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INVESTIGATING THE IMPACTS OF STREAMBED HABITAT HETEROGENEITY ON ECOSYSTEM STRUCTURE AND PROCESSES USING BASIC AND APPLIED PERSPECTIVES

James C. Olson
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INVESTIGATING THE IMPACTS OF STREAMBED HABITAT HETEROGENEITY
ON ECOSYSTEM STRUCTURE AND PROCESSES USING BASIC AND APPLIED
PERSPECTIVES

By

James C. Olson

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Biological Sciences

MICHIGAN TECHNOLOGICAL UNIVERSITY

2014

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This thesis has been approved in partial fulfillment of the requirements for the Degree of
MASTER OF SCIENCE in Biological Sciences.

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Preface

Chapters 2 and 3 of this thesis were completed in collaboration with many authors. I collected and analyzed the data and wrote both chapters. Dr. Amy Marcarelli of Michigan Technological University contributed to the study design, data analysis and the editing of both chapters. Chapter 2 was prepared in collaboration with Drs. Sue Eggert, Anne Timm, and Randy Kolka of the USDA Forest Service, who contributed to the study design and assisted with editing. This chapter is currently in preparation for submission to *River Research and Application*. Chapter 3 was prepared in collaboration with Dr. Casey Huckins of Michigan Technological University, who assisted with the study design and editing. This chapter is currently in preparation for submission to *Freshwater Science*.

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Finally, thank you to my family for your support through my entire education.

Abstract

Stream restoration often focuses on increasing habitat heterogeneity to reverse ecosystem degradation. However, the connection between heterogeneity and ecosystem structure and processes is poorly understood. We looked to investigate this interaction from both applied and basic science perspectives. For the applied study, we examined two culvert replacements designed to mimic natural stream channels, to see if they were better at maintaining ecosystem processes within as well as upstream and downstream of culverts compared to non-replaced culverts. We measured three ecosystem processes (nutrient uptake, hydrologic characteristics, and coarse particulate organic matter retention) and found that stream simulation culvert restoration improved organic matter retention within culverts, and that there were no differences in processes measured upstream and downstream of both restoration designs. Our results suggest that measurements of ecosystem processes are more likely to show a response to restoration if they match the scale of the restoration activity. For the basic science study, we quantified the longitudinal spatial heterogeneity of physical and biofilm characteristics at microhabitat to segment scales on streams with different streambed variability. We found that all physical characteristics and biofilm characteristics were spatially independent at the macro-habitat scale and greater. Together, these studies demonstrate the importance of scale in ecological interactions and the value of incorporating considerations of scale into restoration activities.

Chapter 1: The Impacts of Habitat Heterogeneity on Ecosystem Structure and Processes

Anthropogenic degradation is occurring at an alarming rate in fluvial ecosystems worldwide, resulting in an increasing number of restoration projects (Bernhardt et al. 2005). Negative human influences on streams includes compromising water quality, simplifying the natural flow regime with dams and other barriers, and homogenizing the physical structure of stream channels through straightening, embanking, and removing of debris dams (Lepori et al. 2005). Ecological restoration projects are designed to rehabilitate biological, chemical, and physical conditions to levels prior to disturbance (NRC 1992), and are becoming increasingly common (Bernhardt et al. 2007).

A large proportion of restoration projects in fluvial ecosystems aim to restore connectivity (culverts replacements or dam removals) and/or create habitat to improve fisheries (Roni et al. 2008, Palmer et al. 1997). In the Midwest and nationwide, most of the stream restoration projects are a form of habitat rehabilitation which usually involves adding structures of rock or wood to increase habitat complexity (Moerke and Lamberti 2004, Roni et al. 2008). Despite the prevalence of these projects, they are rarely monitored (Bernhardt et al. 2005) and when they are, success is limited and results are mixed (Roni et al. 2008, Palmer 2009). Habitat rehabilitation projects could affect ecosystem processes either with direct relationships between habitat heterogeneity and ecosystem processes or with an indirect relationship where an increase in habitat heterogeneity increases biodiversity and subsequently improves ecosystem process.

Restoration projects that rebuild physical habitat heterogeneity (physical complexity in rock size, channel form, woody debris, etc.) have expected outcomes to improve both structure and process (Palmer et al. 1997). The first expectation is that restoring physical habitat will increase species abundance or biodiversity (ecosystem structure; Palmer et al. 1997, Lepori et al. 2005). Secondly, the increase in biodiversity will subsequently lead to improved ecosystem processes (Palmer et al. 1997, Brooks et al. 2002, Lake et al. 2007, Palmer 2009). However, these two expectations are rarely observed. Palmer et al. (2010) reviewed restoration projects for habitat heterogeneity and found that only two of 78 were able to conclude increases in invertebrate diversity due to habitat heterogeneity restoration, suggesting a very low success rate. Lepori et al. (2005) also found that restoration of habitat heterogeneity did not promote either fish or invertebrate diversity. Rosi-Marshall et al. (2006) found that a project to rehabilitate habitat for trout in northern Michigan resulted in increased physical habitat quality and an increase in large trout, but observed no response in periphyton abundance, or invertebrate density and diversity. Additionally, Moerke et al. (2004) rehabilitated a stream site by creating meanders and adding substrate heterogeneity. Periphyton abundance appeared to increase relative to an unrestored site but invertebrate diversity showed no response to restoration, and fish biomass increased in one restored site but not another (Moerke et al. 2004). These results indicate that restoration of physical heterogeneity does not consistently support an increase in biodiversity at any trophic level.

Even if an increase in biodiversity was supported, the relationship between biodiversity and stream ecosystem processes is also poorly understood (Lake et al. 2007).

Cardinale et al. (2002a) found facilitative interactions among three caddisfly taxa that increased consumption of suspended particulate matter compared to microcosms with only one caddisfly taxa. However, another microcosm study showed that leaf decomposition rates did not increase with increasing fungal species diversity (Dang et al. 2005). These studies, along with others, have conflicting results on the relationship between biodiversity and ecosystem processes, and most suffer from simplification of physical and biological heterogeneity found in ecosystems (Lake et al. 2007, Hooper et al. 2005). A causal relationship between biodiversity and ecosystem processes is still largely an assumption (Lake et al. 2007) and the mechanisms through which ecosystem processes may depend on biodiversity are not well understood (Hooper et al. 2005).

Finally, a direct relationship between habitat heterogeneity and ecosystem process, without biodiversity as a mechanism for connection, also is not consistently supported. In manipulated, high heterogeneity riffles, biofilm primary production and respiration were shown to recover from disturbance more quickly than in low heterogeneity riffles (Cardinale 2002b). However, Hoellein et al. (2012) monitored a restoration site with improved benthic habitat heterogeneity and found almost no differences between restored and unrestored reaches for multiple ecosystem processes measurements.

Stream restoration has a long history of following the ecological assumption that restoring physical structure and complexity will benefit both ecosystem structure and processes (Palmer 2009). Adequate monitoring of restorations are infrequent (Bernhardt et al. 2005) and results are inconsistent in supporting common restoration activities

(Palmer et al. 2010). My thesis research aimed to understand the relationship between habitat heterogeneity and ecosystem structure and processes with two different approaches. Firstly, we evaluated whether new projects to restore stream connectivity and habitat heterogeneity in the form of culvert replacements improved ecosystem processes. Secondly, we evaluated whether natural patterns of physical heterogeneity of unmanipulated stream reaches are similar to biological structure. The first investigation (Chapter 2) took place in northern Wisconsin streams and compared the effects of culvert replacement designs and non-replaced culverts on three ecosystem processes: nutrient uptake, hydrologic characteristics, and coarse particulate organic matter (CPOM) retention at two different spatial extents: within culverts, and upstream and downstream of culverts. We found that none of the ecosystem processes that we measured changed upstream and downstream of replaced culverts, but that CPOM retention was most similar within replaced culverts to upstream and downstream conditions. These results suggest that restoration and monitoring activities must occur on similar spatial scales. For the second investigation (Chapter 3), three streams in the Upper Peninsula of Michigan were chosen to evaluate the small-scale heterogeneity of physical and biotic structure. We found that physical characteristics (water depth, water velocity, substrate size) in these streams varied at the habitat scale (e.g. riffle-pool; range approx.. 10 m) while chlorophyll *a* and ash-free dry mass (AFDM) varied at the habitat scale and sometimes smaller (range 3-10 m). Additionally, spatial patterns of physical characteristics varied among cobble vs. sand-bottomed streams. Together, these two studies suggest the

importance of scale when studying connections between habitat heterogeneity and ecosystem structure and processes from both basic and applied perspectives.

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Chapter 2: Evaluating the Impact of Culvert Designs on Ecosystem Processes in Northern Wisconsin Streams¹

Abstract

The objective of this study was to investigate the effects of two culvert replacement designs on stream ecosystem processes and how they compare to non-replaced culverts. The two styles of culvert replacements examined were stream simulation design (SSD) culverts, where culverts are designed to accommodate bankfull width and stream bottoms were rebuilt, and bankfull and backwater designs (BBD), where culverts were replaced but stream bottoms were left to fill naturally. We investigated the objective by evaluating three ecosystem processes at two different spatial extents: nutrient uptake, hydrologic characteristics including water velocity and transient storage, and CPOM (coarse particulate organic matter) retention upstream and downstream of replaced culverts, as well as water velocity and CPOM retention through replaced and 4 non-replaced culverts. We found that SSD culverts better maintain CPOM retention rates through culverts compared to non-replaced and BBD culverts. However, we observed no other significant differences in stream ecosystem processes upstream or downstream of replaced culverts, probably because both styles of culvert replacements

¹ The material in this chapter is planned for submission

are likely to fix major hydrologic issues and aquatic organism passage problems, and we avoided non-replaced culverts with significant hydrologic issues in this study.

1. Introduction

Case studies in the Great Lakes region show that 64 % of dams and road-crossings are impassable or only partially passable to aquatic organisms and consequently removal of dams and improvement of road-crossings are a widespread focus of restoration activity in this region (Januchowski-Hartley et al. 2013). Road crossings and associated structures, including culverts, offer a suite of potential negative effects on fluvial ecosystems, and restorations of these structures typically have the goals of improving fish passage and hydraulic conditions. Culverts can act as barriers to fish movement and can cause fragmentation of populations, primarily by impacting the physical structure of stream channels and causing deviations from natural flow conditions (Warren and Pardrew 1998). Bouska et al. (2010) found that box-type culverts in Kansas have deeper mean bankfull depths and smaller width to depth ratios compared to natural stream channels (Bouska et al. 2010). Culverts can also retain large substrates during high discharge events, which then obstruct flow under low discharge conditions (Wellman et al. 2000). Culverts that obstruct natural flow conditions can also increase accumulation of fine sediments downstream or upstream of culverts (Lachance et al. 2008, Wellman et al. 2000, Bouska et al. 2010) and can influence the organic matter content of sediments upstream of culverts (Lachance et al. 2008). Riffle spacing can also decrease in reaches upstream of culverts compared to downstream (Bouska et al. 2010).

Culverts can also act as semi-permeable barriers to aquatic organism movement, with varying degrees of reduced movement based on culvert characteristics (Warren and Pardrew 1998, Bouska and Paukert 2010). For example, using mark-recapture, Bouska and Paukert (2010) found that movement of cyprinid fish increased through culverts with decreased culvert slope, shorter length, and wider width. In Lower Michigan streams, Briggs and Galarowicz (2013) found that culvert design and length affected movement of some species of fish and that species richness was higher downstream of culverts compared to upstream. However, not all fish passage studies have found that culverts reduce fish movements. Pluym et al. (2008) found no reduction in movement of stream fishes through culverts and no significantly different relationships in fish community diversity or a fish index of biotic integrity upstream and downstream of culverts or between culvert designs and bridges. Also, culverts may inhibit upstream movement of invertebrates in the adult stage (Vaughan 2002). For instance, adult caddisfly abundance was lower upstream of culverts compared to downstream, suggesting culverts are a partial barrier to upstream dispersal (Blakely et al. 2006).

Studies that evaluate the ecological effects of culvert replacements are rare but suggest that they can have positive effects on fish and invertebrate assemblages. Roni et al. (2008) surveyed 345 stream rehabilitation projects and of 5 culvert replacement projects, inaccessible stream reaches upstream of culverts were readily recolonized by fish after replacement. However, culvert replacements in the lower peninsula of Michigan have had mixed results for both macroinvertebrate and fish assemblages (Ogren 2014). Of three culvert replacement sites, no differences were observed in a before-after

comparison for fish index of biotic integrity, and significant differences for macroinvertebrate indices were dependent on index type and study site (Ogren 2014). Additionally, only minor differences were found in macroinvertebrate assemblages in a before and after culvert replacement comparison in Northern California (Lawrence et al. 2014).

The use of ecosystem processes in post-restoration monitoring may offer valuable insights that are missed by only monitoring ecosystem structure (Bunn and Davies 2000) and therefore may provide insight into the effects of culvert replacements on stream biota. Some stream restorations that increase or restore physical complexity have been evaluated using ecosystem processes such as coarse particulate organic matter (CPOM; organic particles larger than 1 mm including woody and non-woody debris) retention (Rosi-Marshall et al. 2006, Lepori et al. 2005, Koljonen et al. 2012), transient storage (the temporary delay in downstream movement of solutes in areas outside of the main channel) characteristics (Becker et al. 2013), nutrient uptake, and ecosystem metabolism (Hoellein et al. 2012). There is not always a consistent linkage between ecosystem structure and ecosystem processes but evaluating ecosystem processes may be important in explaining degradation or restoration effects.

The USDA Forest Service has implemented culvert replacement projects with the intent of alleviating some of the possible negative effects to physical processes and stream biota caused by poorly designed or undersized culverts (SSWG 2008). The most intensive style of culvert replacement, called the stream simulation (SSD), is designed to mimic the natural stream dimensions (SSWG 2008), requiring that the culvert be equal or

greater than bankfull width to maintain flow velocities at a similar speed as downstream reaches at a variety of flow conditions (Cenderelli et al. 2011). Additionally, in SSD, the streambed through the culvert is rebuilt to match the natural variability of the stream substrates. An alternative culvert style is the bankfull and backwater design (BBD) where the culvert is fit to have the same bankflow width and depth as the natural channel, but rather than rebuilding the streambed, substrates are allowed to fill in via natural stream flows. Both culvert replacement designs are expected to improve aquatic organism passage and may also better preserve ecosystem processes upstream of, through and downstream of culverts compared to non-replaced culverts.

Our overall goal was to evaluate the effectiveness of two culvert replacement designs for maintaining three ecosystem processes upstream, through, and downstream of culverts. There are two different extents where culverts may affect ecosystem processes. First, culverts may alter ecosystem processes in upstream and downstream reaches by altering hydrologic or geomorphic conditions as described earlier. We hypothesized that the ecosystem processes we measured would be similar above and below both SSD and BBD culverts, as they are designed to mimic natural hydrologic conditions. Second, hydrologic and geomorphic conditions may alter ecosystem processes within culverts, even if these effects do not transmit to upstream and downstream reaches. Therefore, we hypothesized that ecosystem processes measured within culverts would be most similar to those measured in upstream reference reaches in SSD culverts, where streambeds are rebuilt, while they would be less similar in BBD culverts and least similar in non-replaced culverts. To evaluate differences in ecosystem processes at both of these spatial

extents (upstream-downstream reaches and within culverts) we undertook two comparative studies quantifying three ecosystem processes (ammonium uptake, hydrologic conditions including water velocity and transient storage, and CPOM retention) in replaced and non-replaced culverts on northern Wisconsin streams. Together, we hope to inform restoration managers regarding whether these ecosystem processes are altered by culvert replacements and if so, whether incorporating natural substrate heterogeneity as is done in the SSD gains additional improvement in ecosystem processes.

2. Methods

2.1. Study Sites and Design

All study sites were located in northern Wisconsin on the Chequamegon-Nicolet National Forest (Table 2.1, Figure 2.1). The streams were located in northern mesic forests with maple (*Acer saccharum*), hemlock (*Tsuga canadensis*) and yellow birch (*Betula alleghaniensis*) established on predominately loamy or silty soils. Riparian vegetation was dominated by speckled alder (*Alnus incana*). The streams in the Washburn District flow into rivers that reach Lake Superior, the Medford District streams are located in the headwaters of the Mississippi River drainage, and streams in the Eagle River District feed rivers that reach Lake Michigan. These streams generally have peak discharge during snowmelt in early spring and decrease to baseflow during the summer.

Table 2.1: GPS coordinates for all culvert sites.

Site	District	Culvert Type €	Latitude	Longitude
Gasparado	Eagle River	non-replaced	45.96789	-88.74136
Coldwater	Eagle River	BBD	45.83197	-88.69624
Armstrong	Eagle River	SSD	45.64093	-88.44647
Armstrong 2	Eagle River	non-replaced	45.65825	-88.47915
Kingstone	Eagle River	non-replaced	45.84748	-88.74287
Lillypad	Eagle River	BBD	45.93664	-88.77200
Chucks	Eagle River	non-replaced	45.96819	-88.67518
Duck	Eagle River	SSD	45.98114	-88.65333
Wisconsin	Eagle River	BBD	45.97665	-88.60285
Preemption	Washburn	SSD	46.32818	-91.08728
Whiskey	Washburn	BBD	46.30185	-90.91581
John's	Medford	BBD	45.18702	-90.66418
Joseph's	Medford	SSD	45.18215	-90.66065
Popple	Eagle River	SSD	45.79112	-88.68354
Popple	Eagle River	BBD	45.78059	-88.69206

€ SSD = stream simulation design, BBD= bankfull and backwater design

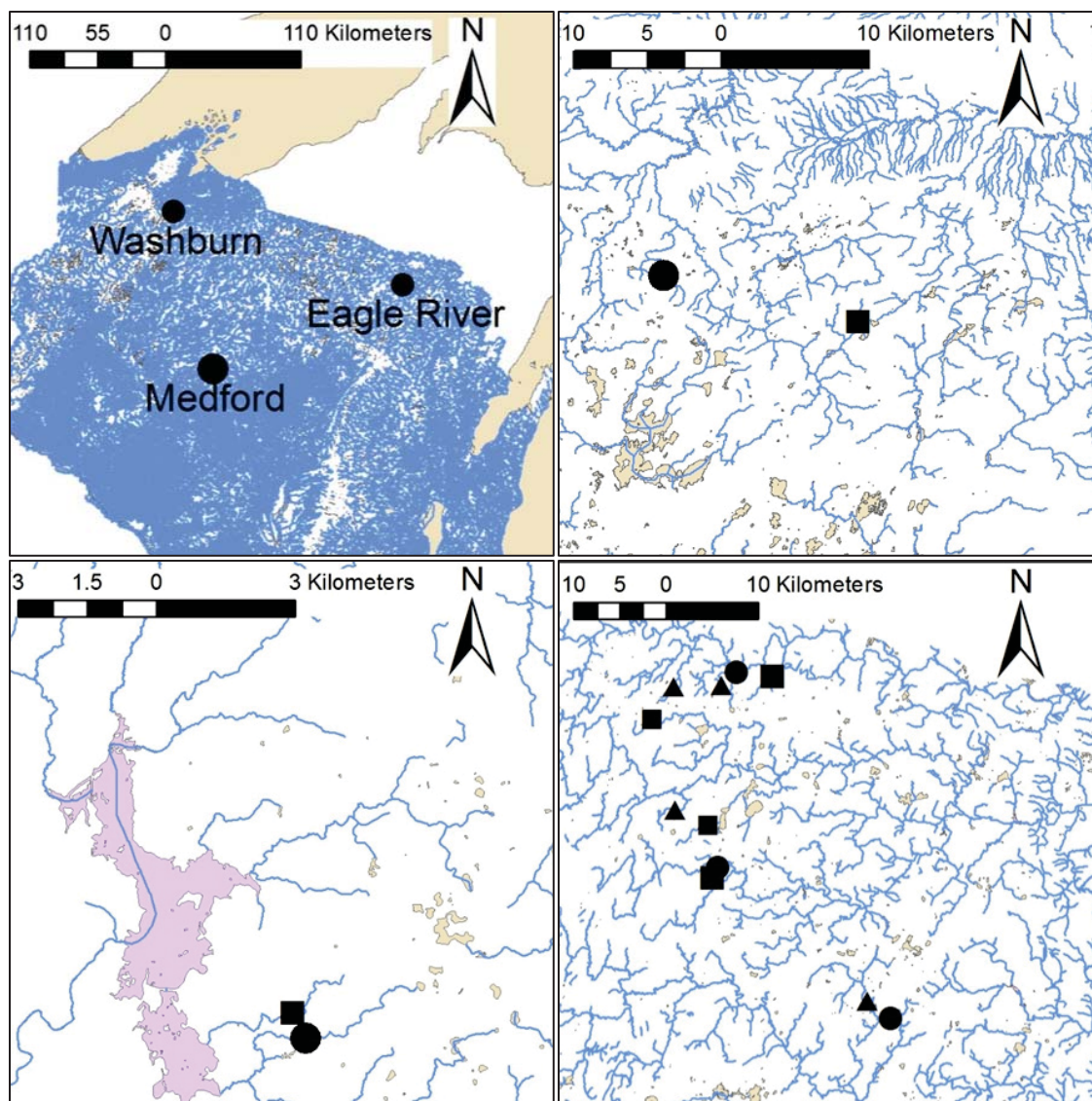


Figure 2.1: Location of study sites. (See table 2.2 for stream sites within each region)
 Top left - Overview map showing location of study districts within Wisconsin, top right-
 Washburn District, bottom left - Medford District, bottom right - Eagle River District.
 Circles - SSD, square - BBD, triangle - non-replaced.

For the first comparison, hereafter referred to as the “culvert replacement comparison,” we selected three pairs of SSD and BBD culverts and quantified 3 ecosystem processes upstream of, through, and downstream of culverts. Each pair had a SSD culvert and a BBD culvert and were chosen based on proximity to each other and by similarity in stream width and depth (Table 2.2). For this comparison, we measured three ecosystem processes: nutrient uptake, hydrologic characteristics, and CPOM retention. These processes were selected to evaluate both our hypotheses regarding upstream-downstream and within-culvert effects on ecosystem processes. To compare upstream-downstream effects, we measured nutrient uptake, transient storage and water velocity as two different aspects of hydrologic conditions, and CPOM retention. Because culvert lengths were too short to apply nutrient uptake and transient storage methods, we used water velocity and CPOM retention which could be measured at smaller spatial extents to compare conditions within SSD vs. BBD culverts.

For the second comparison, hereafter referred to as the “culvert design comparison”, we sought to determine whether ecosystem processes within replaced culverts were different from those within non-replaced culverts. Therefore, we expanded our dataset of the six culvert pairs to include nine additional culverts (two SSD, three BBD, and four non-replaced culverts) with a final total of 15 culverts (Table 2.2). One of the two additional SSD culverts in this comparison was actually a bridge replacement following the same design criteria of SSD (accommodation of bankfull width and a rebuilt streambed). The four non-replaced culverts we added were defined as any culverts

that were at least twenty years old and did not have ponding (hydrologic issues) that would have prevented reach-scale measurement of ecosystem processes. For this comparison, we focused on processes that could be measured within culverts: water velocity as a metric of hydrologic condition, and CPOM retention. For each culvert, we quantified processes within culverts and in an upstream reference reach; removing downstream reference reaches from this comparison allowed us to maximize the total number of culverts in our dataset, but prevented us from evaluating our hypothesis regarding upstream-downstream differences in ecosystem processes using this comparison.

Table 2.2 Physical characteristics and ecosystem processes sampled at the 15 study streams in 2013. Discharge measurements were taken on the same day or within one day of CPOM retention.

Stream	Culvert Type €	Date	Q (L/sec)	Width (m)	Depth (m)	Ecosystem † Processes	Reaches *
Preemption	SSD	28-Jun-13	61	2.2	0.14	Nup, Hyd, CPOM	Up, Thr, Dwn
Whiskey	BBD	28-Jun-13	26	1.6	0.12	Nup, Hyd, CPOM	Up, Thr, Dwn
Joseph's	SSD	7-Jun-13	73	3.3	0.12	Nup, Hyd, CPOM	Up, Thr, Dwn
John's	BBD	7-Jun-13	82.9	2.6	0.10	Nup, Hyd, CPOM	Up, Thr, Dwn
Popple	SSD	26-May-13	447	5.4	0.24	Nup, Hyd, CPOM	Up, Thr, Dwn
Popple	BBD	27-May-13	392	4.3	0.29	Nup, Hyd, CPOM	Up, Thr, Dwn
Armstrong	SSD	25-Aug-13	191	5.8	0.40	Hyd, CPOM	Up, Thr
Armstrong	Non	25-Aug-13	87	3.6	0.34	Hyd, CPOM	Up, Thr
Chuck's	Non	24-Aug-13	18	1.5	0.34	Hyd, CPOM	Up, Thr
Coldwater	BBD	26-Aug-13	50	2.0	0.12	Hyd, CPOM	Up, Thr
Duck	SSD	23-Aug-13	15	1.4	0.09	Hyd, CPOM	Up, Thr
Gasparado	Non	24-Aug-13	39	2.1	0.18	Hyd, CPOM	Up, Thr
Kingstone	Non	26-Aug-13	75	4.2	0.31	Hyd, CPOM	Up, Thr
LillyPad	BBD	26-Aug-13	24	2.9	0.19	Hyd, CPOM	Up, Thr
Wisconsin	BBD	24-Aug-13	45	1.8	0.23	Hyd, CPOM	Up, Thr

€ SSD = stream simulation design, BBD= bankfull and backwater design, Non= non-replaced

† Nup = nutrient uptake, Hyd= hydrologic characteristics, CPOM= coarse particulate organic matter retention

* Up = upstream reach, Thr = through culvert, Dwn = downstream reach

2.2. Field and Lab Methods

2.2.1. Nutrient Uptake

Ammonium uptake velocities (V_f) were estimated upstream and downstream of each culvert using whole-stream nutrient injections (Stream Solute Workshop 1990). Nutrient uptake was evaluated because it is a good metric for nutrient utilization on the streambed and it integrates many biological factors such as primary production and respiration. Therefore any changes in V_f due to restoration may be an indication of changes in controls on nutrient uptake. The upstream and downstream injections at both streams, of each pair, were conducted under similar discharge conditions in June 2013.

Nutrient uptake was measured using standard nutrient spiraling techniques (Stream Solute Workshop 1990, Webster and Valett 2006). A solution of Rhodamine WT (conservative tracer), NH_4Cl and stream water was continuously released at the top of the reach using a fluid metering pump. The pump dripped the solution at ~ 100 mL/min and the concentration in the solution was adjusted accordingly with discharge so that concentrations of $\text{NH}_4\text{-N}$ (ammonium) in the stream were elevated by ~ 10 $\mu\text{g/L}$ above the background concentration in the stream. Reach lengths were adjusted to achieve ~ 45 minutes of travel time when possible (50 - 400 m depending on discharge). Prior to initiating the nutrient release, background water samples were collected from seven sampling stations downstream of the pump and analyzed for background concentrations of $\text{NH}_4\text{-N}$. Water was sampled again at all sampling stations once the conservative tracer

concentrations reached a plateau (no change in rhodamine WT concentrations over ~5 minutes) at the furthest downstream station. Rhodamine WT concentrations were analyzed using a Turner Aquafluor handheld fluorometer. NH₄-N concentrations were determined following the approach of Holmes et al. (1999), as modified by Taylor et al. (2007) using the light sensitive OPA (orthophthaldialdehyde) method. Ammonium and Rhodamine WT samples were processed within 6 hours of collection in the field.

To determine nutrient uptake lengths, the background NH₄-N concentration was subtracted from the plateau concentration and normalized based on rhodamine WT concentrations to account for dilution. Normalized NH₄-N concentrations were then regressed against distance from the injection location; the slope of this regression line (which should be negative) is the overall uptake coefficient (k_c) which is used to calculate uptake length (S_w) (1) and uptake velocity (V_f) (2) using the following equations (Stream Solute Workshop 1990):

$$(1) \quad S_w = 1/k_c$$

$$(2) \quad V_f = \frac{u \cdot h}{S_w}$$

In equation (2), u is average water velocity, h is average depth (m) and S_w is the average distance (m) traveled by a solute before it is removed from solution. V_f (mm/sec) is an estimate of the velocity at which a nutrient atom travels to immobilization in the stream (Stream Solute Workshop 1990, Webster and Valett 2006). Because uptake

velocity incorporates depth and velocity, it accounts for differences in nutrient dynamics due to changes in stream discharge, making it the most useful nutrient uptake characteristic for comparing between streams of different sizes (Davis and Minshall 1999). Alpha values for nutrient uptake regressions were set at 0.15 (Bechtold et al. 2012). If regressions were not statistically significant, nutrient uptake was deemed undetectable and data from the regression was not included in further analysis.

2.2.2. Hydrologic Characteristics

Hydrologic characteristics were evaluated as average velocity and transient storage. Hydrologic characteristics in streams set the physical stage for biological and chemical processes, and hydrologic alterations from poorly designed culverts can affect aquatic organism passage. Transient storage, which is the temporary delay in downstream movement of solutes in areas outside of the main channel, is helpful in understanding flow paths through biologically or chemically active areas as transformation of nutrients can occur both in the main channel and in transient storage zones (Runkel 1998). All field measurements were completed in May/June 2013 for the culvert replacement comparison and in August 2013 for the additional sites in the culvert design comparison.

Hydrologic characteristics were estimated by conducting slug salt pulses and using the resulting conductivity curves to calculate reach-scale water velocity and to model transient storage characteristics using the One-Dimensional Transport with Inflow and Storage (OTIS) model and its modified automated parameter estimation version (OTIS-P) (Runkel 1998).

Salt pulses were completed by deploying one YSI 6920 V2 multiparameter sonde equipped with a 6560 conductivity/temperature probe ~20 meters downstream of the location of the salt release and a second similar sonde at the downstream edge of the reach. The length of the reach was determined to target a travel time of ~45 minutes. 1-1.5 kg of salt per 100 L/sec of discharge was dissolved in a bucket of stream water and this salt solution was then released while sondes recorded conductivity at five second and one minute intervals for the upstream and downstream sonde, respectively. The upstream sonde was given shorter intervals because the salt mass moves through in a shorter length of time as it has not dispersed yet at the upstream edge of the reach. Break-through conductivity curves, created by the moving salt mass, were used to calculate travel times based on the time between peak concentration of upstream and downstream conductivity probes. The sonde setting for wiper blades on conductivity probes caused some upstream conductivity curves to be incomplete with gaps in logging. To compensate for this, all upstream curves were simulated from the point of release by creating a five second (near instantaneous) elevated conductivity where the area of the simulated upstream curve matched the area of the logged downstream conductivity curve. Travel times were then used to calculate average water velocities throughout the reach as travel time divided by reach length.

The conductivity curves collected in reaches upstream and downstream of culverts were used to model transient storage characteristics using the following equations (Runkel 1998):

$$(3) \frac{\partial C}{\partial t} = -\frac{Q}{A} \frac{\partial C}{\partial x} + \frac{1}{A} \frac{\partial}{\partial x} \left(AD \frac{\partial C}{\partial x} \right) + \frac{q_{LIN}}{A} (C_L - C) + \alpha (C_s - C)$$

$$(4) \frac{dC_s}{dt} = \alpha \frac{A}{A_s} (C - C_s)$$

Where

A = main channel cross-sectional area (L^2)

A_s = storage zone cross-sectional area (L^2)

C = main channel solute concentration (M/L^3)

C_L = lateral inflow solute concentration (M/L^3)

C_s = storage zone solute concentration (M/L^3)

D = dispersion coefficient (L^2/T)

Q = volumetric flow rate (L^3/T)

Q_{LIN} = lateral inflow rate ($L^3/T*L$)

t = time (T)

x = distance (L)

α = storage zone exchange coefficient (T^{-1})

These equations take into account the physical processes that affect salt concentrations such as advection (downstream transport of a solute), dispersion (spreading of a solute mass via diffusion and velocity variations due to shear stress), and transient storage (Runkel 1998). For this study, we were particularly interested in the parameters D , A , A_s , α , A_s/A , T_{sto} (turnover time of storage zone), and R_h (hydraulic retention factor). These parameters help to describe how flow paths may change between stream reaches by describing the storage zone, the main channel and interactions between both. The transient storage model works by using an input upstream conductivity curve and applying parameters D , A , A_s , and α to develop a simulated output conductivity curve

that matches the observed downstream conductivity curve. First, D and A are adjusted manually using OTIS until the output conductivity curve is similar to the observed downstream conductivity curve. Then the final estimates of D and A from manual iterations are entered as initial estimates into OTIS-P. OTIS-P then finds the best estimates for parameters D , A , A_s , and α using least squares best fit non-linear regression between the simulated output curve and the observed downstream conductivity curve. To facilitate comparisons in transient storage between reaches, we also derived the ratio of storage zone cross section area to channel cross section area (A_s/A), storage zone residence time (T_{sto} , Thackston and Schnelle 1970) and hydraulic retention factor (R_h , Morrice et al. 1997). Both T_{sto} (s) (5) and R_h (s/m) (6) are metrics of the amount of time an average salt molecule is spends in storage and are determined as follows:

$$(5) \quad T_{sto} = \frac{A_s}{\alpha * A}$$

$$(6) \quad R_h = \frac{A_s}{Q}$$

Hypothesizing how each one of these metrics may potentially respond is difficult because of large natural variations in hydrology and because culverts can cause very different hydrologic problems (high velocities vs. pooling). The flow paths through some culverts, particularly non-replaced culverts, are likely to be less complicated than those in upstream or downstream reaches, with less transient storage and less dispersion because flow is confined to within the culvert structure. However, these differences within

culverts could not be examined because culvert lengths were too short to provide adequate separation between breakthrough curves required for the OTIS model.

2.2.3. Coarse Particulate Organic Matter Retention

CPOM retention was evaluated because it can be indicative of how long organic matter stays in a reach. Organic matter such as leaves and woody debris are key energy sources that support food webs in forested streams (Wallace et al. 1997). CPOM retention using leaf analogs was measured for the culvert replacement comparison in May/June 2013 and for the culvert design comparison in August 2013. CPOM releases were always taken moving from downstream to upstream. CPOM retention was measured by conducting short-term releases of equilateral triangles (sides ~4 cm long) made of computer paper as leaf analogs. A known number of triangles (100-200) were released at the top of a reach for each CPOM release; reach lengths were between 15 and 100 m (~ten times the wetted width beneath the release location) (Lamberti and Gregory 2006). A block seine was stretched across the stream at the bottom of the reach to catch the paper leaf analogs still in transport (Webster et al. 1994). The seine was deployed for about one hour after release or until paper transport ceased (Lamberti and Gregory 2006). The analogs that were captured in the seine at the end of the reach were counted. In addition, the reach was divided into 5-10 meter intervals and searched for retained triangles. The location of retained triangles and the object that retained them were recorded. An exponential decay function was then fit to the data points for each interval so that x values were the distance from the release location and corresponding y values were the number of particles still in transport at that distance. The exponential decay function is as follows (Lamberti and Gregory 2006):

$$(7) P_d = P_0 e^{-kd}$$

Where P_d is the number of particles still in transport at a given distance, P_0 is the number of particles released, d is the distance from release and k is the instantaneous retention rate. The number of triangles in transport at each interval (P_d) was estimated because not all retained triangles could be found within a reach. Therefore, the number of leaves retained at each interval was estimated proportionally based on the ratio of the number of leaves found at a given stream distance over the total number of triangles found. Generally, researchers compare between reaches and streams using instantaneous retention rates. However, discharge was different between sites and sometimes between reaches on the same stream if CPOM releases could not be completed on the same day due to weather or lack of daylight. Therefore, we normalized retention for discharge by calculating a “retention velocity” (V_r), following the same approach to calculate uptake velocity (V_f) for nutrients described in section 2.2.1. Similarly to V_f , V_r describes the speed at which leaf analogs move toward retention and is calculated as:

$$(8) V_r = \frac{u \cdot h}{1/k}$$

2.3. Statistical Analyses

For both the culvert replacement comparison and the culvert design comparison, means of all variables were analyzed using two-way analysis of variance (ANOVA) with reach (upstream, through, and downstream) and culvert type (SSD, BBD, and non-replaced) as fixed factors. Stream was included as a random factor to control for

dependence between process measurements completed on the same stream. For the culvert replacement comparison, V_f , average water velocity, the transient storage parameters (D , A , A_s , α), derived transient storage metrics (T_{sto} , R_h , A_s/A), and V_r were compared for upstream, through and downstream reaches using two-way ANOVAs. For the culvert design comparison, only average water velocity and V_r were compared between upstream and through reaches. Alpha values were set at 0.05 for all two-way ANOVAs. All statistics were completed using R statistical software (R Core Team 2013).

3. Results

3.1. Culvert Replacement Comparison

3.1.1. Nutrient uptake

We hypothesized that nutrient uptake would be similar upstream and downstream of both SSD culverts and to BBD culverts because both are designed to create consistent hydrologic conditions through culverts. Across all streams and study dates, V_f ranged from 0.027 to 0.187 mm/sec (Table. 2.3). Mean V_f increased 55.7% from upstream to downstream reaches for SSD culverts and increased 1.9% for BBD culverts (Figure 2.2). However, these differences were not statistically different either between culvert types or between upstream and downstream study reaches (Table 2.4).

Table 2.3: Nutrient uptake results measured as part of the culvert replacement comparison. SSD= stream simulation design, BBD= bankfull and backwater design, ND = no data because plateau concentrations were not elevated enough above background to achieve a decreasing relationship between ammonium concentration and distance from release.

Stream	Culvert Type	Reach	Discharge	Width	Depth	Bkgd NH ₄ Conc.	Enrichment Factor	S _w	V _f	p-value
			L/sec	m	m	µg N/L	plateau/bkgd	m	mm/sec	
Preemption	SSD	Downstream	72.9	2.0	0.19	13.2	1.6	207	0.19	0.09
Preemption	SSD	Upstream	54.0	1.7	0.18	22.1	1.3	283	0.12	0.41
Whiskey	BBD	Downstream	14.9	1.4	0.09	19.5	1.4	101	0.10	0.11
Whiskey	BBD	Upstream	12.3	1.4	0.09	23.3	1.3	781	0.01	0.60
'opple	SSD	Downstream	255.9	5.0	0.16	10.5	2.1	838	0.06	0.10
'opple	SSD	Upstream	228.5	5.0	0.15	12.8	2.2	342	0.12	0.01
'opple	BBD	Downstream	196.8	4.8	0.19	12.1	2.0	689	0.06	0.07
'opple	BBD	Upstream	196.8	4.8	0.19	8.0	2.6	459	0.09	0.06
Joseph's	SSD	Downstream	3.0	1.7	0.05	14.4	1.0	ND	ND	ND
Joseph's	SSD	Upstream	3.0	1.7	0.05	43.0	1.1	47	0.03	0.02
John's	BBD	Downstream	5.7	2.0	0.10	16.0	1.4	93	0.03	0.05
John's	BBD	Upstream	4.1	1.2	0.06	19.8	1.2	130	0.03	0.08

3.1.2. Hydrologic Characteristics

We hypothesized that hydrologic characteristics (transient storage, water velocity) would be similar upstream of and downstream of both SSD and BBD culverts, but that water velocity within SSD culverts would be more similar to upstream and downstream conditions than those within BBD culverts. The mean water velocity increased 51.8 % through SSD culverts compared to downstream reaches and increased 18.6 % in downstream reaches compared to upstream reaches (Fig. 2.2). Mean water velocity decreased 16.7% through BBD culverts compared to upstream reaches but increased 15.4% from upstream to downstream reaches. However, no statistically significant differences were found for water velocity among reaches and between SSD and BBD culverts designs using a two-way ANOVA (Table 2.4).

Using OTIS modeling, we determined estimates of D , A , A_s and α and derived estimates of T_{sto} , R_h , A_s/A (Table 2.5) Only D (dispersion) was determined to be statistically different between reaches and culvert types ($p = 0.034$) with an observed increase of 48% from upstream to downstream of SSD culverts and a decrease of 78% from upstream to downstream of BBD (Table 2.6).

3.1.3. Coarse Particulate Organic Matter Retention

We predicted CPOM retention would be similar upstream and downstream of both SSD and BBD culverts, but would be more similar to upstream and downstream conditions within SSD culverts than within BBD culverts. Mean V_r decreased 73% through SSD culverts compared to upstream reaches and increased 96% from through reaches to downstream reaches. For BBD culverts, mean V_r decreased 88% through culverts compared to upstream and increased 1,286% from through reaches to downstream reaches (Fig. 2.2). The interaction effect between reach and culvert design was significant using a two-way ANOVA (Table 2.4). Although patterns for SSD and BBD culverts were similar, BBD culverts exhibited greater decreases in retention through culverts compared to their respective upstream and downstream reaches.

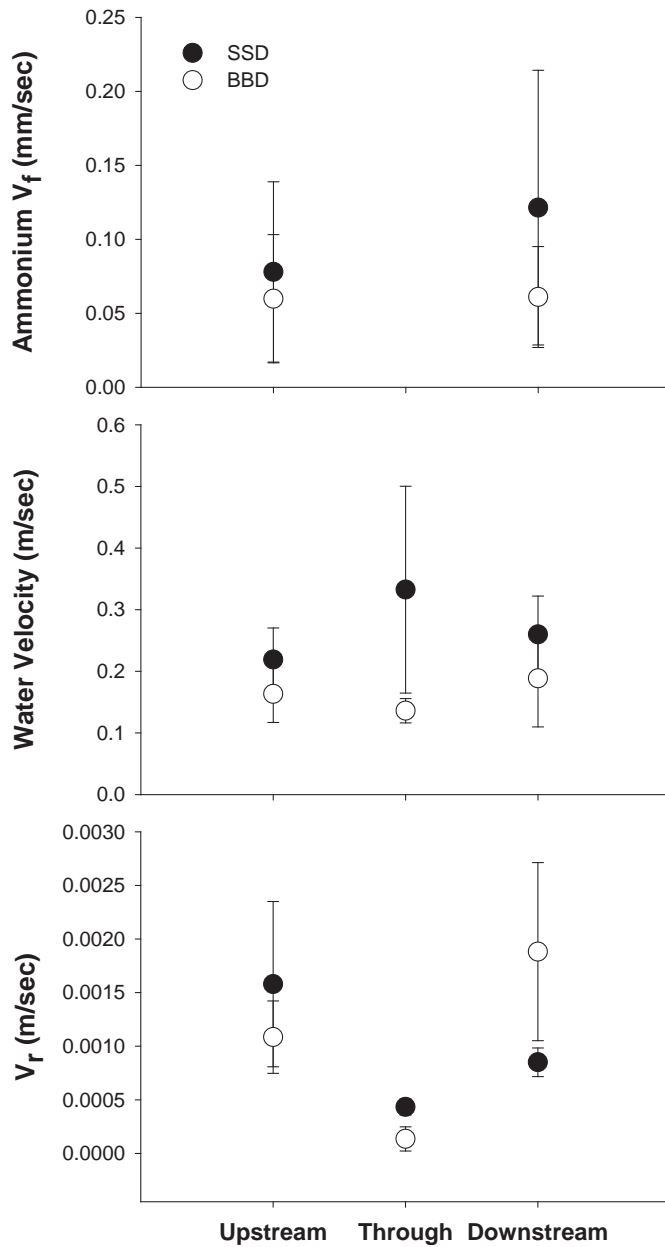


Figure 2.2: Ammonium uptake velocity (V_f) (top), water velocity (middle), and retention velocity (V_r) (bottom) for the culvert replacement comparison. SSD = stream simulation design, BBD= bankfull and backwater design. Error bars ± 1 SE, $n = 2$ for nutrient uptake velocity means for SSD upstream, SSD downstream, BBD upstream, $n = 3$ for all other means).

Table 2.4: Two-way ANOVA results for $\text{NH}_4\text{-N}$ uptake, water velocity and CPOM retention measured for the culvert replacement comparison with culvert type (SSD, BBD) and reach (upstream, through, downstream) as fixed factors and stream as a random factor. All three metrics were log-transformed to meet ANOVA assumptions.

Metric	Culvert Type		Reach		Culvert Type*Reach	
	F_{df}	p	F_{df}	p	F_{df}	p
$\text{NH}_4\text{-N}$ Uptake						
Velocity (V_f)	0.18 _{1,4}	0.70	6.80 _{1,1}	0.23	2.11 _{1,1}	0.38
Mean Water Velocity	4.67 _{1,4}	0.10	0.46 _{2,8}	0.65	1.78 _{2,8}	0.23
CPOM Retention Velocity (V_r)	1.02 _{1,4}	0.37	47.33 _{2,8}	<0.001	13.18 _{2,8}	0.003

Table 2.5: OTIS modeling results measured as part of the culvert replacement comparison.
*SSD = stream simulation design, BBD = bankfull and backwater design

Stream	Culvert*	Reach	Q	Dispersion Coefficient	Storage Zone Cross Section Area	Storage Exchange Coeff	Ratio of transient storage to surface area	Turnover time of storage	Hydraulic Retention factor
			(L/sec)	($D, m^2/sec$)	(A_s, m^2)	(α, sec^{-1})	A_s/A	(T_{sto}, sec)	($R_h, sec/m$)
John's	BBD	Up	65.4	0.295	0.024	0.00039	0.07	186	0.374
John's	BBD	Dwn	82.9	0.099	0.077	0.00411	0.21	52	0.932
Joseph's	SSD	Up	73.0	0.358	0.037	0.00055	0.09	180	0.500
Joseph's	SSD	Dwn	73.0	0.400	0.029	0.00131	0.11	90	0.395
Popple	SSD	Up	447.3	0.345	0.319	0.00159	0.20	127	0.713
Popple	SSD	Dwn	611.8	0.633	0.520	0.00184	0.28	157	0.851
Popple	BBD	Up	392.4	1.044	0.232	0.00007	0.10	1481	0.590
Popple	BBD	Dwn	392.4	0.159	0.344	0.00217	0.22	105	0.877
Preemption	SSD	Up	61.4	0.186	0.050	0.00143	0.15	110	0.815
Preemption	SSD	Dwn	73.0	0.280	0.035	0.00029	0.09	310	0.474
Whiskey	BBD	Up	34.1	0.061	0.063	0.00240	0.22	93	1.840
Whiskey	BBD	Dwn	34.1	0.044	0.081	0.00271	0.28	104	2.380

Table 2.6: Effect sizes (mean % change) and two-way ANOVA results for transient storage metrics from upstream to downstream reaches for both replacement designs (up = upstream, dwn = downstream)

Transient Storage Metric	Stream Simulation Design	Bankfull and Backwater Design	Two-way ANOVA	
	Mean % change up to dwn	Mean % change up to dwn	F _{df}	P-value
D	47.69	-78.44	10.00 _{1,4}	0.034
A_s	44.07	57.67	2.76 _{1,4}	0.172
α	-3.34	215.02	3.69 _{1,4}	0.127
A_s/A	8.53	81.90	3.66 _{1,4}	0.128
T_{sto}	33.32	-85.17	2.38 _{1,4}	0.198
Rh	-15.22	49.37	6.29 _{1,4}	0.066

3.2. Culvert Design Comparison

3.2.1 Hydrologic Characteristics

For the culvert design comparison, we expected that water velocities would be most similar between above and through reaches for SSD culverts, less similar for BBD culverts and least similar for non-replaced culverts. We found that stream water velocity decreased 33.8 % through BBD culverts compared to upstream reaches, but increased 16.7% and 66.1% through non-replaced and SSD culverts, respectively (Figure 2.3). The interaction effect for reach and culvert design was statistically significant (Table 2.7). However, a mean water velocity trend based on a gradient of restoration was not apparent, with velocities being most similar between upstream and through reaches for non-replaced culverts and least similar for SSD culverts.

3.2.2 Coarse Particulate Organic Matter Retention

For the culvert design comparison, we expected that CPOM retention would be most similar upstream and through SSD culverts, less similar for BBD culverts and least similar for non-replaced culverts. The mean retention velocity decreased through all culverts, with 74.0% through SSD culverts, 98.1 % through BBD culverts, and 99.5 % through non-replaced culverts compared to upstream reaches (Figure 2.3). The interaction effect between reach and culvert design was significant using a two-way ANOVA (Table 2.7). For upstream reaches, wood and rocks were the objects that most commonly

retained CPOM analogs, with averages of 24.6% and 7.6% of released triangles found on each object type, respectively (Table 2.8).

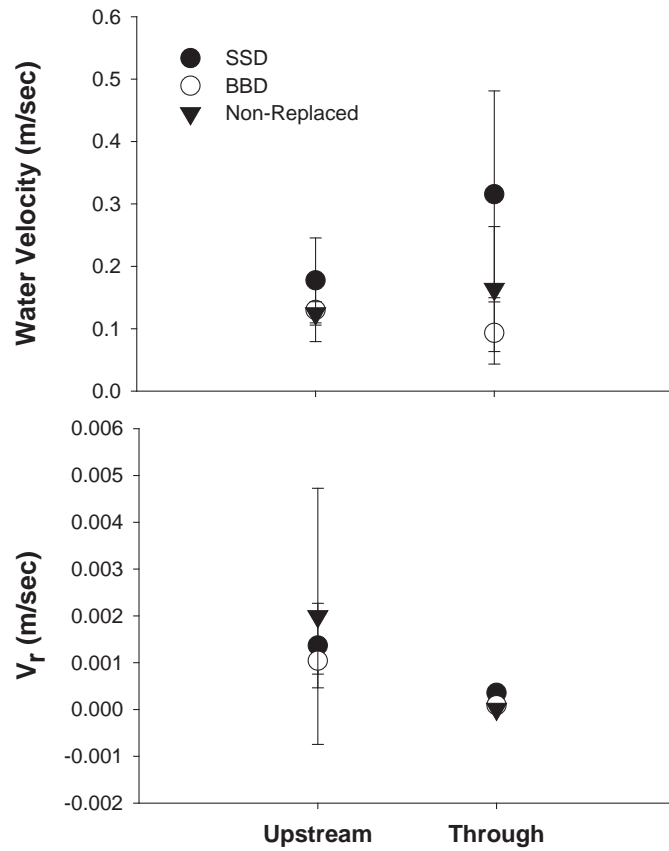


Figure 2.3: Water velocity (top) and retention velocity (V_r) (bottom) measured for the culvert design comparison. SSD = stream simulation design, BBD = bankfull and backwater design. ($n = 5$ for SSD, $n = 6$ for BBD, $n = 4$ for non-replaced, error bars ± 1 SE).

Table 2.7: Two-way ANOVA results for water velocity and CPOM retention velocity measured for the culvert design comparison with culvert type (SSD, BBD, non-replaced) and reach (upstream, through) as fixed factors and stream as a random factor. Both metrics were log-transformed to meet ANOVA assumptions.

Metric	Culvert Type		Reach		Culvert Type*Reach	
	F _{df}	p	F _{df}	p	F _{df}	p
Mean Water Velocity	4.67 _{1,4}	0.10	0.46 _{2,8}	0.65	1.78 _{2,8}	0.23
CPOM Retention Velocity	1.02 _{1,4}	0.37	47.33 _{2,8}	<0.001	13.18 _{2,8}	0.003

Table 2.8: Percent of leaf analogs found retained by each object by culvert type and reach.

Culvert Type*	Reach	Rocks	Roots	Backwater	Bank	Wood	Debris Dam
NR	Upstream	2.0	0.5	3.0	3.0	31.3	3.0
NR	Through	3.8	0.0	0.3	0.0	0.0	0.0
BBD	Upstream	8.8	0.7	2.7	7.3	25.8	0.2
BBD	Through	0.0	0.8	0.8	2.3	0.0	0.0
SSD	Upstream	15.2	3.8	4.6	4.8	22.2	2.6
SSD	Through	7.0	0.0	1.0	1.6	4.4	0.0

*NR= non-replaced, BBD=bankfull and backwater design, SSD= stream simulation design

4. Discussion

Both culvert replacement and habitat improvement projects are common stream restoration activities in the United States (Roni et al. 2008). SSD culverts combine both strategies by trying to improve longitudinal stream connectivity at a road crossing and restoring natural substrates and stream dimensions through culverts (SSWG 2008). Our results suggest that the ecosystem processes we measured (nutrient uptake, hydrologic characteristics, CPOM retention) are similar upstream and downstream of both styles of culvert replacements (SSD and BBD), probably because they are both designed to improve hydrologic conditions that not only improve fish passage but also lead to consistent hydrologic conditions in upstream and downstream study reaches. Moreover, rebuilding the streambed, as is done in SSD culverts, may support more natural ecosystem processes (CPOM retention) within the short reaches within culverts compared to BBD and non-replaced culverts.

Ecosystem processes upstream and downstream of replaced culverts

The main motivation for culvert replacements is that improperly designed culverts can act as semi-permeable or complete barriers to aquatic organism movement (Warren and Pardrew 1998, Bouska and Paukert 2010) and that sedimentation and erosion can exceed natural levels due to hydrologic changes through culverts (Lachance et al. 2008, Wellman et al. 2000, Bouska et al. 2010). Although not directly measured in this study, the design of both the BBD and SSD culverts should alleviate these problems. We did not observe any extreme flow conditions, excessive sediment, pooling or perching around

any of the replaced culverts. Others have found that stream simulation culverts are better at passing high flows associated with culvert failures and ecological consequences such as sedimentation and erosion (Gillespie et al. 2014). Our ecosystem process measurements similarly suggested that conditions were similar upstream and downstream of culverts, probably due to consistent hydrologic conditions allowed by both styles of culvert replacements. All of our ecosystem process measurements upstream and downstream of culverts were within the range of natural variability reported in the literature. For instance, our estimates of $\text{NH}_4\text{-N } V_f$ are within the range of those reported throughout the Great Lakes region (Tank et al. 2008) and near northern Wisconsin (Hoellein et al. 2012). Also, our results for transient storage parameter estimates lie within the range of other studies that have used the OTIS model (Webster et al. 2003, Wondzell 2006, Runkel 2002, Becker et al. 2013), including the high variability in D found in Table 2.5. D was the only metric found to have statistically significant differences between upstream and downstream reaches for any of the transient storage metrics. We believe there is not compelling evidence that hydrologic characteristics were changed in a consistent manner from upstream to downstream for either SSD or BBD culverts. Therefore, we do not see any indication of either culvert replacement negatively affecting ecosystem process upstream and downstream of culverts.

An alternative possibility is that the ecosystem processes we measured upstream and downstream of culverts are not sensitive to culvert effects. Others have found that nutrient uptake and transient storage may or may not be sensitive to stream restoration. For example, Hoellein et al. (2012) measured $\text{NH}_4\text{-N}$ uptake on reaches that were

restored for fish spawning habitat by increasing substrate size with the addition of gravel and boulders and building upstream sediment traps. They found no significant differences in V_f between restored reaches and unrestored reaches. However, there is some evidence that other manipulations of physical complexity such as adding coarse woody debris to streams can increase $\text{NH}_4\text{-N } V_f$ (Roberts et al. 2007). Studies evaluating transient storage characteristics after restorations of physical complexity, similar to SSD culverts, have also reported mixed results. Becker et al. (2013) evaluated the effects of natural channel design restoration, which involved creating physical structures with boulders in stream channels (not a culvert study), and found restored reaches had 33% larger transient storage zones compared to unrestored reaches and a slight decrease in residence time in transient storage zones, yet these differences were not significant. In contrast, Bukaveckas (2007) found that reaches restored from channelization by decreasing bankfull capacity and creating channel meanders, pools and riffles had higher transient storage (A_s/A) compared to channelized reaches. Additionally, Hoellein et al. (2012) found that restored reaches with larger substrate sizes had increased transient storage (A_s/A).

Ecosystem processes within replaced and non-replaced culverts

Of the measurements for our three ecosystem processes, only CPOM retention and water velocity could be measured within culverts in this study. We found that both of these metrics had significant differences among culverts designs and between reaches upstream of and through culverts. Contrary to our hypothesis, we found that non-replaced

culverts were most similar in water velocity between upstream and downstream reaches and that water velocity increased through SSD culverts. This may be because the SSD culverts included in our study were rebuilt to mimic conditions found in riffles, which have naturally faster flow than average water velocities across longer stream reaches that include both pools and riffles. We found that CPOM retention velocity decreased drastically through all three culvert types relative to upstream reaches. However, the decrease in retention velocity was least through SSD culverts and most through non-replaced culverts, likely due to the reconstructed streambed in SSD culverts that retained leaf analogs on rocks and on woody debris, which was also retained in the culvert (Table 2.8). The larger percentage of retention on rock substrates in non-replaced culverts compared to upstream reaches was driven by a few rocks at the exit of one culvert. All other non-replaced culverts had nearly zero retention through and therefore the average retention velocity in non-replaced culverts was the lowest of the three designs.

Rosi-Marshall et al. (2006) also showed that a restoration project where wood structures were added to streams to create pool habitats also increased CPOM retention. Furthermore, Lepori et al. (2005) found that streams restored from channelization by widening channels and adding large boulders had increased CPOM retentiveness channelized reaches. An increase in CPOM retention could influence the distribution of CPOM standing stocks (more within culverts) and subsequently distribution of invertebrates dependent on CPOM as a food source. Other research in these same streams has identified that invertebrate communities within culverts are more diverse in SSD

compared to BBD culverts (S. Eggert, USFS, unpublished), which could be related to CPOM retention and availability.

Although we were unable to measure transient storage within the culverts in this study due to their short length, our visual observations of flow within culverts suggests that there may also be differences between the SSD and BBD designs and non-replaced culverts if methodology existed to measure transient storage at this scale. For instance, non-replaced culverts in particular constrain flow and lack the streambed structure that could increase dispersion and create transient storage. Moreover, SSD and BBD culverts are sometimes constructed with bottomless con-span or bridge structures, while others are constructed with full corrugated or box-type culvert structures, which should limit the potential for surface-subsurface exchange and transient storage relative to bottomless structures.

Limitations and broader implications

It must be noted that culverts vary widely in terms of sizes and designs, even within our groups of SSD, BBD and non-replaced culverts (Table 2.2). Low replication limited our ability to accurately represent variation within groups, particularly non-replaced culverts, where we sampled only two small concrete and two large corrugated culverts. Furthermore, culverts with hydrologic issues such as severe ponding at the inlet or outlet had to be excluded from this study because the three ecosystem processes we measured require predominant water movement downstream through advection, which is not the case in nearly lentic conditions. Measurements of ecosystem processes not

requiring flowing water such as sedimentation or metabolism and nutrient uptake in benthic chambers would be viable options to evaluate process differences across the full breadth of culvert variability.

Varying degrees of success of stream restoration activities for improving ecosystem processes could be due to temporal or spatial scale mismatches between restoration activities and monitoring techniques. Other studies of ecosystem process responses to stream restoration activity have reported limited changes in restored vs. unrestored reaches, even when multiple processes are evaluated as in this study or others (e.g., Hoellein et al. 2012). Lack of changes between restored and unrestored streams could be because measures of ecosystem structure or process vary in responsiveness to particular environmental changes or restoration strategies, suggesting that careful attention needs to be paid to select the best ecosystem processes for monitoring based on site-specific and restoration-specific characteristics (Hopkins et al. 2011, Hoellein et al. 2012). For example, Hopkins et al. (2011) found that ecosystem metabolism was most responsive to nutrient concentrations while macroinvertebrate assemblages were more responsive to hydrologic conditions in a western river, suggesting that restoration activities to restore hydrologic regimes in this river could be best served by focusing on macroinvertebrate community responses. Alternatively, monitoring may occur too soon after restoration activities to allow the ecosystem to recover from the degradation (Palmer et al. 2010). However, Louhi et al. (2011) provided evidence to the contrary, suggesting that nearly 20 years of monitoring streams restored for habitat heterogeneity in Finland,

via addition of boulders and large woody debris, was not enough to see a response in macroinvertebrate diversity.

Lastly, depending on which ecosystem structures or processes are measured, restorations may be too small spatially to detect a change in ecosystem functions (Palmer 2009). As we found in our current study; CPOM retention was significantly different depending on culvert design, possibly because it was an ecosystem process that could be measured at the scale of the restorations (through culverts).

To evaluate the value of SSD culverts we follow the criteria from Palmer et al. (2005) based on both ecological and stakeholder success. In addition to the probable conclusion of improving fish movement and hydrologic conditions compared to improperly designed culverts, we found the ecosystem processes that we measured were similar upstream and downstream of both SSD and BBD culverts, while SSD culverts moved CPOM retention within the culvert closer to natural rates compared to BBD and non-replaced culverts. Furthermore, these culverts may be viewed as a stakeholder success because of the economic benefit of longer life expectancies without costly repairs (Gillespie et al. 2014) and a perception that they are more aesthetically pleasing (Verbal communication with adjacent land-owners, J.M. Kraemer). An important question is whether the additional cost of rebuilding the streambed in the SSD design is worth the improvement in ecosystem processes we observed in these short stream reaches. If restoration activities are aimed at maintaining ecosystem processes in reaches upstream and downstream of culverts, then the BBD design, where bankfull conditions are restored

but the streambed is not rebuilt, may be adequate. On the other hand, if maintaining ecological conditions through the culvert is an important restoration goal, then the investment in the SSD can lead to improvement in ecosystem processes like organic matter retention.

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Chapter 3: Spatial Heterogeneity of Biofilm Biomass and Physical Characteristics in Streams²

Abstract

The concepts in landscape ecology have been applied across many scales in ecosystems, yet little is known about microhabitat to segment-scale spatial patterns in streams. Our objective was to quantify the spatial heterogeneity of biofilm standing crop and the physical factors that might drive this pattern. We hypothesized that physical characteristics (depth, velocity, sediment size) and biofilm standing crop (chlorophyll *a* and ash-free dry mass or AFDM) would show spatial dependence (spatial correlation among sampling points) when sampling points were close in space and that spatial structure of physical metrics would resemble the spatial structure of biofilm metrics. We chose three streams with varying substrate size heterogeneity to explore how spatial patterns differ for physical and biofilm metrics at 1 meter intervals along 200 meter longitudinal transects. We investigated spatial dependence by building semivariograms for each variable. Only depth, velocity and chlorophyll *a* exhibited spatial dependence, while substrate size and AFDM appeared to be spatially independent at all scales within our reaches. Our results suggest that all metrics reach spatial independence at about the

² The material in this chapter is planned for submission.

macrohabitat scale in our study streams and that is likely due to controls on each metric operating at scales beneath or above our sampling scales.

1. Introduction

The investigation of spatial heterogeneity, or spatial variability of biotic and abiotic metrics, has had a profound influence on both our understanding of lotic ecosystems as well as our attempts to restore them. The most common stream restoration projects attempt to increase habitat heterogeneity (Roni et al. 2008) and the motivation behind these projects stems from theory and research in landscape ecology and patch dynamics (Pickett and White 1985). Streams may be conceptualized as a mosaic of units called patches and the number and size of patches may influence ecosystem structure and processes (Naiman et al. 1988, Pringle et al. 1988, Townsend 1989). Research into spatial heterogeneity has contributed to our knowledge across ecological levels including populations, communities and ecosystems (Turner et al. 2001, Pickett and Cadenasso 1995) but further investigations into spatial heterogeneity at many scales will allow us to better understand the important role it plays in lotic ecosystems.

Fluvial ecosystems are naturally hierarchical, which makes them an ideal place to study variance at different scales (Palmer et al. 1997), and spatial patterns across these scales can be both a cause and effect of ecological phenomena. Microhabitats (10^{-1} m) fit into macrohabitats (pools/riffles, 10^0 m), macrohabitats into reaches ($10^1 - 10^3$ m), reaches into segments ($10^3 - 10^5$ m) and segments into stream watersheds ($10^5 - 10^6$ m) (Frissell et al. 1986, Fausch et al. 2002). The physical structure at each of these scales often influences the structure and process of biota. For instance, fine-scale, microhabitat, hydraulic characteristics control macroinvertebrate assemblages within riffles (Brooks et

al. 2005). Surface-subsurface water exchanges and nutrient uptake rates can control the spatial heterogeneity of nutrient concentrations (Dent and Grimm 1999), with positive implications for algal growth (Dent et al. 2001). At larger scales, variability in habitat characteristics controls the distribution and abundance of fish species in river networks (Armstrong et al. 2013, Torgersen et al. 1999, Torgersen and Close 2004). Conversely, biota can create spatial heterogeneity. Grazing by snails can influence the spatial heterogeneity of algal biomass (Sarnelle et al. 1993) and fish grazing intensity can influence the spatial heterogeneity of epilithic biofilms (Flecker and Taylor 2004). Therefore, spatial heterogeneity can be a product of, as well as a driver of, ecological patterns and processes.

A wealth of research has confirmed that spatial heterogeneity has a role in the structure and process of ecosystems but the relevant scales of heterogeneity for individuals, populations, communities, and ecosystems are still poorly understood. In all ecosystems, changing aspects of scale in a study, such as grain (spatial resolution) or extent (spatial area), can change what patterns or processes we observe (Turner 2001, Wiens and Milne 1989). It is critical to understand that biota may respond to or be controlled by ecological phenomena only at particular scales (Wiens 1989). For example, beetle movements were found to be best explained by heterogeneity of vegetation at a very small grain size corresponding to the scale of their perception (Wiens and Milne 1989). Ecologists and other environmental scientists tend to ask questions at the scales that they perceive ecosystems, which means that potentially relevant spatial scales are overlooked (Fausch et al. 2002). Additionally, factors operating at more than one scale

may control ecological phenomena. For instance, Mykra et al. (2004) found that variation in ecoregion and stream size explained variation of macroinvertebrate assemblage metrics while the intermediate-scale variability in drainage basin, had little effect.

Macroinvertebrates are also known to respond to variability at the scale of microhabitats (Brooks et al. 2005). Therefore, macroinvertebrate communities are influenced by small-scale and large-scale heterogeneity, but not all scales in between. The combination of poor understanding of scales beyond our perception and the fact that multiple scales may be important, leaves ample opportunity to study relationships among spatial heterogeneity and many aspects of ecosystem structure and function.

Little is known about the spatial heterogeneity of epilithic biofilms, which are a basal energy resource for stream food webs (Allan and Castillo 2007). In one of the most comprehensive studies to date, Downes et al. (1993) implemented a nested sampling design to investigate the variability of the algal community and algal standing crop. They used a nested hierarchical sampling design for chlorophyll *a* (proxy for algal biomass) by sampling among rocks, microhabitats (groups of rocks within riffles), and macrohabitats (riffles) (Downes et al. 1993). Chlorophyll *a* varied significantly between macro- and microhabitats but not among sites (stream sections on the same 1.5 kilometers of a 4th order stream) or among rocks in the same microhabitat (Downes et al. 1993). In fact, variation in chlorophyll *a* among scrapes on the same rocks was similar to the variation among rocks (Downes et al. 1993). One limitation of this study was that it emphasized scales of observation determined a priori by the researchers and therefore may have missed variability that existed between scales of measurement. Additionally, the study

does not address what could be driving the observed variability, such as the spatial heterogeneity of physical controls on biofilms and the scales at which they operate. For instance, variation between macrohabitats and microhabitats could be due to physical controls on biofilm biomass such as substrate size or velocity playing a role in variability at small scales (Cardinale et al. 2002, Myers et al. 2007).

1.1. Objective & Hypotheses

Our objective was to quantify the spatial pattern of biofilm standing crop and possible physical drivers in streams at the macrohabitat to reach scales (10^0 - 10^3 m). We expected that sampling points would be spatially dependent such that points close in space would be more similar than points farther apart. Yet, we also expected that at large distances (>100 m between points), spatial correlation would be minimal (spatial independence). Therefore, our first hypothesis was that physical characteristics (depth, velocity, substrate size) and biotic characteristics (chlorophyll *a*, AFDM) would show both spatial dependence and spatial independence at these scales.

Substrate size has been thought to influence biofilm biomass because larger substrates are more resistant to scour and therefore have more stable biofilms with more biomass (Myers et al. 2007). Additionally, high (sub-scour) water velocities may enhance nutrient delivery to biofilms, influencing growth (Cardinale et al. 2002). Water depth (generally inversely related to velocity) may influence light availability, which controls algal growth. Therefore, our second hypothesis was that the spatial pattern of sediment

size, water velocity, and depth would be similar to the spatial pattern of chlorophyll *a* and AFDM.

To test these hypotheses, we sampled physical and biofilm metrics in longitudinal transects in three streams. The streams were chosen based on expected streambed heterogeneity with one low heterogeneity stream (predominately sand) and two high heterogeneity streams (predominately gravel/cobble). This allowed us to explore spatial patterns and relationships between physical and biofilm characteristics across a range of physical conditions using semivariogram analysis.

2. Methods

2.1. Study Sites and Design

All three study streams were tributaries of Lake Superior, located on the Keweenaw Peninsula of the Upper Peninsula of Michigan in predominately northern mesic forests with maple (*Acer saccharum*), and yellow birch (*Betula alleghaniensis*). Discharges in streams of this region are generally highest during snowmelt and decrease to baseflow in late summer. Black Creek was the smallest by discharge but had similar width and depth as the other two streams (Table 3.1).

Table 3.1: Physical characteristics of study streams.

Site	Q	Q Transect Width	Mean Thalweg Depth	Mean Thalweg Velocity	Mean Thalweg Substrate Size	NH ₄ Conc
	L/s	m	m	m/sec	cm	µg/L
Hills	270	5.4	0.3	0.46	5.62	3.27
Cole's	215	3.8	0.25	0.50	7.06	5.67
Black	30	3.5	0.45	0.11	0.27	ND

Q = Discharge, ND = no data

Physical and biological characteristics were sampled in one meter increments along a longitudinal transect in the thalweg (area of main flow) of each stream. Additionally, every 30 meters a lateral transect of one meter increments was completed to compare between lateral and longitudinal variation. Together, longitudinal and lateral transects resulted in ~230 sampling points on each stream. Increments of 1 meter were chosen because Downes et al. (1993) showed as much variability in chlorophyll *a* among scrapes on single stones as they did among stones that were 0.5 m apart. If sampling grain size was reduced to 0.5 m or below, we would not expect to obtain any more variability. Therefore, we opted to go with sampling increments of 1 meter to maximize reach length within our sampling efforts and explore variability from the microhabitat to segment scales.

2.2. Physical Metrics

At every sampling point, depth was measured using a discharge staff and velocity was recorded using a Marsh-McBirney Flo-mate flow meter placed at 0.6 x depth. The first substrate encountered adjacent to the discharge staff at each sampling point was

collected and placed in a cooler for later analysis of substrate size, chlorophyll *a*, and AFDM. If the substrate at a sampling station was smaller than gravel, then the sand was collected by submerging a plastic tube with an area of 5.31 cm² to a depth of 1 cm. Beta intermediate axis length (length perpendicular to a substrate's longest axis; Gordon et al. 2004) was recorded for all substrates and used as the metric for substrate size. Sand samples were given a default beta intermediate axis of 0.0625 cm, which was the best estimate of substrate size as determined by sieving. If sand samples were not homogeneous (e.g., contained pebbles and sand grains larger and smaller than 0.0625 cm) we sieved samples using 4.75, 3.327, 2.0, 1.0, 0.25, and 0.125 mm sieves. We then weighed each sieved class and the median size class (by weight) was recorded as the substrate size for that sample.

2.3. Biofilm Standing Crop

We sampled chlorophyll *a* as a proxy for autotrophic standing crop and AFDM as an estimate of total biofilm standing crop, including both living and dead heterotrophic and autotrophic microorganisms using standard methods (APHA 2005). Biofilms were scrubbed from individual rocks and filtered using 0.7 micron GF/F filters, which were frozen until analysis. Chlorophyll *a* was extracted from filters and from entire sand samples in 95 % ethanol for 8-24 hours. Samples were then agitated and the extractant was poured into new falcon tubes, centrifuged, and analyzed for absorbance with a spectrophotometer at 664, 665 and 750 nm wavelengths. Then 0.1 mL of 0.1 N HCl was added to the sample cuvettes and analyzed again at the same wavelengths to determine

phaeophytin. Concentrations of chlorophyll *a* ($\mu\text{g}/\text{cm}^2$) were determined using the equation (Nusch 1980):

$$(1) \frac{29.6 * [(665b - 750b) - (665a - 750a)] * V1 * V3}{V2 * L * A}$$

Where:

V1 = volume of extract, mL

V2 = volume of sample, mL

V3 = volume of scrubate, mL

A = rock surface area, cm^2

L = light path length, cm

665*a*, 665*b*, 750*a* and 750*b* = absorbance at each wavelength before and after acidification

Following chlorophyll *a* analysis, the filters and sand samples were placed on labelled tins and dried overnight in a 60° C oven. Initial weights of both the filters/sand and tins were measured using a Sartorius ED2245 scale and then combusted in a muffle furnace at 550° C. Filters and sand samples were then rewetted with deionized water to rehydrate clays and dried again at 60° C. The filters/sand and tins were then weighed again and the difference between initial and final weights was reported in mg/cm^2 based on the surface area of each substrate. Surface areas for each chlorophyll *a* and AFDM sample was determined by tracing individual rocks onto paper, cutting out the paper, weighing the cutout and calculating the area of the cutout from a standard curve constructed with paper of known surface areas. The surface area for all sand samples was 5.31 cm^2 , determined by the plastic tube size.

2.4. Statistical Analyses

To investigate spatial heterogeneity of all of physical and biotic measurements, we created semivariograms, using the gstat and sp packages in R statistical software (Pebesma 2004, Pebesma and Bivand 2005, R Core Team 2013). Semivariograms are plots of semivariance against a continuous function of common lag distance.

Semivariance for each common lag distance (h) is calculated using the equation:

$$(2) \Sigma(x_i - x_{i+h})^2 / 2n$$

Where:

x = value measured

h = is the lag distance between any two sampling points

n = the number of pairs h distance apart

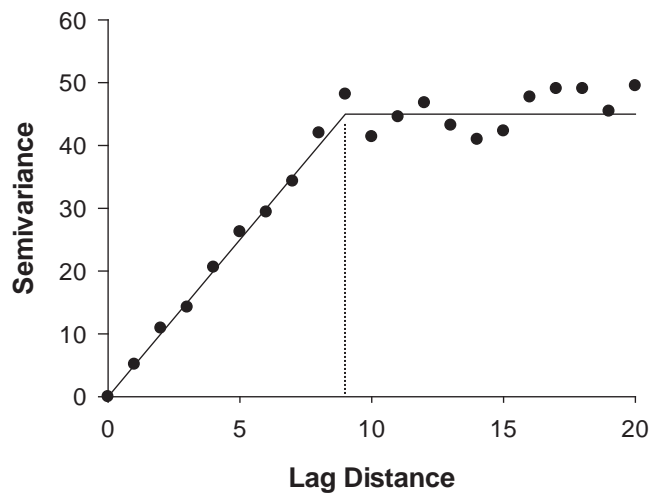


Figure 3.1: Theoretical semivariogram depicting an ascending limb rising from the y-intercept (nugget) to a sill at a semivariance of 45 and a range of 9 meters. Vertical line shows how the range is determined as the x value of the sill.

Semivariograms (Figure 3.1) are a valuable statistical method because hierarchical sampling designs investigate variability at discrete spatial scales while semivariograms investigate variability as a continuous function of distance (Cooper et al. 1997). Semivariances generally increase to an asymptote called the sill. The lag distance at the sill is also known as the range and marks the transition from spatial dependence to spatial independence (Cooper et al. 1997). The nugget, or y-intercept, of the semivariogram is the semivariance attributed to either sampling error or variability that exists at scales beneath one's sampling grain (Cooper et al. 1997). Additionally, the slope between the nugget and the sill represents the strength of spatial dependence so that a steeper slope means the variable is more spatially dependent (Cooper et al. 1997). All semivariograms were plotted to a lag distance of 150 meters to make sure a minimum of 30 sampling pairs were included at every lag distance (Cooper et al. 1997).

We hypothesized that a relationship among spatial patterns of physical and biofilm metrics would exist. Therefore, the shapes of semivariograms and values of nuggets, sills and ranges for biofilm standing crops and physical drivers would be similar within streams. Semivariance is dependent on the units for a given metric. If unit size is decreased, then semivariance will subsequently increase. In order to simplify comparisons among variables and streams, all semivariances were converted to standardized semivariance by dividing each semivariance value by the largest observed semivariance for each metric and stream (Rossi et al. 1992). This means that each semivariogram is given a y-axis with standardized semivariance from zero to one. The advantage of viewing semivariograms with standard semivariance is that comparison of

shapes between metrics and streams is easier because the y-axis is proportional and fit statistics such as residual sum of squares are now standardized between metrics. Spatial heterogeneity was evaluated by the range, or the distance at which spatial independence is reached (Cooper et al. 1997) or the size of a patch (Dent and Grimm 1999). However, range does not take into account how much variance increases as distance between points increases. Therefore, a linear slope from the nugget to the sill was also calculated to give the strength of spatial dependence (Cooper et al. 1997, Dent and Grimm 1999) and this metric was calculated with the original semivariance values (not standardized semivariance). This means that slopes of semivariograms should only be compared among streams for each metric and not among metrics because units for each metric are different. Degree of spatial heterogeneity can be described by both slopes and ranges so that a more heterogeneous stream reach would have steeper slopes and smaller ranges for each metric. We expected that the high heterogeneity streams should have steeper slopes for each metric compared to the slopes of the low heterogeneity stream.

3. Results

3.1 Longitudinal Patterns of Physical and Biofilm Metrics

All of the physical and biofilm standing crop metrics had relatively uniform variances and means within the longitudinal transect for each stream, although differed among streams (Figure 3.2). Depth and velocity generally varied inversely while the other three metrics appear to vary relatively autonomously. There were high outliers in

AFDM on both Hills and Cole's Creek, which are due to sand samples that generally had much higher AFDM. Additionally, substrate size for Black Creek (Figure 3.2) was low and relatively stable compared to the two other streams, which is due to the vast majority of substrate samples that were homogeneous sand. The velocity and depth curve for Black Creek appeared to change between 150 and 200 meters. Depth decreased to less than 0.5 meters for all sampling points greater than 150 meters. Water velocity remained below 0.2 m/sec until 150 meters, where it increased to above 0.5 m/sec.

The coefficients of variation show that longitudinal variation generally was greater than lateral variation for all streams (Figure 3.3). For Black Creek (low heterogeneity), every metric was more variable longitudinally than laterally. For Hills Creek, lateral variation was greater for both depth and substrate size than longitudinal variation, while Cole's Creeks had greater lateral variation for velocity and substrate size. However, meter increments may be too coarse for thorough assessment of lateral variability, limiting this evaluation of directional variability.

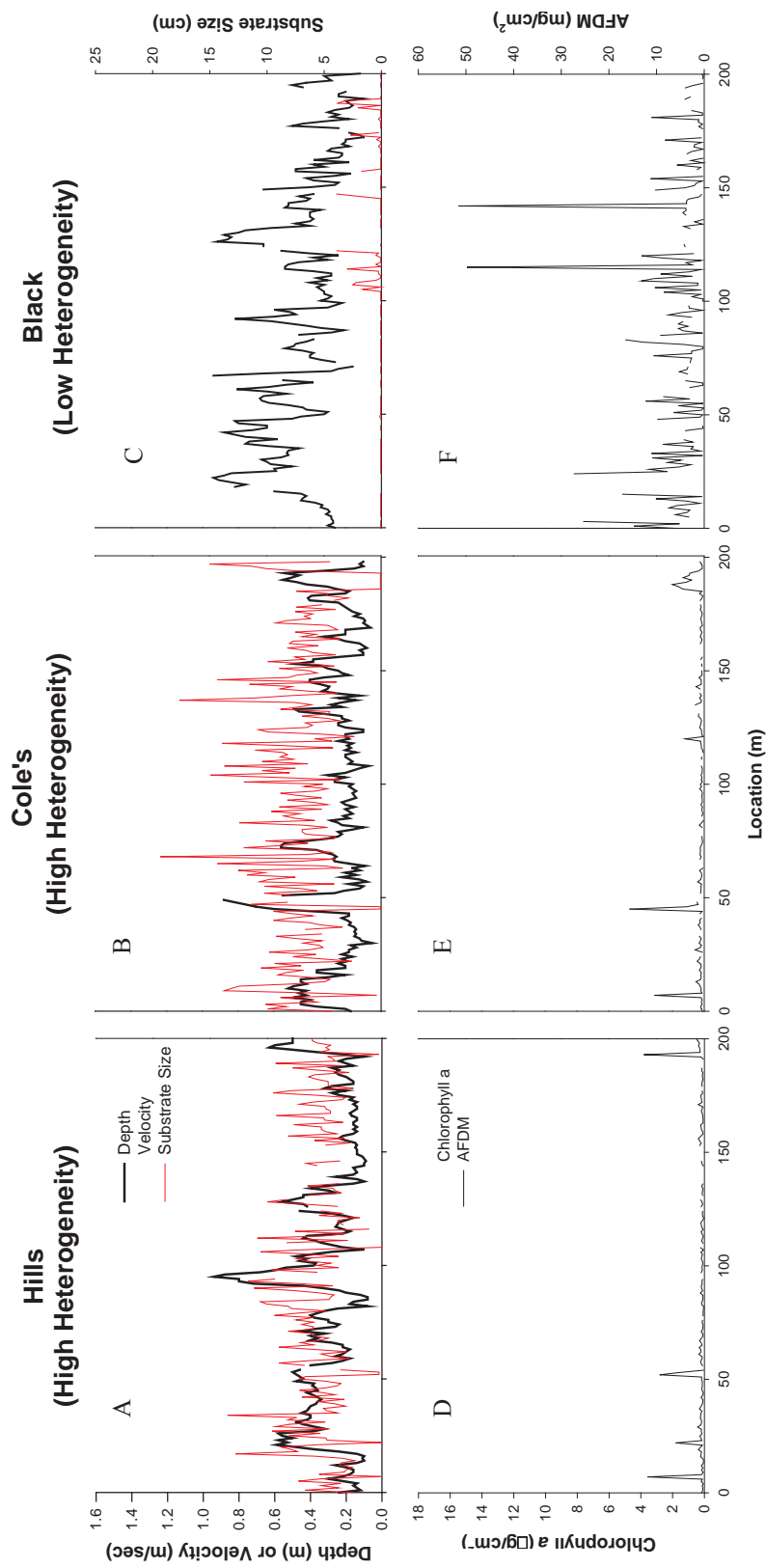


Figure 3.2: Measurements of physical and biotic characteristics along the thalweg of each stream. Missing data (gaps in lines) are due mostly to obstructions in the stream or depths too deep for proper sampling. A-C = depth, velocity and substrate size. D-F = chlorophyll *a* and AFDM.

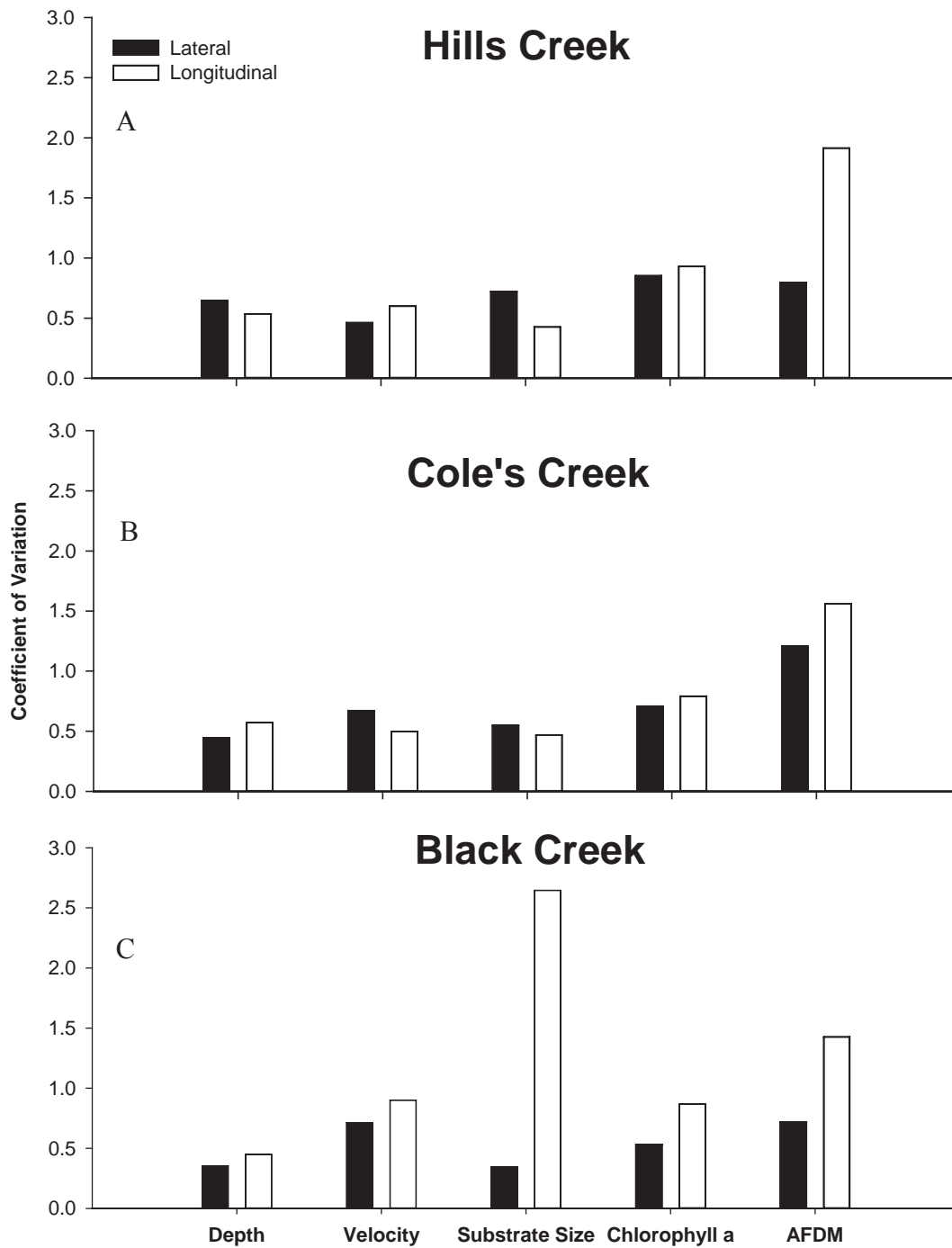


Figure 3.3: Longitudinal and lateral coefficient of variations for each physical and biological variable in all 3 streams. CVs were determined for each lateral transect and then averaged.

3.2. Spatial Analysis

3.2.1 Spatial Structure of Physical and Biofilm Metrics

Overall, only 8 of our 15 semivariograms follow the hypothesized structure of increasing semivariance from the nugget (y-intercept) to a sill (plateau). The remaining seven appear to have random variance from the y-intercept throughout the semivariogram. This prevented a spherical model from being fit because the non-linear optimization iterations returned singular model fits due to an infinite number of inadequate solutions. All depth and velocity semivariograms and two out of three chlorophyll *a* semivariograms were fitted with spherical models successfully (Table 3.2). However, once the sill was reached on the eight fitted semivariograms we often still observed movement around the plateau in a periodic fashion (Figure 3.4).

The semivariograms for depth and velocity on Hills Creek (high heterogeneity) showed an increase in semivariance up to the sill at ~12 and ~11 meters, respectively (Figure 3.4, Table 3.2). Thus, depth and velocity became spatially independent at just above ten meters. Chlorophyll *a* reached a sill faster than depth and velocity with a range of ~4 meters. Both AFDM and substrate size had inadequate fits for models, suggesting that they were spatially independent at all measured lag distances and were exhibiting pure nugget behavior (Cooper et al. 1997).

Spherical models were also fit for depth, velocity and chlorophyll *a* for Cole's Creek, which was also a high heterogeneity stream (Figure 3.4, Table 3.2). Depth and

velocity had a range of 9.4 and 6.1 meters, respectively. The range for chlorophyll *a* was in between at 7.6 meters. Similarly to Hills Creek, both AFDM and substrate size had singular fits for a spherical model suggesting complete spatial independence or nugget behavior.

For the low heterogeneity stream, Black Creek, we were able to fit spherical models to only the depth and velocity semivariograms (Figure 3.4). Despite the visually poor fit, these had low residual sum of squares compared to semivariograms from the high heterogeneity streams (Table 3.2). At high lag distances, semivariance increased away from the sill, demonstrating that more variability was captured at longer distances between sampling points (Figure 3.4). This increase in semivariance appears to be due to increased velocity and decreased depth observed at the downstream end of the transect (Figure 3.2). The range for depth was 10.2 meters and was 4.2 meters for velocity (Table 3.2). Chlorophyll *a*, AFDM and substrate size semivariograms had singular model fits for spherical models.

Table 3.2: Summary of semivariograms. Singular Model – Spherical model could not be fit because non-linear optimization iterations returns an infinite number of inadequate solutions

* Standardized RSS – Residual sum of squares calculated for standardized semivariograms

Site	Metric	Nugget	Sill	Range	Standardized RSS*
		Semivariance	Semivariance	Meters	
Hills	Depth	0.000	0.027	12.230	0.110
Hills	Velocity	0.005	0.079	10.896	0.120
Hills	Chlorophyll <i>a</i>	0.669	1.718	3.867	0.256
Hills	AFDM	NA	NA	NA	Singular Model
Hills	Substrate Size	NA	NA	NA	Singular Model
Cole's	Depth	0.000	0.023	9.434	0.155
Cole's	Velocity	0.020	0.063	6.126	0.073
Cole's	Chlorophyll <i>a</i>	5.391	8.083	7.583	0.440
Cole's	AFDM	NA	NA	NA	Singular Model
Cole's	Substrate Size	NA	NA	NA	Singular Model
Black	Depth	0.002	0.033	10.234	0.094
Black	Velocity	0.003	0.007	4.171	0.118
Black	Chlorophyll <i>a</i>	NA	NA	NA	Singular Model
Black	AFDM	NA	NA	NA	Singular Model
Black	Substrate Size	NA	NA	NA	Singular Model

3.2.2. Comparison of Spatial Structure among Streams

We did not detect any consistent differences in spatial heterogeneity among streams. Hills Creek appeared to be the least spatially heterogeneous for depth as depicted by the largest range, while Cole's was the most heterogeneous. For velocity, Hills Creek appeared to be the least heterogeneous as determined by range, while Black was the most. However, chlorophyll *a* had a smaller range for Hills Creek, so was more heterogeneous than in Cole's Creek.

Spatial heterogeneity can also be compared among streams with the slopes of the ascending limb of semivariograms (Figure 3.5). We found that Black Creek had the steepest slope of all depth semivariograms, which suggests it had the strongest spatial heterogeneity compared to Hills and Cole's Creek. However, Cole's Creek had the steepest slopes for both velocity and chlorophyll *a*. Note that the most heterogeneous stream for depth, velocity and chlorophyll *a*, as determined by range, was not the same as the most heterogeneous determined by ascending limb slopes. Nor was any stream the most or least heterogeneous for all metrics based on either range or slope. However, it is important to note that all ranges are at about the macro-habitat scale, which suggests that at least the physical metrics and biofilm metrics are controlled at the same scale.

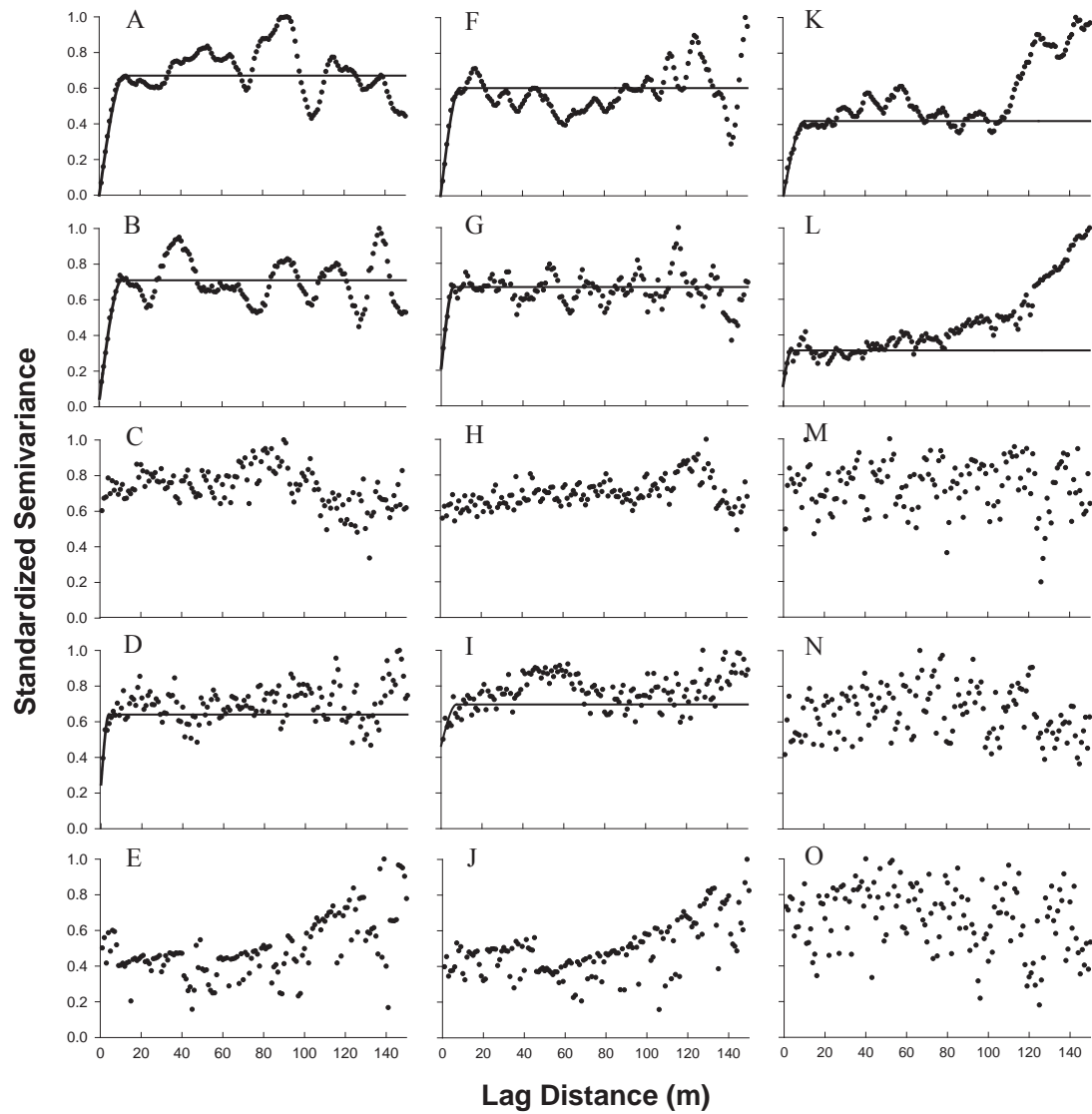


Figure 3.4: Hills Creek (A-E), Cole's Creek (F-J), and Black Creek (K-O) semivariograms plotting standardized semivariance against common lag distance. The trendline depicts the fitted spherical least squares model. A, F, K = depth, B, G, L = velocity, C, H, M = substrate size, D, I, N = chlorophyll *a*, E, J, O = AFDM

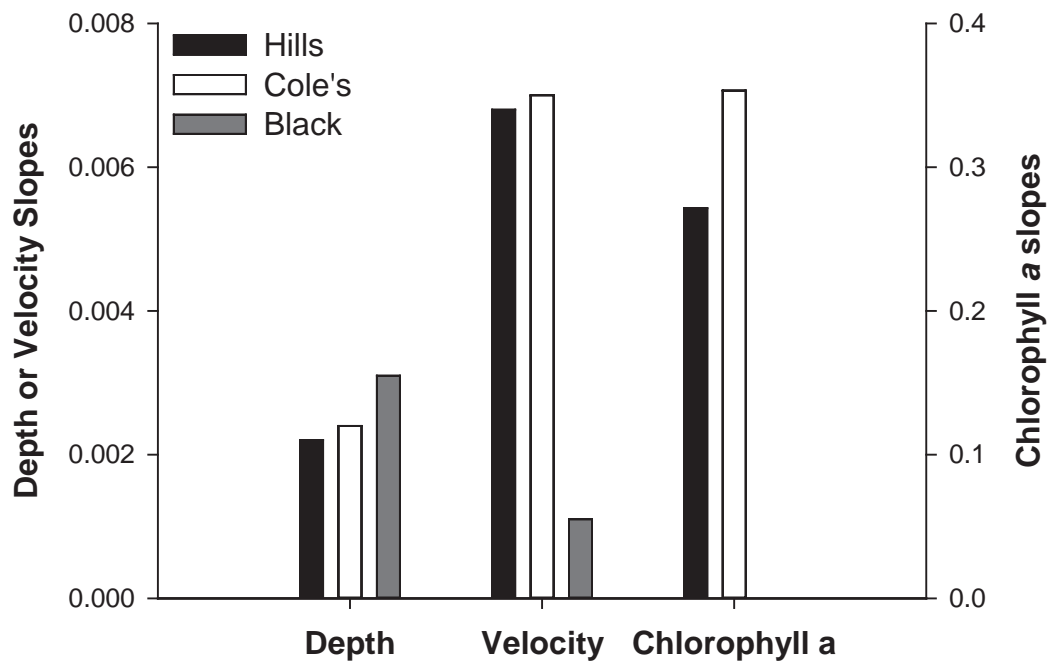


Figure 3.5: Semivariograms summarized with linear slopes of ascending limbs using original semivariance (not standardized). Semivariance is dependent on metric units, therefore comparisons should be made among streams but not among metrics.

4. Discussion

Understanding the spatial heterogeneity of biofilms could advance our understanding of streams because they are a basal food resource for stream food webs and are often responsible for nutrient uptake and chemical transformations. Yet, we know little about the small-scale heterogeneity of biofilm standing crop, the mechanisms that determine it, and the ecological consequences of its spatial structure. We expected that all physical and biofilm metrics would display spatial dependence initially but reach spatial independence within our transects. Therefore, we expected all semivariograms to

increase from the y-intercept (nugget) and reach an asymptote (sill). This was true for depth and velocity for all streams and chlorophyll *a* in two of the streams, but chlorophyll *a* in Black Creek and AFDM and substrate size in all streams did not demonstrate this spatial structure, suggesting that either our sampling transects did not capture their spatial dependence or that it didn't exist. Secondly, we predicted that the semivariograms of physical characteristics would look similar to semivariograms of chlorophyll *a* and AFDM within streams, regardless of the different patterns of benthic heterogeneity in the 3 streams. The shapes of semivariograms show some similarity with ranges occurring at about the macrohabitat scale, but without further statistical analysis anything about potential correlation between physical drivers and biofilm biomass cannot be verified.

Patches are defined as units with a consistent spatial pattern (Pickett and White 1985) but patterns do not necessarily have to be homogeneous within patches (Kotliar and Wiens 1990). Dent and Grimm (1999) used ranges from semivariograms to determine patch size in a stream. For our semivariograms, ranges or patch sizes lie between 3.8 and 12.2 meters (Table 3.2). Riffle-pool sequences were observed while sampling in each stream and can be observed crudely in the longitudinal data of Figure 3.2. A sill is reached at about the size of channel geomorphic units (riffle, pool or run) or at the macrohabitat scale (~10 meters). We suspect that the sill is reached at around ten meters because that is the distance where sampling points are likely to be in different channel geomorphic units, maximizing variance in depth and velocity. Therefore, patches

measured in our study streams using this longitudinal approach resemble channel geomorphic units.

Channel geomorphic units may also explain the periodicity observed in depth and velocity semivariograms in all 3 streams. As lag distance increases, the likelihood of matching channel geomorphic unit types changes. For instance, if the lag distance is such that pairs are both in riffles or both in pools, then semivariance should be low, which would cause a trough on the semivariogram. If the lag distance is such that pairs are in different types of channel geomorphic units, such as one in a riffle and one in a pool, then variance should be high, causing a peak on the semivariogram. This periodicity is not completely consistent because not all channel geomorphic units are the same size and not all channel geomorphic units are riffles or pools. Alternatively, periodicity around the sill of semivariograms can simply be caused by random variation in data (Phillips 1985, Sarnelle et al. 1993).

The depth and velocity semivariograms on Black Creek had a range around the macro-habitat scale similarly to semivariograms from the high heterogeneity streams. However, the semivariance increased above the sill permanently at about 100 meters lag distance for depth and 80 meters lag distance for velocity. All variability at the macrohabitat scale had been gathered because a sill was reached but new variability was introduced at a larger spatial scale. This deviation from the sill could potentially mean that in this transect a boundary between patches at a larger spatial scale was reached. If sills represent patches (Dent and Grimm 1999), then this semivariogram suggests that

there may be two different patch sizes in Black Creek. This could be due to a change in larger-scale geomorphic characteristics, such as stream gradient.

Seven of the fifteen semivariograms exhibited random semivariance starting at the y-intercept, which is known as nugget behavior because semivariance at the nugget is maintained at all lag distances (Cooper et al. 1997). This generally occurs when the sampling grain is too large and all spatial variation occurs at smaller scales (Cooper et al. 1997). Most of our fitted semivariograms had a nugget semivariance of zero, demonstrating that there was no appreciable variation at spatial scales smaller than we measured. However, in both high heterogeneity streams, we found that chlorophyll *a* showed non-zero nugget semivariance that increased to a sill. The fact that chlorophyll *a* exhibited variability beneath our scale of measurement agrees with other work showing that chlorophyll *a* can be as variable on individual rocks as it is among them (Downes et al. 1993). However, the low heterogeneity stream, Black Creek, showed only nugget behavior probably because it was almost entirely uniform sand and had consistently shifting substrates.

Two primary controls on epilithic algal growth in streams are nutrients and light. The spatial structure of these characteristics was not measured as part of this study, but light can vary longitudinally in streams and influence algal biomass (Allan and Castillo 2007, Murphy and Hall 1981). Furthermore, nutrient concentrations vary at the reach (Dent and Grimm 1999), macrohabitat (McIntyre et al. 2008), and microhabitat scales (Dent et al. 2001). Therefore, it is likely that variance in light and nutrients exhibited

some control on the spatial structure of autotrophic biofilm biomass that was not accounted for in our study.

In addition, temporal variability may be a confounding variable that influences algal standing crop. Disturbance in the form of scouring floods is known to have a prominent effect on algal standing crop (Fisher et al. 1982). Therefore, disturbance can drastically change patch structure or spatial heterogeneity of physical and biotic characteristics in streams (Lake 2000) and if temporal variability is not accounted for through repeated sampling, then it may be misattributed as spatial heterogeneity (Wiley et al. 1997). Investigations of spatial heterogeneity, such as this study, would benefit from sampling through time, although the amount of sampling effort required to characterize spatial variability in the level of detail here is often not feasible to also repeat through time. Our finding that the predominately sandy stream displayed nugget behavior is likely due to high levels of disturbance because sand is more mobile than larger substrates (Morisawa 1968). In contrast the high heterogeneity streams with substrates larger than sand likely offered some stable growing surfaces and allowed spatial dependence for chlorophyll *a* to occur at small lag distances.

Substrate size showed nugget behavior in all three streams. Particle size is controlled by available parent material from upstream and substrate sorting controlled by flows (Allan and Castillo 2007). The transport and sorting of substrates are controlled by water velocities (Morisawa 1968) and substrate sizes, therefore, it is common to find finer substrates in depositional zones such as near boulders, around woody debris, or in

large pools (Gordon et al. 2004). Furthermore, studies into longitudinal trends of substrate size in lotic ecosystems have shown that fine sediments become more prevalent in downstream reaches of rivers (Gordon et al. 2004). For instance, Costigan et al. (2014) found that gravel proportions decreased while fine substrates increased over the length of a 200 hundred kilometer study area of the Ninnescah River. Rather than variance in substrate size occurring at scales beneath our measurements, we believe that 200 meters is not a long enough distance to observe spatial structure in sediment size that may be occurring over many kilometers.

Finally, AFDM also demonstrated nugget behavior. AFDM is determined by both heterotrophic and autotrophic microorganisms in biofilms as well as any non-living organic matter. Therefore, factors controlling chlorophyll *a* may also influence AFDM due to the autotrophic component. Additionally, heterotrophic bacteria in biofilms may also be controlled by nutrients, flow conditions, substrate size and even indirectly by light. Romani et al. (2004) found that biofilms grown in light had higher bacterial growth and biomass compared to biofilms grown in dark chambers because heterotrophic bacteria use the labile carbon from algal biomass. Therefore, spatial structure of heterotrophic bacteria in biofilms may still be dependent on spatial heterogeneity of light. Lastly, particulate organic matter may be a large proportion of AFDM. Similarly to small substrates, fine particulate organic matter is most likely to be retained in regions of low water velocity caused by boulders, coarse woody debris or macrophyte beds (Allan and Castillo 2007). This finding is corroborated with our data which showed that sand

samples had substantially greater AFDM than individual rocks, which was likely attributed to particulate organic matter storage in or on sand. The spatial independence we observed for AFDM was probably a consequence of the fact that AFDM integrates a variety of different organisms as well as organic matter storage, each with their own controls and pattern of spatial heterogeneity.

Investigations into spatial heterogeneity have been a fruitful approach to expand our understanding of lotic ecosystems. It has been demonstrated repeatedly that ecological phenomena can both create and depend on spatial heterogeneity. In fluvial ecosystems worldwide, anthropogenic degradation often simplifies the natural stream structure with consequences for patch dynamics (Pringle 1988). As resource managers, our intuitive response is to counteract this simplification by increasing habitat heterogeneity, which remains the main focus of our restoration efforts (Roni et al. 2008). However, a strikingly large proportion of restorations still determine little or no ecological improvement (Roni et al. 2008, Palmer 2009). This suggests that other scales (or controls) may be relevant to biofilms that we did not measure in this study. In regards to restoration, our results also suggest that it may be important to understand not only the controls of the ecological phenomena we are trying to restore, but also the relevant scales of the ecological phenomena. For example, it is not necessarily that restoring streambed/habitat heterogeneity does not benefit biodiversity of invertebrates but that it is not the only driver or the only scale at which an invertebrate responds. Further investigation into spatial heterogeneity in lotic ecosystems, particularly at the

microhabitat and macrohabitat scales at which most restorations occur, will further our understanding of complex ecological connections and will also enable us to better restore degraded lotic ecosystems.

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