Creek (high analog) and Basin Creek (low analog) during September 2011.

Method	High Analog		Low Analog	
	Treatment (h^{-1})	Control (h^{-1})	Treatment (h^{-1})	Control (h^{-1})
Median NT	0.60	0.32	0.62	0.47
E1	2.40	1.55	4.35	4.57
E2	2.25	1.50	4.22	4.32
E3	2.09	1.41	3.88	3.93
E4	1.91	1.28	3.73	3.82
E5	2.16	1.46	3.99	4.03
E6	2.26	1.51	4.00	4.37
E7	1.15	0.91	1.56	1.69
E8	1.87	1.17	3.33	3.61

Table 3.6
Mean daily GPP, ER, NEP, and GPP : ER in control and treatment segments of high analog and low analog streams estimated using the median k_{20} calculated from E1-E8.

Analog Level	Segment	GPP (mg O_2 m^{-2} d^{-1}) ± 1 SD	ER (mg O_2 m^{-2} d^{-1}) ± 1 SD	NEP (mg O_2 m^{-2} d^{-1}) ± 1 SD	P : R
High Analog	Treatment	4.03 ± 0.85	24.12 ± 3.44	-20.05 ± 2.63	0.17
	Control	1.63 ± 0.18	15.55 ± 0.90	-13.92 ± 1.01	0.10
Low Analog	Treatment	2.21 ± 0.59	32.35 ± 0.98	-30.10 ± 1.44	0.07
	Control	2.06 ± 0.60	38.38 ± 4.09	-36.28 ± 4.52	0.05
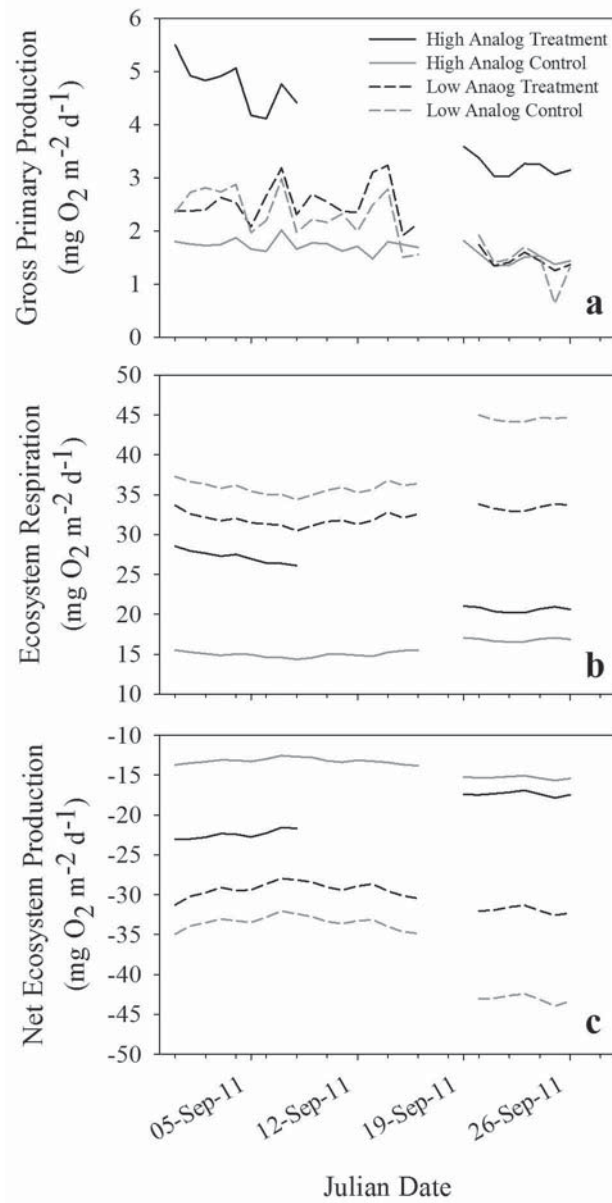
Figure 3.6. Time series of gross primary productivity (*a*), ecosystem respiration (*b*), net ecosystem production (*c*) in control (dark gray) and treatment (black) segments of Basin Creek (low analog; dashed lines) and Cape Horn Creek (high analog; solid lines) near Stanley, ID between 31 Aug 2011 and 26 Sept 2011. All values were calculated from continuous diel oxygen records from YSI 6920 V2 water quality sondes using the median reaeration coefficient (k_{20}) predicted with E1-E8.

3.3.2. Benthic Metabolism

Benthic respiration in light-dark recirculating chambers was three-fold higher in treatment vs. control segments under high analog but did not differ under low analog or no analog levels (Table 3.7, Figure 3.7a). NCP did not respond significantly to any analog level (Table 3.7, Figure 3.7b). We used the mean NCP and R values shown in Figure 3.6a & b to calculate GPP in each segment; because light and dark chambers are not directly paired, GPP values have no replication within analog levels and do not have error bars. It appears that GPP was four-fold higher under high SCA additions, but was not different under no or low analog additions (Figure 3.7c).

Table 3.7
Results of one-tailed *t*-tests on NCP (light chambers) and R (dark chambers) in control and treatment segments under three different analog levels. n=3 for all bars. α was Bonferroni corrected for multiple t-tests and $p = 0.017$ is indicated with an (*).

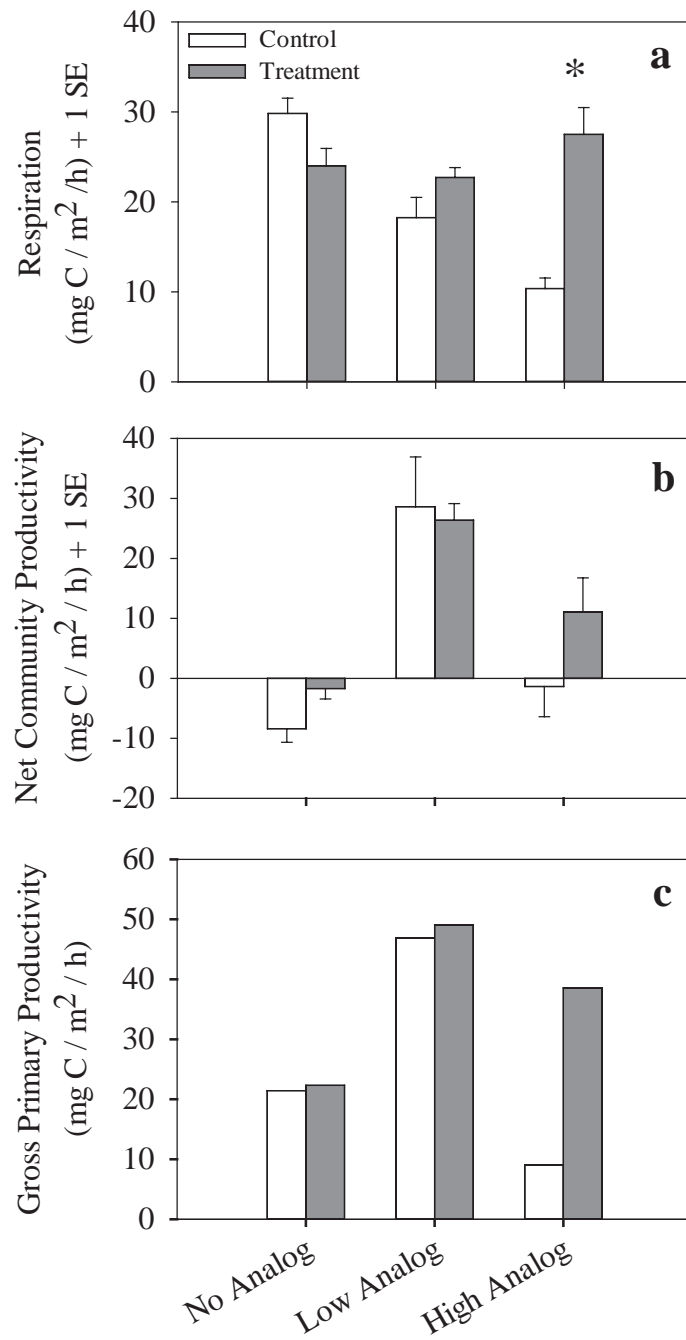| Analog Level	NCP		R	
	T-statistic	*p*	T-statistic	*p*
No Analog	1.63	0.100	2.27	0.946
Low	0.26	0.590	1.79	0.108
High	2.34	0.051	5.33	*0.017

Figure 3.7. Respiration (a), net community productivity (b), and gross primary productivity (c) of benthic biofilms measured in light/dark re-circulating chambers. n = 3 for each bar. (*) indicates statistically significant difference.

4. DISCUSSION

Our objective was to assess changes in three biofilm response metrics to the addition of salmon nutrients in Idaho streams; biofilm standing crop, nutrient limitation and metabolism. We were specifically interested in the strength of the biofilm pathway as a vector for the movement of salmon nutrients into the stream food web, which would require an increase in biofilm production. We added salmon carcass analog in the middle of August to mimic the timing of peak returns of Chinook salmon to the upper Salmon River. Both analog addition and biofilm sampling occurred at a larger extent (3 km) than previous studies (4 -1000 m; Janetski et al. 2009). We found no effect of analog on biofilm standing crop or nutrient limitation, but analog addition may have altered benthic and whole-stream metabolism. Ecosystem respiration and gross primary production responded strongly to high analog addition in one stream. Whole-stream metabolism estimates suggest that high analog addition shifted the stream ecosystem towards greater heterotrophy whereas benthic metabolism measurements suggest that GPP and ER responses were balanced.

In the following, we will first discuss issues associated with our experimental design and the limitations of our whole stream metabolism estimates. We will then compare our results to biofilm responses to other artificial salmon nutrient additions and natural salmon spawning events before we discuss factors that constrain benthic biofilm biomass accrual and evaluate alternative fates of salmon carbon and nutrients in our streams. Finally, we will discuss the implications of our results for salmon nutrient

mitigation efforts and possible future avenues for studying the role of salmon in ecosystem processes.

4.1. Experimental design

Our study was part of a larger effort to examine food web responses to the addition of salmon carcass analog and an extension of previous research (see Kohler et al. 2008; Kohler and Taki 2010; Kohler et al. 2012). In 2010, we sampled biofilm standing crop at the same sites and according to the same stratified design as co-occurring macroinvertebrate sampling. The lack of detectable differences in 2010 biofilm standing crop could be attributed to inadequate within segment sampling because our original sampling design did not account for the great spatial heterogeneity of biofilm standing crop, which varies substantially at scales ranging from individual substrates to kilometers (Stevenson 1997). Equipment failure during chlorophyll analysis caused the loss of all 2010 low analog treatment standing crop samples, which may have also contributed to lowering our statistical power. In 2011, we greatly increased our power to detect differences by changing our sampling design and solving our equipment issues. The change in sampling design between years did not alter our results; we did not find differences in biofilm standing crop in either 2010 or 2011.

A cautious and conservative interpretation of our whole stream metabolism estimates is warranted. First, we were limited in the number of streams in which we could estimate benthic and whole-stream metabolism, which resulted in low replication of our results. Second, our study streams were wide, shallow and turbulent, and these morphologic characteristics made it difficult to estimate gas exchange with the

atmosphere with direct gas evasion. Gas evasion provides the most precise estimates of reaeration coefficients (k; Marzolf et al. 1994; Young and Huryn 1998; Young and Huryn 1999). Young and Huryn (1999) used sulfur hexafluoride (SF_6) gas evasion successfully in streams with wetted width to depth ratios between 10 and 21.3. The width to depth ratio of our high analog control, high analog treatment, low analog control, and low analog treatment segments was 35.6, 40.3, 31.0, and 51.9, respectively. The larger width to depth ratios made it difficult to mix SF_6 evenly across the stream, which precluded the use of this technique in our streams. To overcome this problem, we employed multiple mathematical methods to estimate reaeration including the night-time regression method (Hornberger and Kelly 1975), fitted empirical equations (Melching and Flores 1999; Raymond et al. 2012), and a classic model (Tsivoglou and Neal 1976) suggested by Bott (2006). Conclusions about stream metabolism utilizing reaeration coefficients calculated with generalized equations in streams for which the equations are ill suited should be viewed cautiously (Wallin et al. 2011; Raymond et al. 2012) because GPP and ER are highly sensitive to small differences in reaeration estimates. Estimates of k_{20} from the night-time regression method showed little agreement with generalized equations, and are consistent with Aristegi et al. (2009) who found that agreement between methods was especially low in shallow streams. Most comparisons of reaeration coefficients from gas evasion to mathematical methods find that many empirical equations underestimate gas exchange in high gradient, turbulent streams. As a result of an underestimated k_{20}, we likely underestimated GPP and overestimated ER in our study segments (Young and Huryn 1999).

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4.2. Biofilm responses to salmon nutrients

Biofilm responses to salmon nutrients vary by region, salmon nutrient load, and whether salmon nutrients are added artificially or via natural spawning events (Janetski et al. 2009). In some previous studies live salmon, carcass presence, and carcass analog addition had strong bottom up effects on biofilm chlorophyll *a* and AFDM in natural streams (Chaloner et al. 2007; Kohler et al. 2008; Ruegg et al. 2012) and artificial stream channels (Wipfli et al. 1998; Wipfli et al. 1999; Kiernan et al. 2010; Albers and Petticrew 2012). In contrast, other studies have found that naturally spawning salmon reduced biofilm standing crop in streams with small sediments (Holtgrieve et al. 2010; Verspoor et al. 2010), or that addition of carcasses to natural streams and artificial stream channels did not alter biofilm standing crop (Minshall et al. 1991; Ambrose et al. 2004; Claeson et al. 2006; Cram et al. 2011). Overall, artificial additions lead to higher biofilm standing stocks than live salmon, presumably due to the tendency for artificial additions to exceed the nutrient loads from natural salmon runs and lack the disturbance of substrates associated with natural spawners (Janetski et al. 2009).

Assessments of nutrient limitation in the context of salmon nutrients have also had mixed results. Our nutrient limitation results are consistent with other studies in central Idaho streams that documented nitrogen limitation or N and P co-limitation of benthic biofilms (Marcarelli and Wurtsbaugh 2007; Kohler et al. 2008; Sanderson et al. 2009). Similar to studies where artificial salmon nutrient additions failed to alter patterns of biofilm nutrient limitation in California and Idaho streams (Ambrose et al. 2004; Kohler et al. 2008), biofilms in our current study remained N-limited after analog addition. In contrast, spawning salmon in Alaska alleviated biofilm nutrient limitation

(Ruegg et al. 2011; Tiegs et al. 2011). Ruegg et al. (2011) found that the presence of naturally spawning salmon alleviated autotroph nutrient limitation in 6 of 7 study streams on Prince Wales Island, Alaska. Similarly on Prince Wales Island, Tiegs et al. (2011) found that biofilms were not nutrient limited when live salmon and carcasses were present, but biofilms returned to pre-spawn nutrient limitation shortly after the completion of spawning but while carcasses were still present. These studies suggest that live salmon have greater impact on biofilm nutrient limitation than do carcasses because of spawning disturbance and excretion of metabolic wastes (Tiegs et al. 2011). It is plausible that the inconsistency between our nutrient limitation results and those in Alaskan streams may be due to the inability of salmon carcass analog to mimic the nutrient enrichment and disturbance properties of natural spawning salmon runs. It is important to note, however, that the species of nitrogen used in NDS deployed in our study and that of Kohler et al. (2008) differed from the nitrogen amendment used in the two Alaskan studies above. We used sodium nitrate in our bioassays ($NaNO_3$) whereas Ruegg et al. (2011) and Tiegs et al. (2011) used ammonium chloride (NH_4Cl).

Increased ammonium supply from analog addition may have caused increased chlorophyll *a* standing crop on NDS deployed in high analog streams. In our study, stream water ammonium concentrations increased 40% and 250% in our two high analog treatment segments (Kohler, *unpublished data*). In other studies, similar additions of pasteurized carcasses and salmon carcass analog resulted in elevated stream water ammonium concentrations, but did not alter nitrate levels (Claeson et al. 2006; Kiernan et al. 2010; Wipfli et al. 2010). Natural salmon runs are also associated with strongly elevated ammonium concentrations, yet nitrate concentrations typically remained

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unchanged (Mitchell and Lamberti 2005; Chaloner et al. 2007) or increase just after the initial onset of spawning (Tiegs et al. 2011). Additionally, nitrogen limitation of AFDM on NDS in our study was more frequent in treatment segments than control segments suggesting that total biofilm community biomass shifted from being limited by carbon or to being limited by nitrogen. We used inorganic substrates in our bioassays, and thus heterotrophic microbial populations were supported by algal carbon and used algal cells as their primary substrate for colonization (Rier and Stevenson 2002). Because AFDM is total community biomass (i.e., autotrophs + heterotrophs), this result suggests that heterotrophic biomass on NDS in control segments may have been limited by suitable substrate for colonization rather than nitrogen or phosphorous. Higher NDS algal densities in treatment segments may have alleviated organic substrate limitation.

We expected analog addition to positively influence rates of primary production by increasing nitrogen and phosphorus supply. Both benthic and whole-stream metabolism estimates from this study supported this hypothesis. Not surprisingly, GPP was very low in our study streams due to the highly oligotrophic conditions common in central Idaho streams (Sanderson et al. 2009). Benthic GPP in our study was comparable to estimates obtained in tributaries of the Middle Fork of the Salmon River (Davis 1995). Even after analog addition, whole-stream GPP was an order of magnitude lower than GPP of most other open-canopy stream systems and comparable to GPP in heavily shaded headwater streams (Mulholland et al. 2001). Despite low GPP estimates in our streams, the ratio of production to respiration in all segments fell within the range of GPP : ER commonly observed in streams, albeit at the lower end of that range (Marcarelli et al. 2011). Studies assessing changes in benthic and whole-stream metabolism to

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spawning salmon and nutrients are limited, but suggest that GPP declines when salmon

spawn, and ER increases with the post-spawning disturbance influx of nutrients

(Holtgrieve and Schindler 2011).

The increase in labile organic carbon may have caused changes in ecosystem

respiration, which supports the potential importance of salmon nutrient subsidies to

heterotrophic processes. We found a 70% increase of ER in our high analog treatment

versus control segments during the 10 days of September, and benthic respiration

measured with dark chambers increased by 165%. Holtgrieve and Schindler (2011) found

a similar increase in ER in Alaskan sockeye streams containing a peak salmon abundance

of > 0.6 live salmon per square meter and suggested four mechanisms by which salmon

can increase ER: (1) creating greater access to surface area for colonization and growth of

heterotrophic microbial populations through streambed disturbance; (2) increasing

nutrient concentrations; (3) releasing labile carbon; and (4) directly consuming O_2.

Mechanisms (1) and (4) are not applicable to our streams. Sockeye salmon density in

Holtgrieve and Schindler (2011) was associated with a nutrient and carbon load

approximately equivalent to our high analog level but streambed disturbance by salmon

in our study was negligible relative to streams in Alaskan sockeye streams; redd counts in

our high analog stream suggests a minimum live salmon density of 0.002 salmon per

square meter. Additionally, changes in ecosystem metabolism with increased input of

labile allochthonous carbon observed in our study and by Holtgrieve and Schindler

(2011) are not restricted to salmon-bearing ecosystems. Bernhardt and Likens (2002)

found that the addition of dissolved organic carbon (DOC) resulted in elevated ER, and

increased carbon supply stimulated microbial nutrient demand, shortened nutrient uptake

lengths, and caused a decrease in stream water nitrate concentrations. The similarity between the response of ER to live salmon, salmon carcass analog, and artificial DOC indicates that mechanisms (2) and (3) may be the primary mechanisms by which salmon influence ecosystem respiration.

We observed changes in whole-stream GPP and ER during the third week of September. Decreasing GPP was common to all segments, but the segments exhibited opposite responses in ER. The high analog treatment segment was the only segment to exhibit a decrease in ER whereas ER increased in the other three segments. It is doubtful that fluctuations in stream discharge, velocity, slope, or other physical variables related to reaeration coefficient of treatment segments would have produced the observed change because streams in central Idaho are at baseflow from July onward. Stream temperature can influence ER and GPP (Allen et al. 2005; Demars et al. 2011) and explain up to 50% of seasonal variation in GPP and ER (Uehlinger 2006). Peak day-time water temperature in our streams declined approximately 1°C during the month of September; however, falling temperature fails to explain changes in GPP and ER in treatment segments because stream metabolism in control segments did not decrease with this change in temperature. Re-calibration of dissolved oxygen sensors at the same time as changes in stream metabolism and may have contributed to part of this change. Alternatively, decreasing GPP in all segments may be explained by algal senescence, the increased ER in low analog and control segments to the input of leaves during the rapid senescence of riparian vegetation that occurred at this time (Ebel, personal observation), and the decreasing ER in the high analog segment to a declining effect of analog.

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4.3. Constraints of biofilm standing crop

Like all biota, the growth and reproduction of microbes within benthic biofilms is constrained by a suite of abiotic and biotic environmental pressures that are largely independent of resource availability. Here, we focus our discussion on light availability, macroinvertebrate grazing pressure, and biofilm age as three possible factors that may explain the lack of differences in biofilm standing crop between treatment and control segments despite increases in benthic and whole-stream GPP and ER.

Light intensity synergistically interacts with nutrient availability to control algal standing crop and gross primary productivity (Hill et al. 2011), thus light intensity has been discussed as a major constraint on biofilm response to salmon nutrients (Cedarholm et al. 1999). Photosynthesis and algal biomass is positively associated with light intensity, with higher GPP and chlorophyll *a* concentrations occurring in open canopy streams with abundant light (McIntire and Phinney 1965; Mullholland et al. 2001; Rier and Stevenson 2002; Kiffney et al. 2004). Open canopy streams are also associated with a greater occurrence of biofilm nutrient limitation (Lowe et al. 1986) because algae are no longer constrained by light availability. In our study, we saw a significant positive relationship between canopy cover and chlorophyll *a* on rocks. This result is in direct contrast with what is observed in many other studies and may be explained by differences in canopy cover and chlorophyll *a* between geographic areas (shown in Figure 2.2). Within basins, segments showed no significant relationships between canopy cover and chlorophyll *a*.

Macroinvertebrate grazing may exert a strong negative top-down force on biofilm standing crop and obscure increases in biofilm standing crop associated with salmon nutrients. Many studies have demonstrated positive associations between artificial

additions of salmon nutrients and the abundance and density of macroinvertebrates that feed on algae (i.e., grazers) (Wipfli et al. 1998; Wipfli et al. 1999; Claeson et al. 2006, Kohler et al. 2008; Kohler and Taki 2010; Kohler et al. 2012). Bottom-up effects of inorganic nutrient enrichment on macroinvertebrate production is commonly observed in streams (Peterson et al. 1985; Hart and Robinson 1990; Cross et al. 2006) as is top-down control of biofilm biomass (Lamberti and Resh 1983; Lamberti et al. 1989, Peterson et al. 1993; Hillebrand and Kahlert 2001). During a 5 year nutrient enrichment of a tundra stream, Peterson et al. (1993) observed an initial increase in chlorophyll *a* causing a positive numerical response of grazing macroinvertebrates, which in turn, decreased algal standing crop during the final years of the enrichment. Similarly, if analog addition had bottom up effects on grazer populations, we would not expect to see the full extent of grazing-inhibited biofilm standing crop until the second year of our study. Because we did not observe standing crop differences in either year, we cannot attribute the lack of analog-induced standing crop responses to grazing; however, our power to detect differences in 2010 may have hidden a small increases in biofilm standing crop with analog addition, thus we cannot reject the grazing-constraint hypothesis. To date, few published studies have documented changes in macroinvertebrate density and biofilm standing crop to multiple years of artificial salmon nutrient enrichment. Yet, multi-year studies of grazer-biofilm-salmon nutrient are critical to determining the movement of salmon nutrients into the foodweb via the biofilm pathway and should be vigorously pursued.

Disturbance can determine changes in biofilm production with nutrient enrichment by influencing spatial and temporal patterns in biofilm development (Biggs et

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al. 1999). Biofilms mature rapidly following disturbances (i.e., scouring, grazing) and can obtain maximum biomass for a given stream flow velocity in 30-50 days (Battin et al. 2003; Besemer et al. 2007). In our streams, scouring of the entire streambed during spring snowmelt constitutes the largest disturbance of any given year in both magnitude and extent (Hall et al. 2009) and provides a starting point for biofilm succession. We added salmon carcass analog to treatment segments 61 and 53 days after peak run-off in 2010 and 2011, respectively, which suggests that biofilms in our streams may have reached maturity. Mature, or fully developed biofilms tend to have maximized efficiency (i.e., yield of biomass per unit resource; Kreft 2004) and rely on the internal recycling of nutrients and carbon to support energy requirements (Mulholland et al. 1994). Internal recycling of nutrients is important because the rate of diffusion between the overlying water and microbial cells decreases as biofilm thicken with age (Battin et al. 2003). Nutrient enrichment may lead to higher algal productivity, but rather than incorporate the new carbon into biomass mature biofilms may respond by increasing the release rate of autochthonous DOC (Ziegler and Lyon 2010). In contrast, less developed biofilms tend to maximize the production of new biomass (Kreft 2004) by exhibiting high DOC uptake rates and incorporating more carbon into new biomass (Augspurger et al. 2008). As a result, less developed biofilms respond more strongly to nutrient enrichment than do older biofilms (Sobczak 1996), which is manifested in higher biofilm productivities on frequently disturbed substrates (Cardinale et al. 2005). The difference between the productivity of less developed biofilms vs. mature biofilms may explain why biofilm standing crop was higher after analog addition on unamended NDS in 2011 and on unglazed tiles in 2003 (Kohler et al. 2008), when biofilms on natural substrates in the

same streams did not respond to analog. The hypothesis that disturbance and nutrient enrichment may have a synergistic rather than antagonistic effect on annual biofilm production is underrepresented in salmon-ecosystem and nutrient mitigation literature, but should be considered.

4.4. Alternative fates of salmon carcass analog

The discrepancy between standing crop and metabolism responses suggests that a portion of the carbon and nutrients from salmon carcass analog are assimilated by benthic biofilms in our streams, but are not being accumulated in biofilm standing crop. This raises an important question: Where did the organic material in salmon carcass analog ultimately go?

The positive association of chlorophyll *a* with canopy cover combined with the nearly significant negative relationship between discharge and biofilm chlorophyll *a* is perplexing. Chlorophyll *a* is expected to decrease with increasing canopy cover and decreasing discharge (Vannote et al. 1980). We suspect that this result is due to the interaction between analog addition and the geomorphological characteristics of our streams. Streams with broad, open floodplains may have a larger transient storage zone relative to total stream cross sectional area, thus have a greater capacity to store nutrients in the hyporheic zone (D'Angelo et al. 1993). The larger streams in our study occurred in the Salmon basin and nutrients may have moved into the hyporheic zone rather than being taken up by streambed biofilms. Storage and transformation of nutrients and carbon in the hyporheic zone is another possible fate of salmon carcass analog in our streams as evidenced by the difference between benthic and whole-stream metabolism. Elevated

benthic respiration in the high analog treatment segment was not accompanied by increased NCP indicating that benthic GPP was respired. Meanwhile, whole-stream ER was vastly greater than whole-stream GPP indicating that a substantial portion of ER can be attributed the metabolic activity of hyporheic microbes (Mulholland et al. 1997; Fellows et al. 2001; Hall and Tank 2005). Hyporheic storage may constitute a sizeable fraction of a stream nitrogen budget and exceed uptake and storage in benthic biota (Triska et al. 1989). Conversely, the hyporheic zone may be a nitrogen sink because adequate carbon availability and anoxic conditions can permit denitrification (Pinay et al. 2003; Schade et al. 2001).

The hyporheic zone and streambed may have the capacity to store salmon nutrients over the winter before re-releasing the nutrients the following spring (O'Keefe and Edwards 2002). Recent work on the transport and storage of salmon nutrients suggests flocculation (i.e., the physical, biological, or chemical joining of inorganic and organic particles) can facilitate the transport of salmon nutrients and carbon into the streambed (Rex and Petticrew 2008) where it is retained longer than in surface or interstitial waters (Rex and Petticrew 2010). In our study, movement of analog particulates into the hyporheic zone may have stimulated microbial activity. Because hyporheic exchange is a characteristic of many present and historic salmon spawning streams, the role of the hyporheic zone in storing or removing salmon nutrients from the stream may impact decisions regarding the feasibility of effective nutrient mitigation and conclusions about the importance of salmon nutrient enrichment to stream ecosystem processes.

4.5. Future of the 'biofilm pathway' paradigm

Elucidating the ultimate fate of salmon nutrients is an important step towards the effective and efficient restoration of Pacific salmon populations in the contiguous United States and should be an objective of research on the role of salmon in stream ecosystem processes. In our study, salmon carcass analog had strong effects on respiration and caused higher rates of primary production; however it is unclear whether the increase in primary and secondary microbial productivity will directly stimulate higher trophic levels. Benthic and whole-stream metabolism estimates had a profound effect on our interpretation of our nutrient limitation and standing crop results by highlighting the potential processing and storage of salmon nutrients in the hyporheic zone of our study streams. Additionally, salmon nutrient enrichment during late summer may influence how biofilms respond to changing seasons, with consequences for food web productivity prior to snow-melt scouring in the spring. We suggest that future research on salmon nutrient mitigation utilize measures of ecosystem metabolism, explore the role of the hyporheic zone in storing or removing nutrients and carbon, and consider the physiological implications of organic nutrient supply for the persistence of biofilms through the winter and following spring.

Our study suggests that high levels of salmon carcass analog addition may incite a biofilm response; however, documenting a simple organismal response does not mean that the goals of nutrient mitigation have been achieved. The primary question of mitigation research should shift from "Do stream ecosystems respond to salmon nutrient additions?" to "Where are the added carbon and nutrients going?" This second question can be answered with carbon and nitrogen budgets of target watersheds that identify

whether added nutrients are stored within relevant ecosystem compartments (e.g., biota, particulate organic matter, hyporheic zone, terrestrial vegetation) or exported via respiration, denitrification, or flushing downstream. As salmon populations creep ever closer to complete collapse, it is essential that effort allocated to maintain healthy salmon populations be spent wisely. How much of the money allocated to nutrient mitigation is moving to the ocean as migrating salmon smolts and how much of that investment returns as spawning adults? Alternatively, how much of that money is flushed downstream to be stored and processed behind the same hydroelectric dams that are responsible for the decline of Idaho's salmon in the first place? These questions must be answered for nutrient additions to be accepted as a viable way to lessen the gravity of the socio-industrial assault on the Pacific salmon populations of the contiguous United States.

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