ABSTRACT

Title of Document: TRANSPORT, SOURCES, AND QUALITY OF SESTON IN A PIEDMONT HEADWATER STREAM

David Charles Richardson, Doctor of Philosophy 2008

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Streams transport and process particulate organic carbon (POC) within the suspended load (seston) after terrestrially-fixed (allochthonous) carbon enters lotic ecosystems or as instream (autochthonous) production is suspended from the streambed. POC provides a basal resource for upstream food webs, but can also support heterotrophic metabolism in downstream rivers. Yet, the controls on transport, sources, and biological availability (quality) of POC from headwaters are poorly understood. I examined seston and POC dynamics in a 3rd-order headwater stream in SE Pennsylvania. I studied the temporal controls on seston transport, composition, and sources, and the biological quality of POC.

I present evidence that seasonal patterns in seston and POC transport and composition reflect stream organism activity and cycles of autumnal leaf litter inputs

and vernal algal production. This work also provides the first evidence of recurrent nighttime peaks of seston transport; I attribute this pattern to bioturbation of streambed sediments that suspends particles during stream-organism nest digging, foraging, and movement. I present the first effort to demonstrate that stream salamanders contribute to ecosystem level processes such as modification of seston and organic carbon flow.

Mixing model analyses of seston composition indicate that seston in small streams is predominantly mineral-core particles; however, POC fluxes are primarily organic-core particles. Furthermore, the traditional view of headwater POC as leaf detritus should be expanded to include algal-derived particles, even in forested headwater streams. Finally, I report a new method for measuring the quality of suspended POC from aquatic environments using heterotrophic respiration as a metric for lability. Heterotrophic respiration rates for suspended POC measured using this method were an order of magnitude higher than respiration rates previously reported for benthic POC.

The results from this research highlight the importance of seston for both headwater streams and as a longitudinal linkage of allochthonous and autochthonous organic matter to downstream ecosystems. The magnitude of this connection is controlled by the quality, quantity, and timing of POC delivery.

TRANSPORT, SOURCES, AND QUALITY OF SESTON IN A PIEDMONT HEADWATER STREAM

By

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Preface

This dissertation contains a single introduction section, and four research chapters. Chapter 1, 2, 3, and 4 are presented in manuscript form with introduction, methods, results, and discussion followed by tables, figure captions and figures. A single literature cited section occurs at the end for citations throughout the dissertation.

Dedication

For my grandfather and Maryland alumnus, Pete Sante.

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Table of Contents

List of Tables

List of Figures

Chapter 1 - Temporal dynamics of seston: A recurring nighttime peak and seasonal shifts in composition in a stream ecosystem

Chapter 2 - Contributions of salamanders and macroinvertebrates to seston export in artificial stream ecosystems

Chapter 3 - Modeling sources and seasonal dynamics of seston and suspended particulate organic carbon within a stream ecosystem

Chapter 4 - A method for measuring bacterial mineralization rates of suspended particulate organic carbon in stream ecosystems

Introduction

Each year rivers export approximately 0.2 Pg of particulate organic carbon (POC) to the oceans (Spitzy and Ittekot 1991; Battin et al. 2008). POC within the suspended load (seston) enters lotic ecosystems as terrestrially-fixed (allochthonous) carbon or from instream (autochthonous) production. Streams and rivers not only generate and transport POC, but also play an active role in the processing of POC (Cole and Caraco 2001; Wipfli and Gregovich 2002; Mayorga et al. 2005). Knowledge of the sources of the carbon (allochthonous vs. autochthonous), timing and distance of transport, and biological interaction has implications for the energetic state of aquatic ecosystems (Vannote et al. 1980), the importance of headwaters to downstream ecosystems and management of watersheds (Meyer and Wallace 2001), and understanding the role of lotic ecosystems in the global carbon budget (Cole et al. 2007).

Potential sources of seston include organic-core particles such as algal or bacterial cells, leaf or wood fragments, dissolved organic carbon flocculates, and low-organic content silt or clay particles with adsorbed organic carbon. Seston in streams has historically been considered to be primarily organic-core particles of terrestrial origin, such as fragments of leaves and wood created by macroinvertebrate feeding and egestion upstream (Vannote et al. 1980). However, elemental, radioisotope, and microscopic analyses showed that most seston in streams and rivers is composed of small (<20 μm), old, high mineral content particles with carbon to nitrogen ratios lower than leaves or wood (Meybeck 1982; Cole and Caraco 2001; Dodds and Whiles

2004). These seemingly contradictory results could be reconciled if seston were a mixture of inorganic particles with adsorbed terrestrially-derived organic matter (mineral-core, Aufdenkampe et al. 2001), algal cells, and fragmented leaves or woody debris.

POC dynamics include transport downstream, deposition to and resuspension from the streambed (Thomas et al. 2001; Newbold et al. 2005). Hydrological and biological dynamics control the timing of POC transport from headwaters. Storms are the most important factor regulating variability in the magnitude of annual or monthly seston and POC fluxes (Golladay et al. 2000). However, because small streams are under storm conditions for only a small fraction of time, temporal POC dynamics under baseflow conditions may disproportionately regulate or reflect stream ecosystem processes. At baseflow, POC travels short distances downstream in a series of hops (Cushing et al. 1993; Webster et al. 1999; Thomas et al. 2001) interspersed with periods of retention on the streambed (Newbold et al. 2005). Regulation of suspension from the streambed has been infrequently examined, especially on a diel time-scale. However, seasonal peaks of POC concentration have been attributed to increased macroinvertebrate fragmentation of leaf litter as stream temperature increases (Webster et al. 1990; Molla et al. 2006).

The location of POC mineralization by aquatic organisms depends on the timing and distance of transport, but also on the quality of the organic matter (Webster et al. 1999; Rosi-Marshall 2004). Investigation of CO_2 dynamics in large

rivers indicate that old, terrestrially-derived POC is an important component of heterotrophic river respiration (Cole and Caraco 2001; Richey et al. 2002). However, POC transported under baseflow has been considered metabolically inert (Vannote et al. 1980; Webster et al. 1999), and neither nutritious to macroinvertebrates (Benke and Wallace 1980) nor very important to downstream food webs (Finlay et al. 2002; McCutchan and Lewis 2002). A continuous array of labilities likely exists within the suspended load (seston), with POC ranging from labile (e.g. fresh algal cells) to refractory (e.g. humified soil organic matter) (e.g. Trumbore 2000), but more measurements of suspended POC quality are needed.

My goals for this dissertation were to (1) determine the sources of seston and POC to headwater stream ecosystems, (2) understand controls on seston composition and transport, and (3) determine if suspended POC is biologically available to heterotrophic microorganisms. I used a combination of field surveys, field and lab experiments, and modeling to address these goals and attempted to understand seston dynamics in a 3rd-order Pennsylvania piedmont stream. I provide evidence that seston transport at baseflow is regulated by seasonal and diel patterns in litterfall, instream primary production, and stream organism activity. This work provides the first evidence of recurrent nighttime peaks of seston transport; I attribute this pattern to bioturbation of streambed sediments. I also show that algal carbon is an important component of seston, even in forested headwater streams. Finally, I present a new method for measurement of POC quality, and report some of the few measurements

of suspended POC lability in headwater streams. A brief description of each chapter follows.

Chapter 1 – I measured the baseflow concentration and composition of seston over a one-year period in White Clay Creek, a third-order stream in the southeastern Pennsylvania Piedmont, to assess the temporal variability in seston concentration and quality at seasonal and diel time scales. Each month, I sampled baseflow seston every 1.5 hours over a 24-hour period and measured concentration, carbon composition, pigment content, and ^{13}C isotopic ratios. Seston and particulate organic carbon (POC) concentrations exhibited a strong diel pattern; nighttime concentrations exceeded daytime concentrations by 80% and 43%, respectively. Chlorophyll *a* concentration in seston (1.3 ± 0.8 $\mu g\ L^{-1}$: mean ± SE) did not exhibit a diel pattern. I attribute the diel pattern of seston concentration to bioturbation by the nocturnal stream community, including crayfish, amphibians, eels, and macroinvertebrates. Seasonally, carbon content of seston increased from 9% throughout most of the year to 15% during November, December, and March, while seston $\delta^{13}C$ was depleted in the late fall and enriched in early spring months relative to the rest of the year. Chlorophyll *a* and pheophytin *a* concentrations in seston also peaked during the early spring. Seasonal patterns in seston and POC composition reflect cycles of autumnal leaf litter inputs and vernal algal production. Bioturbation and shifts in organic carbon inputs mediate changes in POC quality and fluxes, which affect the bioavailability of POC and ultimately influence rates of heterotrophic respiration.

Chapter 2 – I tested the hypothesis that bioturbation by salamanders and stream macroinvertebrates is a mechanism for nighttime increases in seston transport. I measured the concentration of seston over four consecutive days and nights in both the presence and absence of macroinvertebrates and salamanders in stream-side flumes. I describe results for seston transport including the outflow concentration of seston, percentage of seston exported from the flumes, average seston transport distance (S_w), and chlorophyll *a* content of seston. During the daytime of the salamander addition, percent seston exported (29.3% ± 2.2: mean ± SE) and S_w (24.5 m ± 1.5: mean ± SE) increased by 44% and 30%, respectively. Macroinvertebrate additions caused no effects on seston transport, and there were no patterns in suspended chlorophyll *a* concentrations. Salamanders caused daytime increases in seston, an opposite trend to the hypothesized nighttime increases. However, this was the first effort to demonstrate stream salamander contribution to ecosystem level processes such as modification of seston and organic carbon flow. Salamander activity, density, and size make them ideal candidates for influencing transport of seston and should be considered further along with other stream organisms such as eels and crayfish.

Chapter 3 – I used a new approach that defines seston end-members by particle type – mineral-dominated, terrestrial plant detritus and algal – with distinct organic carbon and nitrogen contents and used a mixing model analysis to quantify fractions of seston, in a 3rd-order southeastern Pennsylvania stream. I observed that seston mass concentrations were dominated by mineral particles (65% ± 1%: mean ± SE).

However, algal and terrestrial plant particles, because of their high organic content, still represented a large fraction of the particulate organic carbon (89% ± 0.3%: mean ± SE) and nitrogen (87% ± 0.4%: mean ± SE) in transport. Furthermore, seasonal patterns in seston sources reflected cycles of autumnal leaf litter inputs and vernal algal production. The results suggest that the biological view of seston (organic matter) in small streams as fragmented and egested leaf-litter is compatible with the biogeochemical perspective that seston (mass) is mineral-derived. However, the biological view should be expanded to include algal-derived particles. The source of organic matter can indicate lability for biological use and should ultimately influence rates of heterotrophic respiration in downstream rivers.

Chapter 4 – I developed a method to measure heterotrophic respiration rates (quality) of POC. The method involves keeping particles in suspension, correcting for respiration of dissolved organic carbon, and extending rate measurements to 30-40 days. BOD bottles were filled with POC at elevated (5X) concentration using particles collected and concentrated by tangential flow filtration. Glass beads added to the BOD bottles prevented aggregation while the bottles were rotated around an incubation axel to ensure that the particles were suspended. Replicate samples were periodically sacrificed for DO measurements. I observed that, at the beginning of the experiment, suspended particles exhibited POC mineralization rates that were 5-fold higher than particles that were allowed to settle. Mineralization rates were highest at the onset of the experiment at 180 to 300 mg O_2 g initial POC^{-1} day^{-1} and then declined in the following weeks to 10 mg O_2 g initial POC^{-1} day^{-1}. Further, I

compared respiration rates from this study to previous studies of stream POC and found that POC is more labile than previously assumed. Labile POC may be mineralized by bacteria within upstream reaches; more resistant POC may subsidize downstream ecosystems with carbon from the headwaters.

Chapter 1: Temporal dynamics of seston: A recurring nighttime peak and seasonal shifts in composition in a stream ecosystem

Introduction

Particulate organic carbon (POC) provides energy for microorganisms and macroinvertebrates in headwater streams (Hedin 1990), and when transported in the suspended load (seston), supports heterotrophic metabolism in downstream reaches and rivers (Cole and Caraco 2001; Wipfli and Gregovich 2002). POC transport represents a longitudinal linkage between upstream autochthonous and allochthonous production and downstream ecosystems with the magnitude of connection controlled by the quality, quantity, velocity, and timing of POC delivery (Mayorga et al. 2005).

Hydrological and biological dynamics control the timing of POC transport from headwaters. Storms are the most important factor regulating variability in the magnitude of annual or monthly seston and POC fluxes (Golladay et al. 2000). However, because small streams are under storm conditions for only a small fraction of time, temporal POC dynamics under baseflow conditions may ultimately regulate or reflect stream ecosystem processes. At baseflow, seasonal peaks of POC concentration have been attributed to increased macroinvertebrate fragmentation of leaf litter as stream temperature increases (Webster et al. 1990; Molla et al. 2006). However, diel patterns of seston transport have not been extensively examined. Peaks in seston concentration, found in a deciduous European forest reach at mid-morning and evening, were attributed to diurnal rhythms in algal drift (Pozo et al.

1994), but no diel patterns were found in either boreal (Naiman 1982) or glacier-fed alpine streams (Hieber et al. 2003). Knowledge of temporal patterns can help evaluate the sources of POC (Kendall et al. 2001), quality of the energetic subsidy to downstream biota (Rosi-Marshall 2004), controls on transport (Pozo et al. 1994), and lead to better estimates of annual POC fluxes, especially in streams with pulsed inputs of organic matter (Fisher et al. 2004).

Potential sources of seston include organic-core particles such as algal or bacterial cells, leaf or wood fragments, dissolved organic carbon flocculates, and low-organic content silt or clay particles with adsorbed organic carbon. Seston in streams has historically been considered to be primarily organic-core particles of terrestrial origin, such as fragments of leaves and wood created by macroinvertebrate feeding and egestion upstream (Vannote et al. 1980). Stable isotope and chlorophyll *a* (Chl *a*) analyses supported the idea that stream POC is terrestrial (Finlay et al. 2002) with minimal contributions from autochthonous production (Angradi 1993). However, elemental, radioisotope, and microscopic analyses showed that most seston in streams and rivers is composed of small (<20 μm), old, high mineral content particles with carbon to nitrogen ratios lower than leaves or wood (Meybeck 1982; Cole and Caraco 2001; Dodds and Whiles 2004). These seemingly contradictory results could be reconciled if seston were a mixture of inorganic particles with adsorbed terrestrially-derived organic matter (Aufdenkampe et al. 2001), algal cells, and fragmented leaves or woody debris.

I report results from intensive field surveys of seston quantity and quality in a 3rd-order piedmont stream in southeastern Pennsylvania, U.S.A. I measured seston concentration and composition using a comprehensive array of measurements through monthly diel measurements over a one-year period to assess the temporal variability in seston transport and quality at both seasonal and diel time scales. Here, I report (1) a consistent and repeatable diel pattern of nighttime peaks in seston and POC concentrations that I attribute to bioturbation and (2) seasonal shifts in composition associated with annual cycles of autumnal leaf-fall and vernal algal production prior to canopy closure.

Methods

Site description – The upper east branch of White Clay Creek (WCC: N39°51'33", W75°46'59") is a 3rd order watershed that drains 7.3 km^2 of eastern piedmont land. The watershed has 23% of total area as temperate deciduous forest, 52% as pastures or hay fields for horses or cattle, and 22% as row crop agriculture, but has a largely intact riparian forest and livestock have minimal access to the stream (Newbold et al. 1997). The mean daily baseflow discharge averaged 122 L s^{-1} over the past five years with a high annual mean discharge of 174 L s^{-1} and low of 59 L s^{-1}. At baseflow, WCC has high nutrient concentrations (3-5 mg NO_3-N L^{-1}; 4-33 μg PO_4-P L^{-1}) from agricultural land use and dissolved organic carbon (DOC) concentrations of 1.5 mg DOC L^{-1} (Newbold et al. 1997). Most of the particulate organic matter input (predominantly leaves) occurs from late October to mid-November (J. D. Newbold unpubl.).

Temperature and discharge – Throughout the sampling period, stream water temperature was recorded using automated temperature loggers (Onset Computer Corporation) and stream stage was recorded using an automated pressure transducer (Telog Instruments), both at fifteen minute intervals. Stream stage was converted to stream discharge using a 10-year rating curve, according to the method of Dunne and Leopold (1978), and averaged to hourly discharge. I used the discharge record to identify the antecedent storm discharge and defined storms as two times baseflow discharge because such flows typically result in bed mobilization in WCC (Newbold et al. 1997).

Sampling protocol – Under baseflow conditions, stream water was collected every 1.5 hours during one 24-hour period each month, from August 2005 to July 2006 (Fig. 1.1) using an automated water sampler (Teledyne Isco), with the collection tube (9.5 mm inner diameter) at 2/3 depth in the middle of the stream. Sampling days (11 Aug 05, 21 Sep 05, 01 Nov 05, 07 Dec 05, 12 Jan 06, 24 Feb 06, 31 Mar 06, 27 Apr 06, 24 May 06, 20 Jun 06, 26 Jul 06) were not evenly spaced; however, I delineated names based on the sampling month (Aug, Sep, Nov, Dec, Jan, Feb, Mar, Apr, May, Jun, Jul). On 22 August 2006, sampling (see spatial sampling section) included three additional sites, all 2nd-order streams within the watershed upstream of the WCC site. Simultaneously at each of the four locations, three samples were taken at night and three were taken during the day to confirm that diel patterns were not unique to the main sampling location.

The automated sampler was set to collect four 1-L samples at each time period. The first sample was considered a rinse of the inflow tubing and discarded, the second was used for pigment analysis, and the final two samples were used for replicate seston and POC analysis. Only the first replicate of seston and POC analysis is reported; the second replicate was used to ensure the precision of the automated sampling or as a substitute if the first replicate was lost. Once or twice each sampling month, three replicate grab samples were taken by submerging 1-L bottles at 2/3 depth to ensure that automated sampling was unbiased in selection of particles and accurate in representing stream seston.

Seston – Seston concentration was determined gravimetrically using the dry weight of total suspended solids. Entire 1-L stream water samples were filtered, after recording precise volume, through stacked pairs of pre-weighed and pre-ashed (480ºC for 4.5 hours) 47 mm glass fiber filters (Millipore AP40 with nominal pore-size of 0.7 μm). The sample was filtered through two filters to check analytical accuracy, assess the integrity of the top filter and ensure even distribution of seston across the filter. The sample was discarded if the bottom filter gained or lost more than 0.2 mg. The filters were dried for 12-18 hours at 60ºC, re-weighed to calculate seston mass on the filter, and divided by filtered volume to calculate seston concentration (mg seston L^{-1}).

Pigments – Samples for pigment analysis were collected on a glass fiber filter (Millipore AP40), frozen and processed within 30 days using a modified hot ethanol extraction method (Biggs and Kilroy 2000). The samples were heated to 78ºC for 10

minutes in 90% ethanol and then stored frozen for 24 hours. Chl *a* and pheophytin *a* (Pheo *a*) concentrations (μg L^{-1}) were calculated using measurements of absorbance on a spectrophotometer (Beckman Coulter DU-640) before and after acidification (Biggs and Kilroy 2000). Pheo *a*, a degradation product of Chl *a*, suggests dead or senescing algal cells (Peterson and Stevenson 1992).

Elemental and stable isotope analysis – Subsamples of each seston filter were wet with nanopure water and fumed with hydrochloric acid vapors for 18 hours, effectively removing carbonates (A. K. Aufdenkampe unpubl.). Subsamples were analyzed in batches on an Elemental Analyzer (Costech ECS 4010) interfaced with an Isotope Ratio Mass Spectrometer (Thermo DeltaPlus XP). Standard curves for the elemental and isotopic analysis were created for each batch using 8 or 9 isotopically enriched (+37.63‰) and depleted (-26.39‰) L-glutamic acid standards over a range of carbon masses that encompassed the subsample masses. Stable carbon isotope ratios, reported using $\delta^{13}C$ notation, were normalized relative to the Vienna Peedee Belemnite standard (Coplen et al. 2006). POC concentration (mg C L^{-1}) was calculated from the mass of C analyzed, the fraction of total sample analyzed, and the volume of stream water filtered. Weight percent organic carbon (%OC) of seston was calculated as the ratio of POC (mg POC L^{-1}) to seston (mg seston L^{-1}) concentrations and POC flux (mg s^{-1}) was calculated by multiplying the POC concentration (mg L^{-1}) by the average hourly discharge (L s^{-1}).

End member analysis – Four species of dried, senesced leaves (American beech: *Fagus grandifolia* Ehrh., northern red oak: *Quercus rubra* L., red maple: *Acer rubrum* L., and tulip poplar: *Liriodendron tulipifera* L.) were collected from the forest floor, dried, and analyzed for stable carbon isotopes as potential allochthonous sources of POC. In May 2007, epilithon (predominantly *Melosira varians*), collected from six rocks in riffles in WCC and rinsed carefully to remove sediments and trapped leaf pieces, was analyzed for stable carbon isotopes as potential autochthonous sources of POC.

Mixing model – I modeled seston composition as a mixture of two distinct particle types: organic-core particles composed of algal cells, leaf fragments, and bacterial cells (carbon content for mixture = 45%); and, mineral-core particles with adsorbed organic carbon and bacterial cells (carbon content = 1.5%) (Meybeck 1982; Hedges et al. 1986). For each sample, I simultaneously solved equations (1.1) and (1.2)

Equation 1.1 $S = P_o + P_m$

Equation 1.2 $POC = 0.45 \times P_o + 0.015 \times P_m$

using measured seston (S) and POC concentrations to determine the concentration of organic-core (P_o) and mineral-core (P_m) particles in seston and the concentration of organic-core ($0.45 \times P_o$) and mineral-core ($0.015 \times P_m$) particles in POC. Mixing model results are reported as the fraction of total concentration by particle type (e.g.

P_o/S as the organic-core particle fraction of seston by weight). Results were split into two groups: (1) months with increased organic inputs from allochthonous (Nov, Dec) and autochthonous sources (Mar) and (2) lower organic inputs (all other months).

Environmental scanning electron microscopy (ESEM) – Seston was collected on 16 January 2006 from WCC and images of particles in stream water were captured at 1300X to14500X magnification using an environmental scanning electron microscope (Electro Scan) with an accelerating voltage of 28 kV.

Statistical analyses – All statistical analyses were conducted using SAS v9.1 Level 1M3 (2007, SAS Institute Inc., Cary, North Carolina, United States of America). I performed two-way ANOVAs (PROC MIXED) for seston, POC, Chl *a* and Pheo *a* concentrations, POC flux, percent carbon of seston, and $\delta^{13}C$ data with month and day or night as treatments. Samples collected at 1.5 hour intervals throughout each day or night were considered replicates. For the spatial sampling, I performed two-way ANOVAs for seston, POC and Chl *a* with station and day or night as treatments. To ensure homogeneity of variances, it was necessary to log-transform several variables (seston, POC); these are reported as back-transformed geometric means. Following each ANOVA, I made multiple mean comparisons using the Tukey's test. Tests of auto-correlation (Durbin-Watson) revealed a violation of ANOVA assumptions of independence of residuals for seston, POC, carbon content, POC flux, and stable isotopes within each month. Therefore, I used the interaction mean squares (MS) as the denominator in the F-statistic, rather than the residual MS, for the

test of diel main effects. I did, however, use the within-month mean squared error in conjunction with the Tukey's test to identify specific months in which the diel effect was observed, reasoning that most or all of the serial auto-correlation reflected real (mechanistic) trends over the course of the day or night, rather than over-sampling of the random error.

Linear correlation (PROC REG) was used to identify relationships between dependent and explanatory variables of interest including temperature, discharge, and antecedent storm timing and magnitude. Outliers were identified (PROC ROBUSTREG) using a prespecified residual value (3.0). For quality control, I analyzed the correlation between the automated sampler and grab samples and compared the slope and intercept to the 1:1 line using *t*-tests (PROC TTEST). For the mixing model results, fractions of seston and POC as mineral-core and organic-core particles were compared between the grouped months using paired *t*-tests (PROC TTEST).

Results

Over the year, one hundred eighty-three samples were taken during baseflow conditions on 11 monthly sampling days over 24 hours to 25.5 hours (Fig. 1.1). Mean water temperatures ranged from 2°C in December to 20°C in August and July, while mean daytime temperature exceeded nighttime temperatures between 0.15°C (November) and 1.5°C (April). WCC discharges, during sampling, ranged from 23 L s^{-1} in June to 120 L s^{-1} in February (Fig. 1.1), with minimum discharge in late summer, maximum discharge in winter. Daytime discharge was not different than

nighttime discharge ($F_{1,10}$=0.94, p=0.35). A series of storms occurred during the winter; one large summer storm, on 28 June 2006, reached twice the maximum discharge of any other storm (Fig. 1.1).

Precision and accuracy of automated sampler – There was a positive correlation in seston concentrations between grab and automated samples that explained almost 90% of the variance (r^2=0.88, $F_{1,13}$=106.5, p<0.001, n=14). One observation was excluded from the regression because it was identified as an outlier with a standardized robust residual (6.3) greater than the prespecified value (3.0). The line was statistically identical to the 1:1 line, (intercept = 0, p =0.68; slope = 1, p=0.68) and all automated samples were within ±10% of grab samples. Furthermore, I found low coefficients of variation for duplicate automated seston (6.5% ± 6.5: mean ± SD, n=183 pairs) and POC samples (8.8% ± 9.8: mean ± SD, n=104 pairs).

Seston and POC – Over the year, nighttime seston concentrations (3.0 mg L^{-1} ± 0.07: mean ± SE) exceeded daytime concentrations (1.7 mg L^{-1} ± 0.07: mean ± SE) by 80% (Fig. 1.2a, $F_{1,10}$=76, p<0.001). While mean nighttime concentrations were always greater than daytime concentrations, the difference was not significant during winter months (interaction effect: $F_{10,163}$=2.4, p=0.01). Seasonally, seston concentrations were lowest during the late fall and winter months and highest in the summer ($F_{10,163}$=67, p<0.001). I observed the largest diel variation of seston in June, when the nighttime concentration of seston (6.4 mg L^{-1} ± 0.43: mean ± SE) exceeded the daytime concentration (2.5 mg L^{-1} ± 0.21: mean ± SE; p<0.001) by 155% (Fig. 1.3).

POC followed the same trends as seston, both on the diel and seasonal scales. Nighttime POC concentrations (0.24 mg C L^{-1} ± 0.005: mean ± SE) exceeded daytime concentrations (0.17 mg C L^{-1} ± 0.005: mean ± SE) by 43% (Fig. 1.2b, $F_{1,10}$=15, p=0.003). These differences, while observed each month, were significant only during August, April, May, and June (interaction effect: $F_{10,156}$=8.6, p<0.001). POC concentrations varied by month and were lower in the winter months ($F_{10,156}$=42, p<0.001). The largest diel change for POC also occurred in June with a 105% increase in POC concentration (Fig. 1.3) from day (0.21 mg C L^{-1} ± 0.013: mean ± SE; p<0.001) to night (0.43 mg C L^{-1} ± 0.017: mean ± SE).

Both seston (r^2 = 0.32, p=0.07) and POC (r^2 = 0.17, p=0.21) concentrations, averaged by month, were not correlated with stream discharge. However, seston concentration correlated with other physical changes in the stream. Water temperature was positively correlated with both day (Fig. 1.4: n=11, p=0.02, r^2=0.45) and night (Fig. 1.4: n=11, p=0.02, r^2=0.45) seston concentrations. The slopes for day (0.07 ± 0.03: mean ± SE) and night (0.18 ± 0.07: mean ± SE) regression fits were not statistically different from each other (Fig. 1.4, t=1.55, df=8, p=0.13). Mean nighttime POC concentrations were also positively correlated with water temperature (n=11, p=0.05, r^2=0.37) but daytime concentrations were not (n=11, p>0.05, r^2=0.26). The maximum discharge from the antecedent storm was negatively correlated with the percentage of increase of seston from day to night (Fig. 1.5: n=11, p=0.005,

r^2=0.61). However, the antecedent storm discharge did not explain diel differences in POC concentrations (n=11, p=0.09, r^2=0.28).

Carbon content – Overall, the daytime carbon content (11.4% ± 0.2: mean ± SE) was greater than the nighttime carbon content (9.3% ± 0.2: mean ± SE) by 1.2% (Fig. 1.2c, $F_{1,10}$=15, p=0.003). Although the mean carbon content was higher in the daytime than in the nighttime in each month, the main effect was driven by significant differences in November and March. The percent carbon varied seasonally, with the highest values in November, December, and March ($F_{10,156}$=18, p<0.001).

POC flux – The downstream flux of POC had both diel and seasonal trends. The nighttime POC fluxes (Fig. 1.2d: 17.3 mg s^{-1} ± 0.9: mean ± SE) were 45% greater than daytime fluxes (11.9 mg s^{-1} ± 0.6: mean ± SE; $F_{1,10}$=20, p=0.001). The magnitude of this effect was buffered in the winter, when both the day-night differences in POC concentration were lower and the stream discharge was higher (interaction effect: $F_{10,155}$=3.7, p<0.001). POC flux varied seasonally, peaking during the late winter and early spring ($F_{10,155}$=45, p<0.001).

Pigments – Chl *a* concentration (1.26 μg L^{-1} ± 0.8: mean ± SE) was less variable than seston or POC concentrations. Chl *a* varied by month (Fig. 1.6a, $F_{10,165}$=5.32, p<0.001), but there were neither diel ($F_{1,10}$=0.04, p=0.77) nor interaction effects ($F_{10,165}$=1.33, p=0.22). The mean Chl *a* concentrations for March were significantly

greater than all other months ($p<0.05$). Pheo *a*, unlike Chl *a*, showed a diel difference (Fig. 1.6b, $F_{1,165}=28$, $p<0.001$), with nighttime Pheo *a* concentrations (2.10 μg L^{-1} ± 0.07: mean ± SE) greater than daytime concentrations (1.56 μg L^{-1} ± 0.07: mean ± SE). Pheo *a* concentrations also varied by month ($F_{10,165}=122$, $p<0.001$), and could be divided into three groups: the greatest concentrations during February and March, intermediate during late spring and summer and lowest during the late fall and early winter. Pheo *a* concentrations were more variable than Chl *a*, and included monthly means both lower than and double the average Chl *a* concentration (Fig. 1.6a, b).

Stable carbon isotopes – The highest $\delta^{13}C$ value of POC occurred during March, while the lowest values occurred during December and January (Fig. 1.7: $F_{10,142}=28$, $p<0.001$). For potential allochthonous end-members, all four species of leaves had similar $\delta^{13}C$ values (Fig. 1.7, box b: -28.2‰ ± 0.6: overall mean ± SD), but the epilithic algae were more enriched (Fig. 1.7, box a: $p=0.03$, -26.5‰ ± 1.6: overall mean ± SD). $\delta^{13}C$ was positively correlated with mean pigment (Chl *a* + Pheo *a*) concentrations (Fig. 1.8: $n=11$, $p=0.016$, $r^2=0.49$); this trend was driven by the February and March peaks in pigment concentrations. The $\delta^{13}C$ of March samples (-25.7± 0.24: mean ± SE) was greater than the end-member for epilithic algae, but was within 1 SD of the end-member mean. $\delta^{13}C$ value of POC had neither diel ($F_{1,142}=0.43$, $p=0.52$) nor interaction effects ($F_{10,142}=1.48$, $p=0.15$).

Mixing model – Throughout the year, mineral-dominated or mineral-core particles contributed to >70% of seston concentration, with organic-core particles contributing

<30% of seston concentration by weight. However, organic-core particles consistently contributed the majority of POC concentration by weight (Table 1.1). The contribution of organic-core particles to both seston and POC increased during periods of increased carbon influx (Nov, Dec, and Mar) from leaf fall and vernal algal blooms compared to other months (Table 1.1).

Spatial sampling – In all four streams sampled within the WCC watershed in August, nighttime concentrations of seston (9.0 mg L^{-1} ± 5.5: mean ± SE) and POC (0.64 mg L^{-1} ± 0.37: mean ± SE) were greater than daytime concentrations (2.2 mg L^{-1} ± 0.4: mean ± SE) and (0.19 mg L^{-1} ± 0.02: mean ± SE), respectively (p<0.05), but there were no differences across the streams. Suspended Chl *a* concentrations were low in all four streams and there was neither diel nor spatial differences among streams.

Discussion

Diel patterns – Possible mechanisms for the consistent nighttime increases in seston and POC concentrations (Fig. 1.2a, b) include diel cycles in stream flow, primary production, feeding-related particle processing (e.g. shredding, suspension of non-ingested food particles, egestion), and bioturbation. Stream flow can be higher at night due to decreases in evapotranspiration in deciduous forests (Czikowsky and Fitzjarrald 2004). In WCC, nighttime baseflow is <5% greater than daytime baseflow, but only during the growing season (J. D. Newbold unpubl.); I observed diel trends for seston and POC concentration in all seasons. Primary production in WCC causes diurnal increases in dissolved organic matter concentrations (Kaplan and Bott 1982) and could be expected to cause similar diurnal increases in the Chl *a*

portion of seston due to algal drift (Pozo et al. 1994). However, I observed the opposite pattern with nighttime increases in seston concentration, and I saw no diel variations in Chl *a* in any of the sampling months (Fig. 1.6a) or across the watershed. Feeding-related particle processing should increase at night due to increased nocturnal macroinvertebrate activity (Wagner 1991), and could contribute to seston through particle shredding (Wallace et al. 1982), suspension of non-ingested food particles ("sloppy feeding"), or suspension of egested particles. I would expect, however, that all of these processes would preferentially contribute carbon-rich particles to seston, whereas the carbon content of seston actually declined at night (Fig 1.2c). Thus, none of these explanations prove satisfactory. The remaining explanation is bioturbation, activity that suspends streambed particles during nest digging, foraging, and movement (Moore 2006).

Several lines of evidence suggest that the diel pattern is caused by bioturbation. The diel pattern appears to be biologically driven because rising water temperatures, and subsequent increases in organism activity (Wallace et al. 1991), may result in greater nighttime suspension (Fig. 1.4). Furthermore, in this study, seston and POC concentrations increased after twilight without a lag (Fig. 1.3), which matches the activity patterns of nocturnal aquatic organisms (Schloss and Haney 2006). For example, mayfly and stonefly larvae are most active from sundown to sunrise to minimize risk of fish predation (Cowan and Peckarsky 1994; Elliott 2000). While I cannot exclude the activities of aquatic or terrestrial mammals, such as deer or raccoons, as a mechanism for the diel patterns, I suspect that those organisms

would generate intermittent pulses of seston resulting from isolated movements, such as fishing for food, drinking, or crossing the stream. Rather than intermittent pulses, nighttime increases of seston and POC concentrations appear to be maintained throughout the night (e.g. Fig. 1.3).

Furthermore, Pheo *a* concentrations, which indicate dead or senescing algae, increased during the nighttime (Fig. 1.6b); it is feasible that these algae are more susceptible to be suspended into the water column through bioturbation or nighttime sloughing. I did not see concomitant increases in suspended Chl *a* which would have been evidence for excess production or grazing.

Bioturbation is known to affect ecosystem function in marine, freshwater, and terrestrial ecosystems (Palmer et al. 1997; Meysman et al. 2006; Moore 2006). Studies, within freshwater aquatic ecosystems of various biomes, have identified nocturnal species of macroinvertebrates and amphibians as drivers of bioturbation (Zanetell and Peckarsky 1996; Statzner et al. 2003). Predaceous stoneflies increased sediment removal from the streambed while searching the rocks and sediments for prey (Zanetell and Peckarsky 1996). Crayfish and amphibians, such as tadpoles, suspend particles through predator avoidance, conspecific aggression, and feeding at night (Creed and Reed 2004; Ranvestel et al. 2004; Usio and Townsend 2004), but spend much of the day in burrows or under rocks and other refugia (Petranka 1984; Statzner et al. 2000). Key fish species, in both tropical and Alaskan streams, cause appreciable sediment and POC suspension and downstream export through

movement, migration, spawning, feeding, and egestion (Moore et al. 2004; Taylor et al. 2006).

In WCC, the nocturnal community that could contribute to bioturbation of the stream sediments includes 104 species of mayflies (Ephemeroptera), 45 species of stoneflies (Plecoptera) (J. K. Jackson unpubl.), two species of crayfish (D. A. Lieb pers. comm.), and three species of salamanders (E. H. Grant unpubl.). Fish probably do not drive the diel pattern because, of all 20 fish species in WCC, only eels are nocturnal (R. J. Horwitz pers. comm.). Furthermore, the diel patterns occurred throughout the watershed, including several 2^{nd} order streams (<10 cm deep) that would exclude large fish such as trout or sunfish.

Seasonal patterns – Warm season peaks of POC and seston concentrations have been attributed to increased macroinvertebrate fragmentation of leaf litter in other deciduous forest streams in North Carolina (Webster et al. 1990; Wallace et al. 1991) and Spain (Molla et al. 2006). Similarly, I observed peak concentrations during the warm seasons in addition to the consistently observed diel patterns (Fig. 1.2a, b). The peaks, in spring and summer, can be explained by stream organism activity, such as feeding and bioturbation, because maximum macroinvertebrate activity and biomass occurs during the warm seasons (Wallace et al. 1991). Bioturbation likely helps control seasonal patterns because the organic content of seston decreased during the warm months (Fig. 1.2c), indicative of bioturbation rather than feeding behaviors (sloppy feeding, egestion). Filter feeding macroinvertebrates could decrease the

carbon content of seston by selectively feeding on high organic content particles (Parkes et al. 2004), but are likely not responsible for ecosystem-level decreases in POC content. Monaghan et al. (2001) showed that filter feeders are only responsible for 11% of particulate organic matter removal from the water column in an Idaho stream.

Seasonal changes within the deciduous riparian vegetation appeared to drive seasonal patterns of seston quality. In late October and early November, WCC receives 40-50% of annual organic matter fall-in (313 g m^{-2} yr^{-1}, Newbold et al. 1997), while in late winter and early spring, the open canopy, increasing hours of daylight and warming temperatures are conducive to the growth of long algal filaments on rocks and sediments (Kaplan and Bott 1982). The two seasonal peaks in carbon content coincided with autumnal leaf litter inputs and vernal algal blooms (Fig. 1.2c). Despite the seasonal changes in quality, the overall carbon content (9% to 15%) was still low compared to organic-core material like leaves, wood or algae (~45%), but higher than seston in large rivers with high sediment loads (~1-3%; Hedges et al. 1986; Onstad et al. 2000). Seston is likely a mixture of these two types of particles (organic-core and mineral-core, Meybeck 1982), with peaks in organic-core particles occurring in autumn and spring (Table 1.1). Mineral-core particles comprised most of total seston mass (Table 1.1); microscopic analysis showed that most seston particles are clays or empty diatom frustules (Fig. 1.9). However, organic-core particles, despite being a relatively small fraction of total seston mass

and rarely seen in microscopic analysis (Fig. 1.9), still represent a large fraction of carbon flux because of their high carbon content (Table 1.1).

Autochthonous sources provide a fraction of organic-core particles in seston; it is necessary to convert pigment concentration to algal carbon to determine the proportion of POC from autochthonous carbon. If a fixed C:Chl *a* ratio of 30 (Smith 1980) is assumed, algal carbon would vary between 70% of POC during early spring and 30% during the summer. However, C:Chl *a* ratios vary with growth rate, irradiance, temperature, and nutrient concentration (Cloern et al. 1995) and pheopigments, degradation products of Chl *a* including Pheo *a*, are not included in these calculations. This is problematic because pheopigments represent algal-derived carbon from old, dying cells sloughed from epilithon (Peterson and Stevenson 1992), or are otherwise suspended by stream organism activity including bioturbation, herbivory, and filter-feeding egestion (Downs and Lorenzen 1985). Microscopic examination of WCC seston revealed that, of the few particles that were algal cells, most were individual diatoms and relatively few of these had retained their chloroplasts (Fig. 1.9). Thus, two types of algal cells appear to exist in the suspended load: fresh algal cells or older, senesced and grazed cells which likely have different algal carbon:pigment ratios (Downs and Lorenzen 1985). Therefore, algal contributions to POC may exceed the preliminary estimate of 30-70%. More work is needed to accurately estimate the contribution of algal carbon to downstream POC transport by determining the temporal variation of C:pigment ratios in seston and epilithon on the streambed.

Studies from large rivers (Kendall et al. 2001) and streams (Walters et al. 2007) are consistent with observations that POC is isotopically enriched in spring and depleted in the late fall months (Fig. 1.7). Autumnal POC-δ^{13}C was even depleted relative to whole deciduous leaves (-28.2‰, Fig. 1.7) suggesting that there was a selective preservation of lignin (~-30‰) relative to proteins and cellulose (~-27‰) (Benner et al. 1987). As much as 25% of leaf carbon leaches within the initial 24 hours of wetting (Lush and Hynes 1973) with lignin remaining as a considerable portion of leaf carbon available for physical and biological fragmentation (Benner et al. 1987). The spring samples reflect a greater δ^{13}C epilithon signal (Fig. 1.7). However, algal δ^{13}C is known to vary spatially as a function of the concentration of dissolved free CO_2, the δ^{13}C of dissolved inorganic carbon, water-velocity effects on cell-wall CO_2 gradients, and primary production rates, which in turn depend on position within the watershed relative to carbonate lithologies and on reach morphology (Finlay et al. 1999; Walters et al. 2007). In this study, I sampled an autochthonous source from only one riffle at one time. Temporally and spatially explicit samples of epilithon are necessary to determine the algal δ^{13}C contributions throughout the WCC watershed.

Hydrologic patterns – Most POC and seston export from WCC occurs during storms (Newbold et al. 1997), as is typical of small streams (Golladay et al. 2000). However, during storms, seston is transported long distances downstream (Mosisch and Bunn 1997) and carbon content decreases, lowering POC quantity and quality available for

local biological use. Baseflow transport, though comprising a smaller proportion of the total load, delivers upstream POC to relatively close reaches downstream (Thomas et al. 2001). Furthermore, seston and POC concentrations were not correlated with baseflow discharge. From measurements of particle deposition velocities (0.1 to 0.4 mm s^{-1}; Thomas et al. 2001), WCC mean baseflow water velocity (0.12 m s^{-1}), and depth (0.10 m), I can calculate that, at baseflow, particles travel between 31 to 124 meters on average before they are deposited on the streambed. Furthermore, WCC is at baseflow 90% of the time (Newbold et al. 1997), when most heterotrophic respiration occurs. Therefore, I focused on baseflow transport of seston and POC.

Hydrology can still influence baseflow seston concentrations. A large storm or series of successive storms can deplete the seston supply by removing readily suspended particles from the streambed, and clearing rocks and sand of epilithon (Carling 1983; Van Sickle and Beschta 1983). As a result, POC concentrations are higher during baseflow periods following infrequent storms rather than frequent scouring floods (Pozo et al. 1994). In this study, the magnitude, duration and time since the antecedent storm could not adequately explain the baseflow concentrations or characteristics of seston. However, the time since the antecedent storm did influence the diel variation in seston suggesting that large antecedent storms reduced the amount of seston available for nighttime increases in concentration in the days following the storm (Fig. 1.5).

Implications for energy flow – In a tropical stream, bioturbation increased downstream POC flux by 60% (Taylor et al. 2006), similar to the observation of 45% increases in nighttime POC fluxes in WCC (Fig. 1.2d). For extrapolation to annual POC fluxes, many studies typically use flow-weighted concentrations or rating curves derived from baseflow samples (Golladay 1997). However, I demonstrate here that this approach is inappropriate for estimating temporal dynamics or magnitudes of POC fluxes at baseflow. For example, using only daytime POC concentrations in WCC, the annual baseflow POC transport fluxes for the sampling period would be 337 kg POC yr^{-1}; however, integrating over day and night concentrations, the annual baseflow POC transport flux is 414 kg POC yr^{-1}. Without consideration of nighttime differences in POC concentration, annual baseflow POC flux would be underestimated by 23%.

My quantitative and qualitative geochemical analyses of particles in transport suggest that temporal patterns in seston and POC concentration and composition were generated by nocturnal bioturbation and seasonal cycles of leaf fall and algal production. These results argue that the traditional ecological perspective of POC as fragmented and egested leaf-litter (Vannote et al. 1980) requires modification to include mineral-core and algal-derived particles as components of the suspended load. Furthermore, the results detail variability in POC fluxes and quality (i.e. carbon content of seston), which controls carbon bioavailability (Baisden et al. 2002) and should ultimately influence rates of heterotrophic respiration in downstream rivers (Cole and Caraco 2001; Mayorga et al. 2005). Future work is needed to advance the

understanding of the role of river networks in controlling the transformations of allochthonous and autochthonous POC transported from inland waters to the oceans.

Tables

Table 1.1. Mixing model results presented as the percent of seston and POC concentration by mass (mean ± SE), where *m* represents mineral-core and *o* represents organic-core particles, and $p<0.05$ indicates significant difference between high organic matter input months (Nov, Dec, Mar) and the rest of the year from paired t-tests (df=75 for each test).

% of mass	Type of particle	Nov, Dec, Mar	Other months	*t*-test *p*-value
seston	*m*	70% ± 1.4%	81% ± 0.4%	<0.001
seston	*o*	29% ± 1.4%	18% ± 0.4%	<0.001
POC	*m*	5% ± 0.4%	9% ± 0.2%	<0.001
POC	*o*	94% ± 0.4%	90% ± 0.2%	<0.001

Figure Captions

Figure 1.1. Hourly discharge of White Clay Creek over the sampling period. Arrows and circles indicate sampling days (11 Aug 05, 21 Sep 05, 01 Nov 05, 07 Dec 05, 12 Jan 06, 24 Feb 06, 31 Mar 06, 27 Apr 06, 24 May 06, 20 Jun 06, 26 Jul 06). Note the break in the y-axis scale to account for one large storm in June 2007.

Figure 1.2. Seasonal changes in (a) seston concentration, (b) particulate organic carbon concentration, (c) seston carbon content, and (d) particulate organic carbon flux for day and night samples. The means were plotted against julian day because of uneven intervals between sampling periods, but were identified as the month in which they were sampled. Error bars are standard errors; some cannot be seen when smaller than the symbol.

Figure 1.3. Example diel curve from June sampling of seston concentration (left vertical axis) and particulate organic carbon (POC) concentration (right vertical axis).

Figure 1.4. Least squares linear regressions between temperature and seston concentration for both day (seston = 0.07 Temperature + 0.78, r^2=0.45, p=0.02) and night (seston = 0.18 Temperature + 0.83, r^2=0.45, p=0.02) samples.

Figure 1.5. Least squares linear regressions between the maximum discharge of the antecedent storm and the increase of total seston concentration from day to night for each sampling month (% Increase = – 0.09 Max Discharge + 119, r^2=0.61, p=0.005).

Figure 1.6. Seasonal changes in (a) Chl *a* and (b) Pheo *a* concentrations for day and night samples. The means were plotted against julian day because of uneven intervals between sampling periods, but were identified as the month in which they were sampled. Error bars are standard errors; some cannot be seen when smaller than the symbol.

Figure 1.7. Seasonal changes in $\delta^{13}C$ of POC averaged across day and night samples. The means were plotted against julian day because of uneven intervals between sampling periods, but were identified as the month in which they were sampled. Error bars are standard errors; some cannot be seen when smaller than the symbol. Boxes (mean ± 1 SD) represent the range of $\delta^{13}C$ of (a) epilithic algae as autochthonous and (b) four species of senesced leaf species as allochthonous end-members.

Figure 1.8. Least squares linear regressions between total pigment concentrations (Chl *a* + Pheo *a*) and $\delta^{13}C$ of POC ($\delta^{13}C$ = 0.35 pigments – 29.2, r^2=0.49, p=0.015).

Figure 1.9. Environmental scanning electron microscope images of seston. (a) Most particles were small angular clays and silts with adsorbed bacteria or organic matter, while (b) other particles were recognizable as diatoms cells (e.g. Pinnularia).

Figures

Figure 1.1

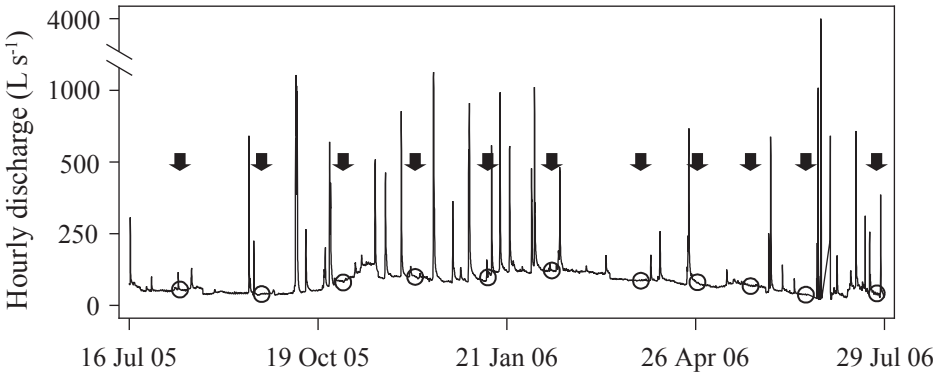


Figure 1.2

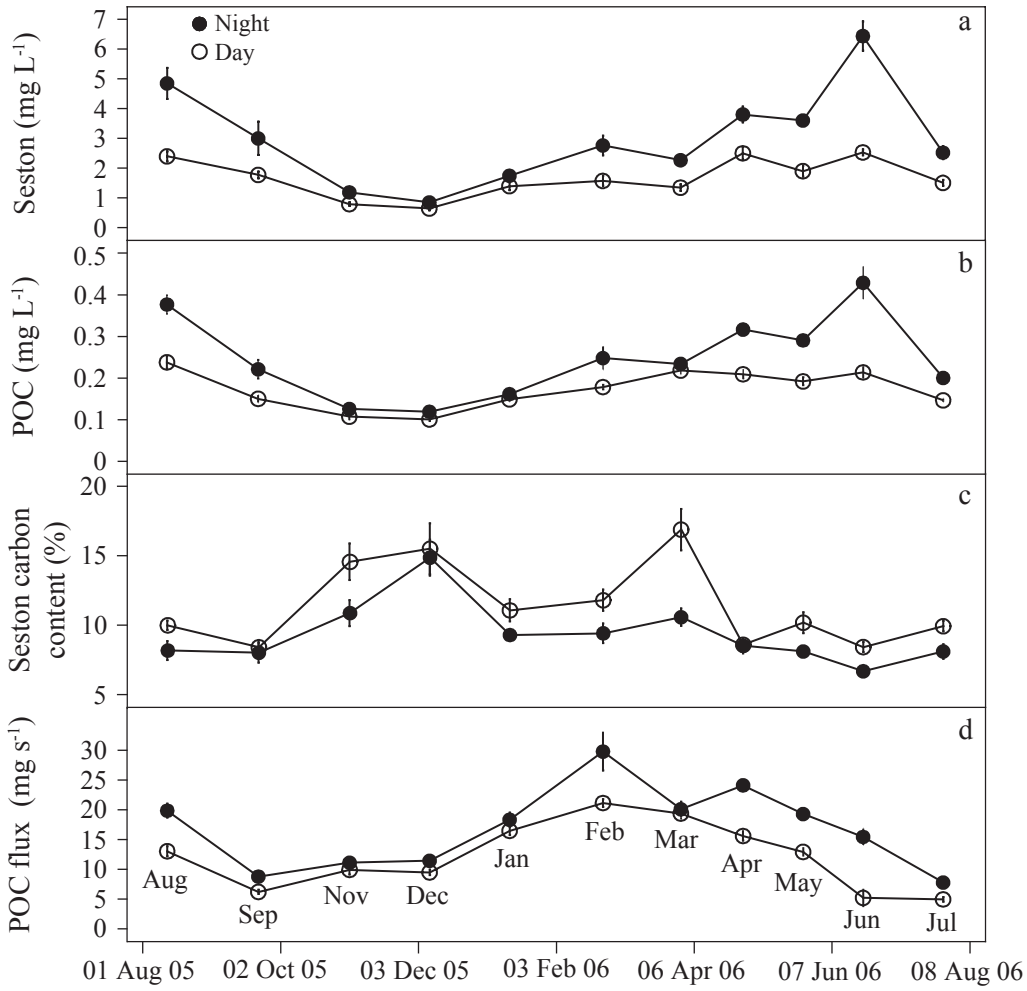

Night
Day
a
b
c
d
Seston (mg L-1)
POC (mg L-1)
Seston carbon content (%)
POC flux (mg s-1)
Aug
Sep
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
01 Aug 05
02 Oct 05
03 Dec 05
03 Feb 06
06 Apr 06
07 Jun 06
08 Aug 06

Figure 1.3

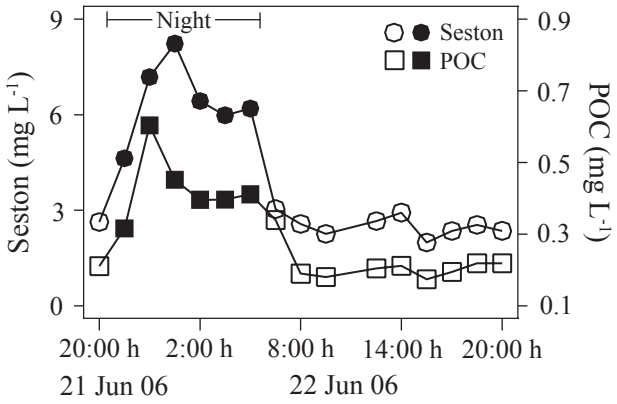

Night
Seston
POC
Seston (mg L-1)
POC (mg L-1)
9
6
3
0
0.9
0.7
0.5
0.3
0.1
20:00 h
2:00 h
8:00 h
14:00 h
20:00 h
21 Jun 06
22 Jun 06

Figure 1.4

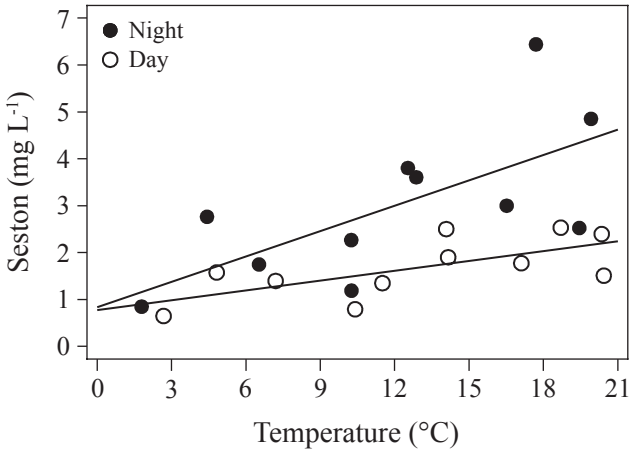

Night
Day
Seston (mg L-1)
Temperature (°C)
0
1
2
3
4
5
6
7
0
3
6
9
12
15
18
21

Figure 1.5

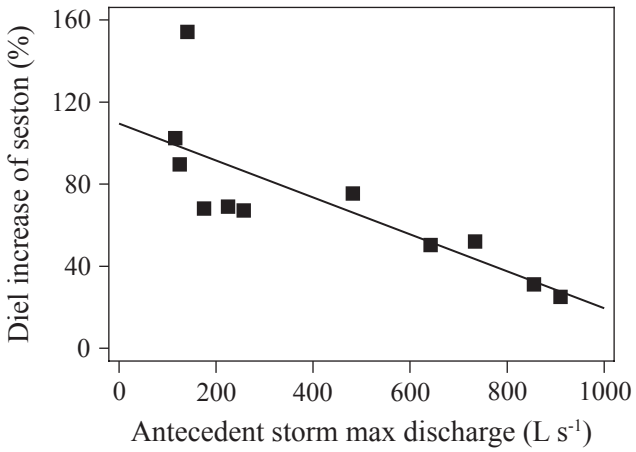

Diel increase of seston (%)
160
120
80
40
0
0
200
400
600
800
1000
Antecedent storm max discharge (L s-1)

Figure 1.6

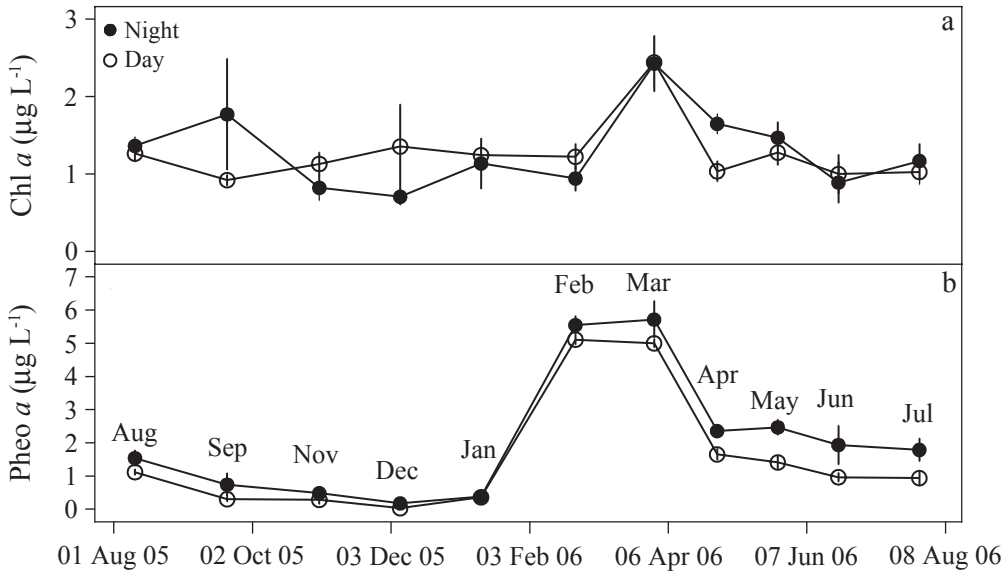

Night
Day
a
b
Chl a (µg L-1)
Pheo a (µg L-1)
Aug
Sep
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
01 Aug 05
02 Oct 05
03 Dec 05
03 Feb 06
06 Apr 06
07 Jun 06
08 Aug 06

Figure 1.7

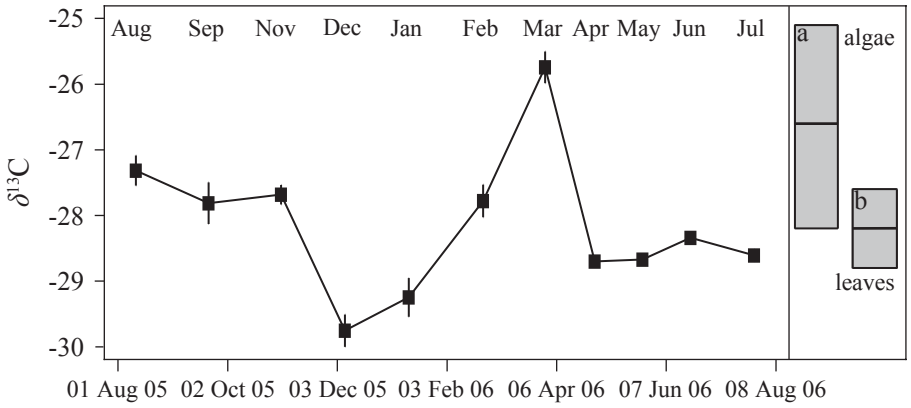

-25
-26
-27
-28
-29
-30
$\delta^{13}C$
Aug
Sep
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
01 Aug 05
02 Oct 05
03 Dec 05
03 Feb 06
06 Apr 06
07 Jun 06
08 Aug 06
a
algae
b
leaves

Figure 1.8

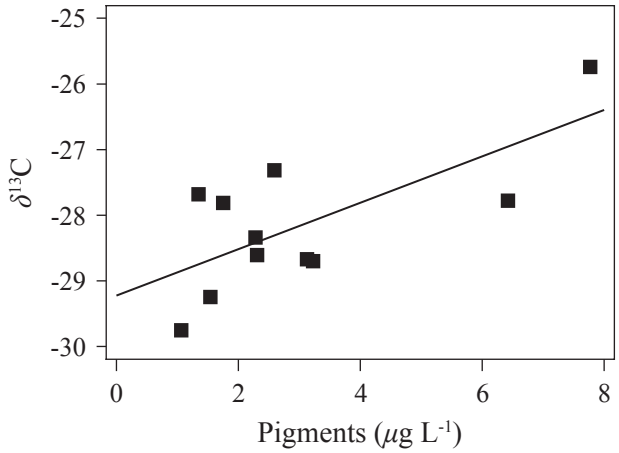
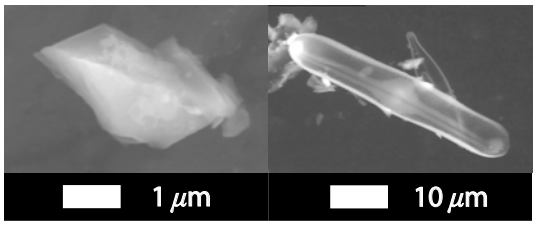


Figure 1.9



Chapter 2: Contributions of salamanders and macroinvertebrates to seston export in artificial stream ecosystems

Introduction

Particulate organic carbon (POC) provides energy for microorganisms and macroinvertebrates in headwater streams (Hedin 1990; Mulholland et al. 2000), and when transported in the suspended load (as seston), supports heterotrophic metabolism in downstream reaches and rivers (Wipfli and Gregovich 2002; Cole and Caraco 2001). Stream organisms can control the movement of seston through retention by filtration (Wotton et al. 1996), generation of particles by sloppy feeding and egestion (Wallace et al. 1991), or bioturbation that suspends particles during nest digging, foraging, and movement (Moore 2006). While the ecosystem-level effects of bioturbation have been studied extensively in soft-sediment environments of oceans, lakes, and rivers (Mermillod-Blondin and Rosenberg 2006; Meysman et al. 2006; Nogaro et al. 2006), relatively few studies have addressed this issue in streams, despite the fact that stream organisms are known to impact bottom sediments (Jones et al. 1997; Palmer et al. 1997). For example, predaceous stonefly larvae can disturb streambed sediment while searching for prey (Zanetell and Peckarsky 1996). Crayfish and amphibians, particularly tadpoles, may suspend particles through predator avoidance, conspecific aggression, and feeding (Creed and Reed 2004; Ranvestel et al. 2004; Usio and Townsend 2004). Through their movement, migration, feeding, and spawning activities, key fish species suspend sediment and

POC which is then exported downstream in both tundra and tropical streams (Moore et al. 2004, Taylor et al. 2006).

In a southeastern Pennsylvania piedmont stream, daily fluctuations in seston transport included 80% increases in nighttime seston concentrations and 43% increases in nighttime POC concentrations (Chapter 1). The macroinvertebrate and salamander communities may contribute to the observed nighttime increase of seston through bioturbation of bed sediments because of their nocturnal behavior and high streambed population densities (Moore 2006). Macroinvertebrates and salamanders both exhibit diel patterns in their activities on the streambed. Many macroinvertebrates are nocturnal, moving to the surface of streambeds to feed on detritus, periphyton, and other organisms at night (e.g. Schloss and Haney 2006) and often exhibiting peak downstream drift during nighttime hours (Brittain and Eikeland 1988). Similarly, many salamanders are most active at night as they search for macroinvertebrate prey on the sediment surface (Petranka 1984). Macroinvertebrates, excluding crayfish, are small in body size relative to other stream organisms such as fish and amphibians, but their densities exceed those of larger organisms by several orders of magnitude, suggesting they could have a larger cumulative effect on seston transport. Previous work has linked amphibian activity with bioturbation of streambed sediments (Ranvestel et al. 2004), and salamander larvae can reach considerable densities in small streams, where they often replace fish as dominant predators (Davic and Welsh 2004). However, I have not found any work that has focused on the effect of salamander feeding and foraging activity on ecosystem level

processes, despite their high diversity, density, and predator position in the food chain of small order streams.

Here, I test the hypothesis that bioturbation by salamanders and stream macroinvertebrates is a mechanism for nighttime increases in seston transport. Using stream-side flumes, I measured the concentration of seston over four consecutive days and nights in both the presence and absence of macroinvertebrates and salamanders. I also measured suspended chlorophyll *a* to determine if the seston was a byproduct of grazing or sloughing from within the autochthonous biofilm (Peterson and Stevenson 1992). Throughout the experiment, I describe the percentage of seston export and average seston transport distance as metrics for bioturbation and particle suspension within the artificial streams. I show significant daytime increases in seston transport in experimental treatments with salamanders. However, macroinvertebrates did not affect seston transport and there were no patterns in suspended chlorophyll *a* (Chl *a*) concentrations.

Methods

Experimental streams – I conducted a four day experiment in two stream-side flumes at the Stroud Water Research Center, in southeastern Pennsylvania in September 2006. The two flumes (30m long; 0.3 m wide; 0.04 m water depth; approx. 3 cm s^{-1} water velocity; surface area = 9 m^2) were filled with stones (approximately 5.7 cm median diameter) that overlaid a sand bed (8 mm median diameter, "hyporheic zone") (Fig. 2.1a; Battin et al. 2003). Prior to the experiment, all the stones were power-washed and the streambed sand was stirred while the flumes were running to remove

particles and macroinvertebrates and to ensure minimal differences in sediment accumulation between flumes at the start of the experiment. The flumes were gravity-fed stream water from White Clay Creek (WCC) from one shared header tank and run in continuous through-flow mode at ~0.5 L s^{-1} for 21 days before the experiment began to allow periphyton to colonize the rocks and seston, from storms and baseflow within WCC, to fill interstitial spaces (Battin et al. 2003).

Experimental design – The experiment was run for four consecutive days with different treatments in each flume and during each day (Table 2.1). The first treatment was a control, with no added macroinvertebrates or salamanders to either flume to compare the behavior of the two flumes. For the second treatment, I added macroinvertebrates freshly collected from WCC to flume 1 (see *macroinvertebrate and salamander collection*) to examine the effects of the diel behavior of macroinvertebrates on particle transport. For the third treatment, I added a macroinvertebrate community to flume 2, without removing any macroinvertebrates from flume 1 to ensure both flumes had macroinvertebrates when I added the salamanders. For the fourth and final treatment, I added salamanders (see *macroinvertebrate and salamander collection*) to flume 1 to examine the effect of diel behavior of salamanders on particle transport. After organism additions, 16:00 to 18:00 on the afternoon preceding nighttime sampling, salamanders and macroinvertebrates could be observed moving on and between the rocks. Between 4-6 hours passed before I began sampling for each treatment. I collected water samples at three time points per night (22:00, 1:00, 4:00) and day (10:00, 13:00 and 16:00) to

measure seston and suspended Chl *a* (see below for sampling procedure). The sampling design allows for two experimental comparisons of day/night and treatment differences (Table 2.1); thus, hereafter Experiment A includes the initial 48 hours (treatments 1 and 2) and Experiment B the final 48 hours of manipulations (treatments 3 and 4).

Macroinvertebrate and salamander collection - WCC drains a temperate deciduous basin (7.3 km^2) of agricultural and forested piedmont land and harbors an abundant biotic community (Newbold et al. 1997). For each of the two macroinvertebrate additions (treatments 2 and 3), I collected macroinvertebrates from Surber samples (50 x 50 cm opening, 85 cm net length, 250 μm mesh size) in 3 WCC riffles with a total sample area approximately equal to half of the bed area of a flume (4.5 m^2). I supplemented the Surber samples with 18+ D-net jab samples collected along the stream banks, debris, and pools. The Surber and D-net samplers are two standard collecting devices for stream macroinvertebrates involving disturbing the bottom sediments and catching organisms in a net held downstream. Surber samplers are used for quantitative analysis of macroinvertebrate density using a square sampling frame to border the sampling area. For each of the two macroinvertebrate additions (treatments 2 and 3), I added ~17,000 individuals for a final density of 1800 individuals m^{-2}. Families that appeared the most frequently included water pennies (Coleoptera: Psephenidae), riffle beetles (Coleoptera: Elmidae), chironomids (Diptera: Chironomidae), crawling mayfly larvae (Ephemeroptera: Ephemerellidae), clinging mayfly larvae (Ephemeroptera: Heptageniidae), portable-case caddisfly

larvae (Trichoptera: Brachycentridae) and net-spinning caddisfly larvae (Trichoptera: Philopotamidae; Trichoptera: Hydropsychidae).

Macroinvertebrates from all samples were held for a maximum of 8 hours at stream water temperature before they were added to the flumes. I used a swirl and decant technique (Palmer et al. 2006) to collect organisms from a 5 gallon bucket and then distribute them at 3 meter intervals along the length of each flume. Crayfish were excluded from the macroinvertebrate additions because of their size and to limit the experiment to testing differences between salamander and small macroinvertebrate additions. Flume outflow passed continuously through a 250 μm sieve to collect drifting macroinvertebrates and salamanders.

For the salamander addition treatments, two-lined salamander larvae (Fig. 2.1b: *Eurycea bislineata*) were collected by turning over rocks and wood within several 2nd order tributaries to WCC and in the 3rd order stream itself. Salamanders were placed individually into bags filled with stream water and held in coolers streamside for <8 hours. At sunset following treatment 3, I released 89 salamanders at intervals of 0.1 m from the first meter to the 29th meter of flume 1 for a final salamander density of 9.9 individuals m^{-2}. I added realistic densities of two-lined salamander larvae to the artificial flumes; similar salamander species such as the Blue Ridge Two-lined salamander (*Eurycea wilderae*) have been shown to have densities between 8.6 and 10.1 individuals m^{-2} (Davic and Welsh 2004).

Seston – At each sampling time, water samples were taken from the inflow to the flumes (header tank outflow) and the outflows of flume 1 and flume 2. In the laboratory, seston concentration was determined gravimetrically using the dry weight of total suspended solids. Aliquots of stream water were filtered through stacked pairs of pre-weighed and pre-ashed (480ºC for 4.5 hours) glass fiber filters (Millipore AP40 with nominal pore-size of 0.7 μm). The sample was filtered through two filters to check analytical accuracy, assess the integrity of the top filter and ensure even distribution of seston across the filter. The sample was discarded if the bottom filter gained or lost more than 0.2 mg. The filters were dried for 12-18 hours at 60ºC and re-weighed to calculate seston concentration (mg seston L^{-1}).

Seston outflow concentration (S_{out}) was divided by inflow concentration (S_{in}) to determine the percentage of seston (P_{out}) exported from each flume (eq. 2.1). The net seston transport distance (S_w), the average length of travel before deposition, was calculated using S_{in}, S_{out} and the length (L) of the flume (eq. 2.2; Battin et al. 2003).

Equation 2.1 $$P_{out} = 100 \times (S_{out} / S_{in})$$

Equation 2.2 $$S_w = L / \ln(S_{in} / S_{out})$$

Pigments – Measured volumes of water for suspended Chl *a* concentrations were filtered through a glass fiber filter (Millipore AP40). The filters were frozen and processed within 30 days using a modified hot ethanol extraction method (Biggs and

Kilroy 2000). The samples were heated to 78ºC for 10 minutes in 90% ethanol and then stored frozen for 24 hours. Chl *a* concentrations ($\mu g\ L^{-1}$) were calculated using measurements of absorbance on a spectrophotometer (Beckman Coulter DU-640) before and after acidification (Lorenzen 1967; Biggs and Kilroy 2000).

Statistical analysis – All statistical analyses were conducted using SAS v9.1 (Level 1M3, 2007, SAS Institute Inc., Cary, NC, USA). I analyzed the data as two separate experiments (Table 2.1) testing the effects of macroinvertebrate additions in experiment A and the effect of salamander additions in experiment B. For each experiment, I performed BACI three-way ANOVAs (proc GLM) for seston outflow concentration, percent seston exported, transport distance, Chl *a* outflow concentration and percent Chl *a* exported with day or night, treatment and flume as fixed effects (Skalski and McKenzie 1982; Smith 2002). Three- and two-way interaction terms were removed from the model if the effects were non-significant. I acknowledge limitations in the statistical analysis due to potential serial auto-correlation and lack of independence between samples, treatments, and experiments (see Smith et al. 1993). However, the BACI model is most effective if the variance in the difference between flumes does not change over sampling time (Smith et al. 1993) and I only saw random fluctuations in the variance.

Results

Macroinvertebrate and salamander additions – Within 3 hours of each addition, I captured a small number of macroinvertebrates (<50) in the outflow, but added those immediately back to the top of the flume. In the flumes without macroinvertebrates

additions, I captured no individuals in the outflow. After the initial drift, fewer than 4 individuals hour^{-1} were captured in the flume outflow. Throughout the course of the experiment, no salamanders were recovered in the flume effluent and following the experiment, I recovered >95% of the salamanders during a thorough search of the flume.

Flume conditions – The inflow seston concentration was greater at night than day during both experiments (Exp 1: $F_{1,20}$=8.1, p=0.0099; Exp 2: $F_{1,20}$=23.6, p<0.0001) and there was a trend of decreasing inflow concentrations throughout experiments A and B (Fig. 2.2a). WCC experienced storm flows 36 hours before the start of the treatment 1 and discharge within the creek slowly decreased from 80 L s^{-1} to 65 L s^{-1} during the four day experiment. Inflow seston concentrations were lowest in the day during treatment 4 and highest in the night during treatment 2 (Fig. 2.2a). Flume 1 discharge was constant (0.4 L s^{-1}); however, flume 2 discharge declined by 40% during the last two treatments (0.5 L s^{-1} to 0.3 L s^{-1}) (Fig. 2.3).

Seston, experiment A (treatments 1 and 2) – Nighttime seston outflow concentration (0.68 mg L^{-1} ± 0.02: mean ± SE) exceeded daytime seston outflow concentration (0.46 mg L^{-1} ± 0.02: mean ± SE) by 48% (Fig. 2.2b; $F_{1,20}$=38, p<0.0001) following the diel patterns of the flume inflow concentrations. However, there were no other significant effects for seston outflow concentration, % seston exported or transport distances when considering diel macroinvertebrate activity (Fig. 2.2b, c, d).

Seston, experiment B (treatments 3 and 4) – All three-way interactions were non-significant and removed from the ANOVA model; I examined 2-way interactions and main effects (flume, day/night, treatment 3 vs. 4) for seston outflow concentration, percent seston exported, and transport distances. Seston outflow concentration (Fig. 2.2b) treatment effects are superimposed on inflow concentration effects resulting in significant treatment by flume ($F_{1,17}$=8.1, p=0.01), day/night by flume ($F_{1,17}$=4.9, p=0.04), and treatment by day/night ($F_{1,17}$=10.0, p=0.0006) interaction effects. However, because the inflow concentration had a diel pattern with nighttime peaks, examining metrics that correct for inflow concentration (seston transport distance and percent seston exported) was a more effective approach when examining the diel effects of salamander additions on seston transport. Both seston transport distances and percent seston exported still had significant treatment by flume (Fig. 2.4a, b) and treatment by day/night (Fig. 2.4c, d) interactions. These interaction effects were driven by a 44% and 30% increase in percent seston exported (29.3% ± 2.2: mean ± SE) and S_w (24.5 m ± 1.5: mean ± SE), respectively, during the daytime results of the salamander addition (Fig. 2.2c, d).

Chlorophyll a – Chl *a* outflow concentration (Fig. 2.5a; 0.79 μg L^{-1} ± 0.05: mean ± SE) was more variable than seston data, but with few clear patterns throughout experiments A and B. Chl *a* only had a significant main effect in experiment B when daytime outflow concentration was greater than nighttime concentration ($F_{1,20}$=4.9, p=0.04), but there were neither treatment nor flume effects. On average, the flumes

exported 4% more suspended Chl *a* than they received (Fig. 2.5b) with percentage of Chl a export averaging 104 % ± 8 (mean ± SE).

Discussion

S*alamander effect* – Salamanders did not increase the nighttime export of seston as I predicted, but did increase daytime export of seston from the artificial flumes (Fig 2c, 2d). Salamander activity is likely to result in the suspension of particles because they are large stream organisms, cover considerable areas in their movement and actively search for macroinvertebrate prey (Petranka 1984). However, the 9% increase in seston export was only a fraction of the nighttime increase in seston concentration (80%) that was seen in WCC (Chapter 1) or in the inflow concentration during the day and night of treatment 4 of this experiment (Fig. 2.2a: 39% nighttime increases of seston concentration).

The increase in seston transport due to salamander additions during the day in treatment 4 was surprising given nocturnal salamander activity patterns measured in other studies (Petranka 1984). Salamanders might have increased daytime activity because they were released from visual stimulus of predatory fish. Stream and pond macroinvertebrates and amphibians will be nocturnal in the presence of predatory fish and diurnal when released from this visual pressure (Flecker 1992; Hampton and Duggan 2003). Alternatively, it is possible I did not capture normal nocturnal salamander activity because the salamanders were added to the flume only four to five hours before the first nighttime measurement. After experiencing handling stress for up to 8 hours and removal from the stream (translocation), the salamanders may

not have resumed typical behavior (Matthews 2003; Artigas et al. 2005). Salamanders could have been recovering from handling during the daytime and been digging under rocks and in the sediment to establish refugia or search for prey. Allowing more time for salamanders to recover might have been needed for a realistic reproduction of normal diel behavior patterns.

Macroinvertebrate effect – I added ~1800 individuals m^{-2} to the flumes, densities that are comparable to lower ranges of densities of macroinvertebrates found in northeastern deciduous streams and WCC (Kratzer et al. 2006; J.K. Jackson pers. comm.). I added a diverse array of functional feeding groups representative of the stream community including predators, scrapers, shredders, collectors-filterers, and collector-gatherers (Merritt and Cummins 1995). Despite the addition of a diverse macroinvertebrate community and particles available for suspension to the flumes, I found no significant effect of macroinvertebrates on seston concentration. The only small macroinvertebrates (i.e. excluding shrimp and crayfish) that have been documented to cause particle suspension in stream ecosystems are large predatory stonefly larvae that actively search for their prey on stream sediments (Zanetell and Peckarsky 1996). The additions included very few of these macroinvertebrates (0.1% of all individuals). Furthermore, in the study conducted by Zanetell and Peckarsky (1996), the stonefly larvae were examined in small in-stream experiments designed to maximize bioturbation effects. Therefore, given the low density of predatory macroinvertebrates that may have contributed to bioturbation, macroinvertebrates

additions did not increase seston concentration, export or transport distances (Fig. 2.2b, c, d: Experiment A).

Non-treatment effects – Other than the treatment effects, inflow concentrations of seston, flow velocity and discharge can also affect seston transport within the artificial streams. I saw patterns in the inflow concentrations that reflect the diel patterns of seston in WCC (Fig. 2.2a; Chapter 1). The inflow concentrations, in turn, caused diel variation in the seston outflow concentration (Fig. 2.2b). The inflow concentrations also declined throughout the experiment (Fig. 2.2a) due to decreasing discharge in WCC following a storm ~36 hours prior to the beginning of treatment 1. The outflow seston concentrations mirror inflow concentrations resulting in nighttime peaks and overall decline in outflow concentration in both experiments A and B (Fig. 2.2b). However, I was able to correct for inflow effects by using the percent seston exported and transport distance metrics.

The flow within each artificial stream could also affect the deposition and outflow concentrations. For example, seston transport distances were closely correlated with discharge throughout a range of different stream sizes (Thomas et al. 2001; Paul and Hall 2002). Unfortunately, the flume discharge decreased throughout experiment B in flume 2 (Fig. 2.3). If the discharge in flume 2 had remained constant, the percent of seston exported and S_w for flume 2 might have been higher, especially in treatments 3 and 4. However, there seemed to be little effect of flow because the outflow concentration of seston of flume 2 continued to match flume 1

(Fig. 2.2b). There was only real divergence between measurements in the two flumes in the daytime of treatment 4 (Fig. 2.2b). Regardless, the BACI statistical design cannot account for external effects other than treatment effects (Smith et al. 1993), so the statistical results should be viewed with caution.

Chlorophyll a – I used Chl *a* as an indicator of algae in the suspended load (Elliott et al. 2004). In previous studies in these flumes, the biofilm grew for ~25 days before the algal biomass was limited by biofilm detachment or grazing (Battin et al. 2003). In both experiment A and B, Chl *a* outflow often exceeded Chl *a* inflow (Fig. 2.5b) indicating production and senescence of algal particulate matter within the flumes. Chl *a* export did not increase even during the daytime of treatment 4 (Fig. 2.5b) indicating that the additional particles from salamander bioturbation were more likely derived from stream sediments than grazed or sloughed periphyton.

Directions for future research – Although previous work has demonstrated amphibian bioturbation (Ranvestel et al. 2004), this is the first effort to demonstrate stream salamander contribution to ecosystem level processes such as modification of seston and organic carbon flow. Stream-associated species account for approximately 35% of total amphibian diversity in the United States (Davic and Welsh 2004), and up to 45% of all salamander species rely on streams for part or all of their life cycle (Lannoo 2005). Despite the significance of stream-associated amphibians (Davic and Welsh 2004; Lannoo 2005), knowledge of their ecosystem-level importance is

lacking (Corn et al. 2003). Their activity, population density, and size make them ideal candidates for influencing transport of seston and should be considered further.

I could modify future experiments to test whether results from salamander and macroinvertebrate additions were artifacts of experimental design or true effects. I could improve the statistical power by using replicated flumes for each treatment, allow the flumes to equilibrate for 6 months or more (inflow concentration = outflow concentration; J. D. Newbold pers. comm.), and maintain constant discharge throughout the experiment. Furthermore, I could add more macroinvertebrates, to better mimic the higher densities measured in WCC (up to 25,000 individuals m^{-2}; J. K. Jackson pers. comm.). The 10-fold increase in individuals would include more relevant individuals (i.e. mobile predators; Zanetell and Peckarsky 1996), although at similar proportions to the community in this study. Also, it is probably necessary to allow the salamanders more time to acclimate to the artificial streams and recover from handling stress and translocation. Finally, other nocturnal stream organisms might contribute to the bioturbation of the sediments. For example, crayfish suspend particles from the streambed through movement related activity, including predation, conspecific aggression, and feeding activities (Creed and Reed 2004, Usio and Townsend 2004). Including crayfish additions in the experiment might allow me to further understand mechanisms for nighttime increases in seston transport occurring in WCC.

Tables

Table 2.1. Experiment design for the four day experiment. 'Macroinverts' represents the macroinvertebrate community excluding crayfish.

	Experiment A				Experiment B			
	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Night	Day	Night	Day	Night	Day	Night	Day
Flume 1	No Macroinverts No Salamanders		Macroinverts No Salamanders		Macroinverts No Salamanders		Macroinverts Salamanders	
Flume 2	No Macroinverts No Salamanders		No Macroinverts No Salamanders		Macroinverts No Salamanders		Macroinverts No Salamanders	

Figure Captions

Figure 2.1. Artificial streams and salamander additions. (a) Evan Grant adding salamanders into the artificial streams. (b) Salamander (*Eurycea bislineata*) following addition to artificial stream.

Figure 2.2. Seston inflow and outflow for Experiments A and B. (a) Inflow seston concentration from header tank and (b) outflow seston concentration, (c) % seston exported and (d) transport distance (S_w) in flume 1 and flume 2.

Figure 2.3. Discharge over the course of the experiment for flumes 1 and 2. Discharge was measured prior to each treatment and once following treatment 4.

Figure 2.4. Interaction effects for Experiment B: (a) treatment by flume interaction for Sw ($F_{1,17}$=4.9, p=0.04), (b) treatment by flume interaction for % seston exported ($F_{1,17}$=4.9, p=0.04), (c) treatment by day or night interaction for Sw ($F_{1,17}$=4.4, p=0.05), and (d) treatment by day or night interaction for % seston exported ($F_{1,17}$=4.4, p=0.05).

Figure 2.5. Chl *a* for experiments A and B. (a) Chl *a* outflow concentrations and (b) % Chl *a* exported.

Figures

Figure 2.1

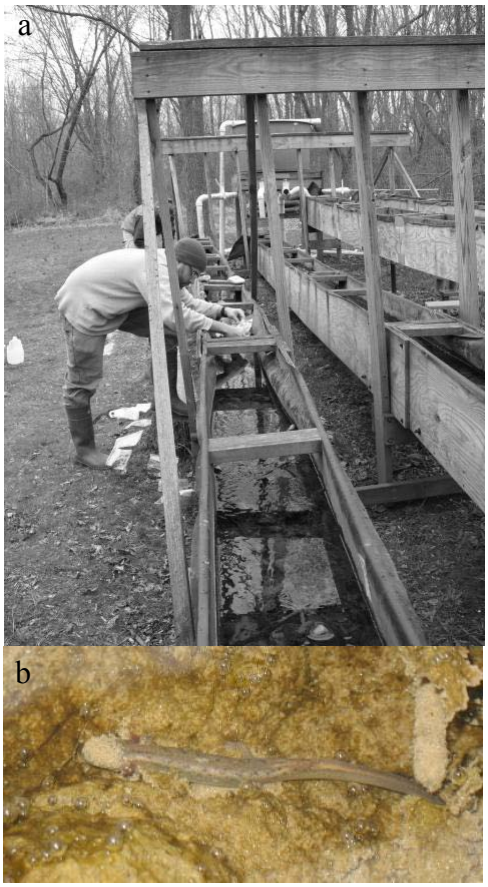


Figure 2.2

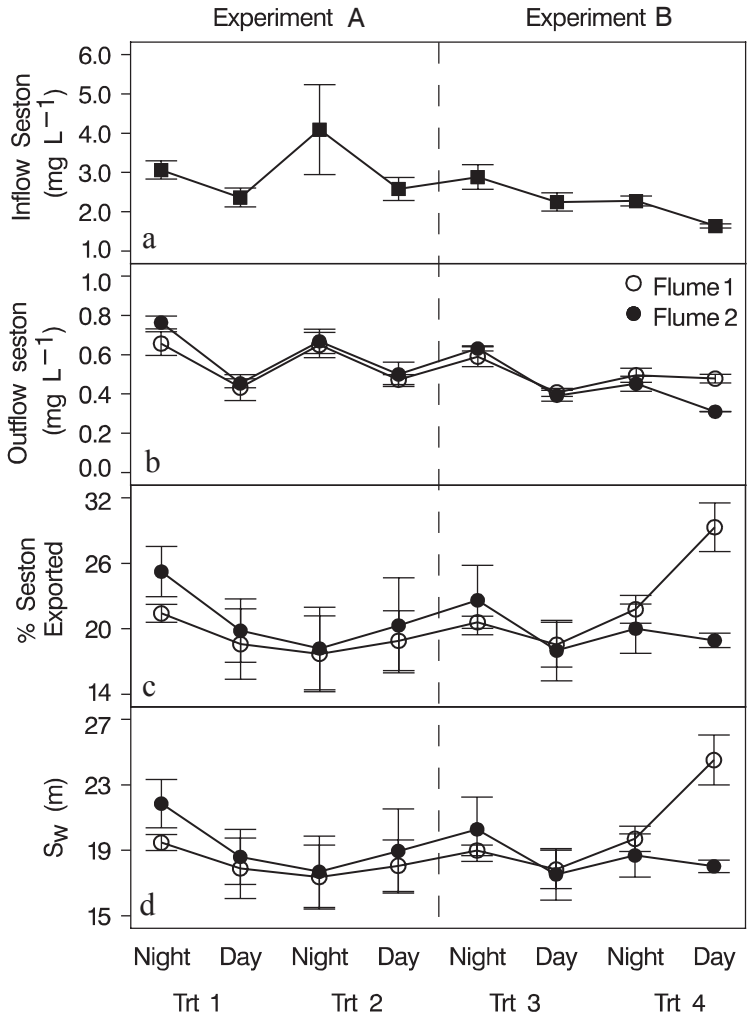

Experiment A
Experiment B
Inflow Seston (mg L−1)
6.0
5.0
4.0
3.0
2.0
1.0
a
Outflow seston (mg L−1)
1.0
0.8
0.6
0.4
0.2
0.0
b
Flume 1
Flume 2
% Seston Exported
32
26
20
14
c
Sw (m)
27
23
19
15
d
Night
Day
Night
Day
Night
Day
Night
Day
Trt 1
Trt 2
Trt 3
Trt 4

Figure 2.3

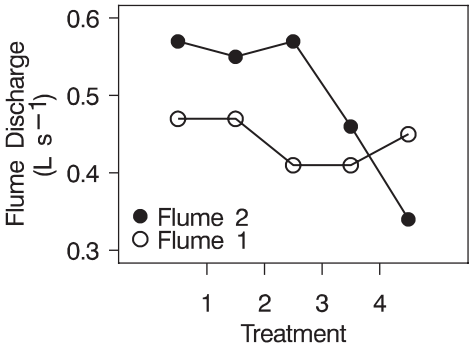

0.6
0.5
0.4
0.3
Flume Discharge
(L s −1)
Flume 2
Flume 1
1
2
3
4
Treatment

Figure 2.4

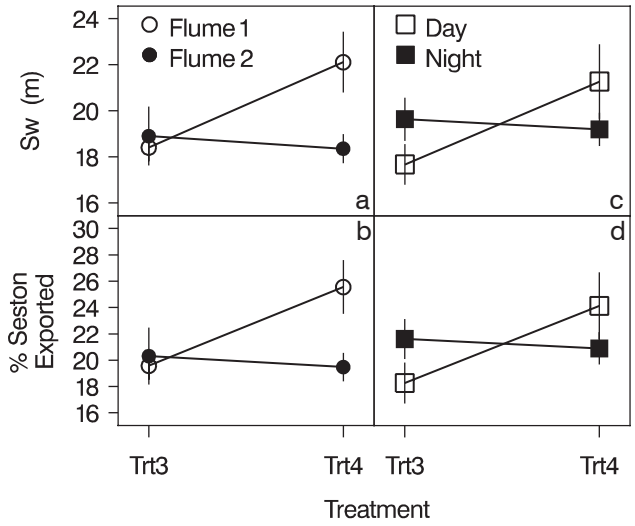
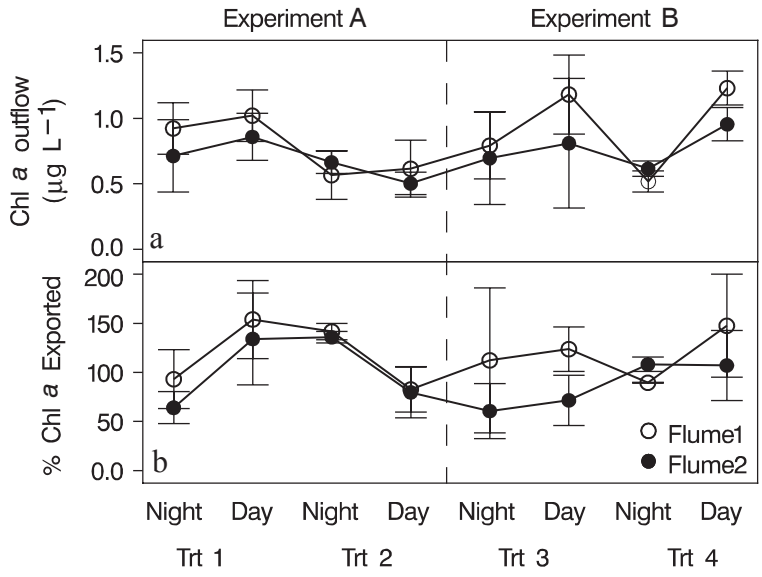

Flume 1
Flume 2
Day
Night
Sw (m)
% Seston Exported
24
22
20
18
16
30
28
26
24
22
20
18
16
a
b
c
d
Trt3
Trt4
Trt3
Trt4
Treatment

Figure 2.5


Experiment A
Experiment B
Chl a outflow (µg L−1)
1.5
1.0
0.5
0.0
a
% Chl a Exported
200
150
100
50
0.0
b
Flume1
Flume2
Night
Day
Night
Day
Night
Day
Night
Day
Trt 1
Trt 2
Trt 3
Trt 4

Chapter 3: Modeling sources and seasonal dynamics of seston and suspended particulate organic carbon within a stream ecosystem

Introduction

Particulate organic carbon (POC) provides energy for microorganisms and macroinvertebrates in headwater streams (Hedin 1990), and when transported in the suspended load (seston), supports heterotrophic metabolism in downstream reaches and rivers (Cole and Caraco 2001; Wipfli and Gregovich 2002). POC transport represents a longitudinal linkage between upstream autochthonous and allochthonous production and downstream ecosystems with the magnitude of connection controlled by the quality, quantity and timing of POC delivery (Mayorga et al. 2005). Knowledge of the sources of POC and temporal patterns of POC transport can help evaluate the quality of the energetic subsidy to downstream biota (Mayorga et al. 2005), controls on transport (Pozo et al. 1994), and lead to better understanding of the metabolic state of freshwater ecosystems (Cole et al. 2007).

Potential sources of seston include organic-core particles such as algal or bacterial cells, leaf or wood fragments, and dissolved organic carbon flocculates or mineral-core particles such as silts or clays with adsorbed organic carbon. Seston in small streams has historically been considered to be primarily fragments of leaves and wood created by macroinvertebrate feeding and egestion upstream (Vannote et al. 1980). Stable isotope and chlorophyll *a* analyses support the idea that stream POC is

terrestrial with minimal contributions from autochthonous production (Finlay et al. 2002; Kaiser et al. 2004). However, elemental, radioisotope and microscopic analyses showed that most seston in streams and rivers is composed of small (<20 μm) particles with high mineral content and elemental compositions closer to algae (C:N<10) than leaves or wood (C:N>30) (Meybeck 1982; Cole and Caraco 2001; Dodds and Whiles 2004). These seemingly contradictory results could be reconciled if seston were a mixture of algal cells, fragmented leaves or woody debris, and mineral-core particles (Aufdenkampe et al. 2001); especially given that POC associated with mineral-core particles has a C:N ratio lower than its terrestrial source because of preferential adsorption of high nitrogen content amino acids (C:N<10; Hedges et al. 1986; Aufdenkampe et al. 2001). The composition and source of seston could influence the array of POC quality (lability) that exists within seston. For example, POC quality of different particle types could range from labile (e.g. fresh algal cells) to semi-labile (e.g. leaf detritus) to refractory (e.g. humified soil organic matter). Understanding the diversity of POC quality by identifying its source and composition could help reveal the magnitude of the link between headwater streams and downstream aquatic ecosystems (Thorp et al. 2006).

In addition to POC quality, hydrological and biological dynamics regulate the longitudinal linkage of POC by controlling the timing and distance of seston transport. Storms are the most important factor regulating variability in the magnitude of annual or monthly seston and POC fluxes (Golladay et al. 2000), and transport particles long distances downstream during high flow events (Verhoff et al.

1979). However, because small streams are under storm conditions for only a small fraction of time (4% for Appalachian mountain streams: Buffam et al. 2001), temporal POC dynamics under baseflow conditions may play a disproportionately large role in regulating stream ecosystem processes. At baseflow, riparian vegetation and aquatic organisms may regulate seasonal patterns in seston transport more than hydrological patterns. Seasonally, the composition of seston at baseflow can vary with autumnal peaks in leaf litter inputs from deciduous riparian vegetation or vernal increases of algal production (Chapter 1). Webster et al. (1990) and Molla et al. (2006) attributed baseflow peaks of POC during warmer months to increased macroinvertebrate fragmentation of leaf litter as community biomass and activity increased. However, these studies did not verify that seasonal increases in seston concentration resulted from increased concentrations of leaf fragments. In some large temperate streams and rivers, autochthonous production dominates seston during seasons with high primary production (Pozo et al. 1994; Walters et al. 2007).

My goals for this study were to (1) identify the sources of seston, (2) determine if increased seston concentrations during warmer months result from increased processing of leaf detritus, and (3) determine if algal carbon is an important component of seston in headwater streams. I used a mixing model to examine baseflow seasonal fluctuations in seston composition and sources from an intensive field survey (Chapter 1) and from 14 years of sampling in a 3rd-order piedmont stream in southeastern Pennsylvania, U.S.A. The mixing model identified contributions to seston concentration from three distinct particle types (algal cells, detrital leaf

fragments and mineral-core particles) using carbon and nitrogen contents of seston. When only carbon data were available, seston was identified as one of two distinct particle types (organic-core and mineral-core particles). I also present data from a watershed habitat survey of seston, soils, and vegetation and identify potential patterns in seston sources from ponds, seeps, riffles and runs.

Methods

Site description – The upper east branch of White Clay Creek (WCC: N39°51'33.9", W75°46'59.6") is a 3rd order watershed draining 7.3 km^2 of the southeastern Pennsylvania Piedmont. The watershed has 23% of total area as temperate deciduous forest, 52% as pastures or hay fields for horses or cattle, and 22% as row crop agriculture, but has a largely intact riparian forest and livestock have minimal access to the stream (Fig. 3.1; Newbold et al. 1997). The dominant tree species are American beech (*Fagus grandifolia* Ehrh.), red maple (*Acer rubrum* L.), northern red oak (*Quercus rubra* L.), black oak (*Quercus velutina* Lam.), and tulip poplar (*Liriodendron tulipifera* L.) (Wiegner et al. 2005). Herbaceous vegetation in the riparian zone of streams includes Japanese honeysuckle (*Lonicera japonica* Thunb.), multiflora rose (*Rosa multiflora* Thunb.), knotweed (*Polygonum aviculare* L.), skunk cabbage (*Symplocarpus foetidus* L.), and jewelweed (*Impatiens capensis* Meerb.). The soils in the WCC watershed are typic hypludults, however, in the riparian zone, aquic fragiudults dominate (Wiegner et al. 2005). The mean daily baseflow discharge averaged 122 L s^{-1} over the past five years with a high annual mean discharge of 174 L s^{-1} and low of 59 L s^{-1}. At baseflow, WCC has high nutrient concentrations (3-5

mg NO_3-N L^{-1}; 4-33 μg PO_4-P L^{-1}) from agricultural land use and moderate dissolved organic carbon (1.5 mg DOC L^{-1}) concentrations (Newbold et al. 1997). Streambed sediments are composed of clay-, silt-, and sand-sized particles in runs with gneiss- and schist-derived gravel and cobble in riffles (Kaplan et al. 1980). Most of the particulate organic matter input, predominantly leaves, occurs from late October to mid-November (J. D. Newbold unpubl.).

WCC 2005-2006 baseflow sampling – Under baseflow conditions, stream water was collected every 1.5 hours during one 24-hour period each month, from August 2005 to July 2006 using an automated water sampler (Teledyne Isco), with the collection tube (9.5 mm inner diameter) at 2/3 depth in the middle of the thalweg (see Chapter 1 for complete sampling design). The automated sampler was set to collect four 1-L samples at each time period. The first sample was considered a rinse of the inflow tubing and discarded, the second was used for pigment analysis, and the final two samples were used for replicate seston and POC analysis. Only the first replicate of seston carbon and nitrogen analysis is reported. Samples were processed within 9 hours of collection. I use carbon (Fig. 1.2b) and pigment (Fig. 1.6) concentrations as well as carbon content of seston (%, Fig. 1.2c) from Chapter 1 for modeling and analysis of modeling results.

WCC historical baseflow sampling – From Jan 1993 to Dec 2006, WCC was sampled weekly, monthly, or bimonthly at one or two sites by submerging a 5 L sample bottle at 2/3 depth in the thalweg of the stream. Samples were carried to the lab and stored

for <1 week at 4°C before they were processed for seston (see *seston analysis* section).

Watershed habitat site descriptions (2004 and 2007) – In Aug 2004, three ponds outflows (Fig. 3.1: P1, P3, P4) and four seeps were sampled for seston concentration and carbon and nitrogen content. P1 (area = 9409 m^2) and P4 (pond area = 340 m^2) are ponds in agricultural and residential grass fields with connections to tributaries of WCC by pipe outflows. P3 (area = 631 m^2) flows to a 2^{nd}-order tributary of WCC through a small stream. Seston samples were also taken from four zero-order streams (seeps) flowing from groundwater springs that emerge within forests (Fig. 3.1: S1, S2, S3, S4). In Jun 2007, I sampled 2 ponds (P1, P2), 3 seeps (S1, S2, S3), 3 riffles (R1, R2, R3) and 3 runs (N1, N2, N3) within the WCC watershed for seston, riparian soils from the O-horizon, bank and stream sediments, coarse particulate organic matter (CPOM) and herbaceous vegetation (Fig. 3.1). The two ponds, located in agricultural and residential grass fields, P1 and P2 (area = 4808 m^2), had direct outflows to WCC that were approximately 3 L s^{-1} or <10% of the stream discharge. Samples from three forested groundwater seeps were collected just downstream of where groundwater emerged and formed a small channel (<1 L s^{-1}). Three riffles and three runs were randomly selected along a 1 km stretch of the 3^{rd}-order branch of WCC with discharges ranging from 28 to 39 L s^{-1}.

Watershed habitat sample collection and processing – For both 2004 and 2007 sampling, triplicate water samples for seston analysis were collected at 2/3 depth in

the water column at each riffle and run site, and directly from each pond outflow. Sample water was collected from each seep using a small sampling container, taking care to avoid disturbing the seep bed sediments because of the shallow depths (<5 cm). For the 2007 watershed habitat sampling, three bank sediment samples were collected above water level in the stream channel, and three soil samples were collected in the riparian zone within 2-10 meters of the channel. Both bank sediments and soils were collected at each site using a trowel, stored in a plastic bag, dried at 60-100°C, sifted by hand to remove vegetation, visible roots, and animals, and then ground into a fine powder using a mortar and pestle. At each site, three composites of CPOM were collected from in-stream leaf packs and rinsed in the water column to remove sediment and stream organisms. Also, three composites of herbaceous vegetation and grasses (herbs) were cut from live plants growing next to the water, within the channel and often hanging into the stream. The vegetation samples (CPOM and herbs) were immediately dried at 60-100°C and then ground in a Wiley Mill through a 250-µm sieve. All samples were processed for carbon and nitrogen content (%), ^{13}C and ^{15}N stable isotopic ratios and C:N elemental ratios (see below). The three replicates for each measure at each site were averaged together and the mean was used for statistical analyses.

Seston analysis – Seston concentration was determined gravimetrically using the dry weight of total suspended solids. Stream water samples were filtered, after recording volume, through stacked pairs of pre-weighed and pre-ashed (480ºC for 4.5 hours) 47 mm glass fiber filters (Millipore AP40 or Whatman GF/F with nominal pore-size of

0.7 μm) and dried for 12-18 hours at 60ºC. Seston concentration (mg seston L^{-1}) was calculated by dividing the dry mass of seston collected on the filter by the filtered volume. WCC 2005-2006 baseflow and watershed habitat seston samples for 2004 and 2007 sampling were processed for carbon and nitrogen content and isotopes (see below), while seston samples from WCC historical baseflow were ashed (480ºC for 4.5 hours) and reweighed to determine ash-free dry mass which was converted to POC concentration (mg POC L^{-1}) by assuming AFDM was 45% carbon and dividing by the filtered volume (Webster et al. 1999).

Carbon and nitrogen content and stable isotope analysis – Subsamples of each seston filter, soil, bank, and stream sediment sample were wet with nanopure water and fumed with hydrochloric acid vapors for 18 hours, effectively removing carbonates (A.K. Aufdenkampe unpubl.). Subsamples were analyzed in batches on an Elemental Analyzer (Costech ECS 4010) interfaced with an Isotope Ratio Mass Spectrometer (Thermo DeltaPlus XP). Standard curves for the elemental and isotopic analysis were created for each batch using 8 or 9 isotopically enriched (+37.63‰) or depleted (-26.39‰) L-glutamic acid standards over a range of carbon masses that encompassed the subsample masses. Stable carbon isotope ratios, reported using $\delta^{13}C$ notation, were normalized relative to the Vienna Peedee Belemnite standard, while stable nitrogen isotope ratios, reported using $\delta^{15}N$ notation, were normalized relative to air N_2 as the standard. Carbon and nitrogen content (%) were calculated as the ratio of measured carbon or nitrogen mass to dry mass of subsample for all seston, soil, sediment and vegetation samples.

Two-compartment mixing model – For the historical WCC baseflow data, because I only had carbon content for seston, I modeled seston composition as a mixture of two distinct particle types: organic-core and mineral-core particles. Organic-core particles are composed of algal cells, leaf fragments and bacterial cells (carbon content for mixture = 34%), while, mineral-core particles are clays and silts with adsorbed organic carbon and bacterial cells (carbon content = 1.5%) (Meybeck 1982; Hedges et al. 1986). For each sample, I simultaneously solved equations (3.1) and (3.2):

Equation 3.1 $S = P_o + P_m$

Equation 3.2 $POC = 34\% * P_o + 1.5\% * P_m$

using measured seston (S) and POC (POC) concentrations to determine the concentration of organic-core (P_o) and mineral-core (P_m) particles in seston and the concentration of organic-core (34% x P_o) and mineral-core (1.5% x P_m) particles in POC (Chapter 1). Modeling results were averaged by month.

Three-compartment mixing model – For the WCC 2005-2006 baseflow, I used carbon and nitrogen content (%) to model seston composition as a mixture of three distinct particle types: mineral-core (m), terrestrial plant detritus (d) and algal (a) particles. I define mineral-core particles as small clays and silts with adsorbed organic matter and microorganisms (mineral-associated POC) (Aufdenkampe et al. 2001) and terrestrial

plant detritus particles as small fragments of deciduous leaves. I define algal particles as individual diatoms because using microscopy, I can clearly identify individual diatom cells, but few other products of instream production. Each end member has a distinct combination of carbon and nitrogen contents that were calculated by averaging a range of literature values (Table 3.1). Mineral-core particles and algal cells have low C:N ratios, but, mineral-core particles have low carbon and nitrogen content while algal particles have higher carbon and nitrogen content. Leaf detritus particles have the highest carbon content, but also the highest C:N ratio resulting in low nitrogen content organic matter.

For each sample, I simultaneously solved equations (3.3), (3.4), and (3.5)

Equation 3.3 $S = P_d + P_a + P_m$

Equation 3.4 $POC = C_d * P_d + C_a * P_a + C_m * P_m$

Equation 3.5 $PN = N_d * P_d + N_a * P_a + N_m * P_m$

using measured seston (*S*), POC (*POC*), and particulate nitrogen (*PN*) concentrations carbon contents and nitrogen contents for each end member derived from literature values (Table 3.1). P_x represents seston concentration from detrital (*d*), algal (*a*) and mineral-core (*m*) particles while C_x and N_x are the carbon and nitrogen contents (%) of each particle type (Table 3.1). Because of the standard deviation associated with the end member means (Table 3.1), I used a Monte Carlo simulation where I solved

equations (3.3), (3.4), and (3.5) for 1000 randomly selected combinations of the end members (C_x and N_x) for all three particle types for each sample (Manly 2006). I report the mean contribution (P_x, mg L^{-1}) of detrital, algal, and mineral-core particles to seston concentration from this simulation. Furthermore, I can use these solutions and the end members to get the contribution of each particle type to POC ($C_d * P_d$, $C_a * P_a$, $C_m * P_m$; mg L^{-1}) and PN ($N_d * P_d$, $N_a * P_a$, $N_m * P_m$; mg L^{-1}) concentration.

Statistical analysis – All statistical analyses were conducted using SAS v9.1 Level 1M3 (2007, SAS Institute Inc., Cary, NC, USA). For WCC 2005-2006 baseflow, I performed one-way ANOVAs (proc MIXED) for C:N ratios and modeling results, P_d, P_a, and P_m, concentrations with month as the treatment. Since the contributions of detrital, algal, and mineral-core particles to POC and PN are linear scalars of P_d, P_a, and P_m, results from seston ANOVAs for each type of particle can be applied to seasonal trends in both POC and PN contributions. For WCC historical baseflow, I performed one-way ANOVAs for carbon content (%), seston, and POC concentrations with month as the treatment. I performed one-way ANOVAs on modeling results for seston contributions from mineral-core and organic-core particles. Since the contributions of mineral-core and organic-core fractions to POC are linear scalars of their respective contributions to seston, I applied ANOVA results from the contributions of seston to the POC contributions. Seasonal differences were examined by making multiple mean comparisons using the Tukey's test for all pair-wise differences between months and describe the non-significant differences between months by using seasons (winter, spring, summer, autumn). For seston

samples from the Jun 2007 watershed habitat sampling, I performed one-way ANOVAs for seston concentration, carbon and nitrogen content, $\delta^{13}C$, $\delta^{15}N$, and C:N ratios with habitat (riffle, run, pond, seep) as the treatment. I performed two-way ANOVAs for carbon and nitrogen content, $\delta^{13}C$, $\delta^{15}N$, and C:N with habitat (riffle, run, pond, seep) and type (soil, bank sediments, CPOM, herbs) as the treatments. To ensure homogeneity of variances, it was necessary to log-transform several variables (seston, POC, modeling results); these are reported as back-transformed geometric means. Following each ANOVA, I made multiple mean comparisons using the Tukey's test. For seston samples from the watershed habitat 2004 sampling, I performed paired t-tests between ponds and seeps for seston concentration, carbon and nitrogen contents, and C:N ratios.

Results

WCC 2005-2006 baseflow sampling – Over the year, one hundred eighty-three samples were taken during baseflow conditions on 11 monthly sampling days. The carbon and nitrogen contents of these samples were contained by the mixing model space made by the end members (Fig. 3.2). Low seston C:N ratios (Fig. 3.3a : 9.2 ± 0.1: mean ± SE) caused the particles to fall along the axis created by algal and mineral-core end members (Fig. 3.2). Seasonally, seston C:N ratios peaked in Aug and Dec, and were lowest in Mar ($F_{10,169}$=9.3, p<0.001). Throughout the year, mineral-core particles dominated seston concentration, followed by algal and detrital particles, respectively (Fig. 3.3b). However, algal particles consistently contributed the most to POC concentration, followed by detrital and mineral-associated POC, respectively (Fig. 3.3c). Algal particles also consistently contributed the majority of

PN concentration, followed by mineral-associated nitrogen and detrital particles, respectively (Fig. 3.3d). Mineral-core particles showed seasonal trends for seston, POC, and PN with high concentrations in the summer and low concentrations in the winter (Fig. 3.3b, c, d: $F_{10,153}$=20.2, p<0.001). Algal particles also showed seasonal trends for seston, POC, and PN with the highest concentrations in spring and summer and lowest concentrations in the late autumn and winter (Fig. 3.3b, c, d: $F_{10,153}$=34.8, p<0.001). Detrital particles showed seasonal trends for seston, POC, and PN with the lowest concentrations in March and July (Fig. 3.3b, c, d: $F_{10,153}$=8.5, p<0.001). Algal POC was positively correlated with pigment (chlorophyll *a* + pheophytin *a*) concentration (Fig. 3.4; r^2=0.36; $F_{1,160}$=92, p<0.001; algal POC = 0.09 + 0.01 x pigments).

WCC historical baseflow sampling – Over the 14 years of sampling, from 1993 to 2006, 502 total seston samples were taken on weekly, bimonthly, or monthly sampling intervals with 26 to 50 samples taken for each month. Seasonally, seston concentration was highest in late spring and summer months (April-July), and lowest in late autumn and winter months (Oct-Dec) (Fig. 3.5a: $F_{11,490}$=24.0, p<0.001). Similarly, POC concentration was highest in spring and summer months (April-August) and lowest in late autumn and winter (October-Feb) (Fig. 3.5b: $F_{11,490}$=11.4, p<0.001). Stream discharge was not correlated with seston (r^2=0.05, p = 0.75) nor POC concentration (r^2=0.03, p = 0.77). Carbon content of seston increases through the summer until a peak in November at 17% ± 0.8 (mean ± SE) (Fig. 3.5c: $F_{11,490}$=13.1, p<0.001). Mineral-core particles showed seasonal trends for seston and

POC with high concentrations in the summer and low concentrations in the winter (Fig. 3.5d, e: $F_{11,489}$=27.8, p<0.001). Organic-core particles also showed seasonal trends for seston and POC with high concentrations in the summer and low concentrations in the winter (Fig. 3.5d, e: $F_{11,489}$=9.5, p<0.001). Mineral-core particles dominated seston concentration for most of the year, except in late autumn when mineral-core and organic-core particles made up equal proportions of seston (Fig. 3.5d). Organic-core particles comprised most of POC concentration for the entire year (Fig. 3.5e).

Watershed habitat sampling – For the Aug 2004 sampling, seep seston C:N ratio was higher than pond outflow seston C:N ratio, but there was no difference between seep and pond outflow seston concentration, carbon content, or nitrogen content (Fig. 3.6). For the Jun 2007 sampling, seston concentration and carbon content were highest in the pond outflows and seeps and lowest in riffles and runs (Table 3.2). Seston nitrogen content was highest in the pond outflows; while δ^{13}C was lowest in pond outflows (Table 3.2). Seston C:N ratios were highest in seeps, and there was no difference in δ^{15}N among the habitats (Table 3.2).

CPOM and herbs had higher carbon content ($F_{3,27}$=115.1, p<0.001) and nitrogen content ($F_{3,27}$=53.4, p<0.001) than soils or bank sediments (Fig. 3.7). However, there were no effects of habitat on carbon or nitrogen content. Herbs had lower δ^{13}C than CPOM, soils or bank sediments ($F_{3,27}$=17.9, p<0.001), but the was no effect of habitat on δ^{13}C (Fig. 3.8). Riffle and run δ^{15}N was lower than seep and pond

δ^{15}N ($F_{3,27}$=7.5, p<0.001), but there was no significant effect of sample type (Fig. 3.8). CPOM had higher C:N ratios (26.7 ± 0.9: mean ± SE) than herbs, soils or bank sediments ($F_{3,27}$=4.0, p=0.02).

Discussion

Seston source – Seston in streams has historically been considered to be primarily terrestrial leaf fragments (Vannote et al. 1980), however, modeling results indicate mineral-core particles dominate total seston concentration (Fig. 3.3b, 3.5d). Microscopic analysis confirms that most particles are amorphous clay or silt particles that are unidentifiable as leaf fragments or algal cells (Meybeck 1982; D.C. Richardson pers. obs.). However, algal cells and leaf detritus, with high carbon content, dominate POC concentrations (Fig. 3.3c, 3.5e; Chapter 1). A small percentage of organic-core particles can dominate POC concentration (by mass) because of their high carbon content (Table 3.1), which is congruent with microscopic analysis of seston where few particles (by number) can be identified as having an organic-core. Algal POC was always greater than detrital POC indicating the importance of primary production to POC flux downstream, even in forested headwater streams (e.g. Elliott et al. 2004). Using similar mixing models, Aufdenkampe and Buckaveckas (unpubl.) found that Amazon and Mississippi river seston is also comprised mostly of mineral-core particles, while POC concentration is predominantly algal and detrital leaf particles. These results explain the importance of both mineral-core particles to the mass and number of stream particles (e.g. Meybeck 1982; Cole and Caraco 2001), but also the importance of organic-core

(algal and detrital leaf) particles to the flux of carbon transported downstream (e.g. Vannote et al. 1980; Elliot et al. 2004).

Seasonal trends in seston composition – Most POC and seston export from WCC occurs during storms (Newbold et al. 1997), as is typical of small streams where seston and POC concentrations are positively correlated with stream discharge (Golladay et al. 2000). However, in this study and others in WCC at baseflow, seston and POC concentrations are not correlated with stream discharge (Newbold et al. 1997; Chapter 1). Thus, alternative hypotheses are necessary to explain seasonal patterns of seston concentration and composition (Fig. 3.3, 3.5). Warm-season peaks of seston and POC concentrations are attributed to increasing stream organism biomass and activity including feeding and bioturbation (Wallace et al. 1991; Molla et al. 2006; Chapter 1). I attribute this pattern to an increase in all types of activity, not simply shredding, because while leaf detritus and organic based particles peak in the summer, so do algal particles and mineral-core particles (Fig. 3.3, 3.5). I hypothesize that increases in seston and POC concentrations during the warm months (Fig. 3.5a, b) result from shredding and egestion of leaf detritus, increases in periphyton growth and grazing on the streambed (Mulholland et al. 1991; Hill et al. 2001) and increases in stream organism activity (bioturbation) that suspends bed sediments (Moore 2006). In autumn, the seston concentration decreased dramatically at the same time as litterfall (Fig. 3.3, 3.6), which could have resulted from the filtration of particles by leaf packs instream (Wallace et al. 1993). This filtration could occur simultaneously with increased fragmentation of leaf litter both by

physical and biological degradation (Swan and Palmer 2004). These two simultaneously occurring mechanisms are supported by trends in October through December where seston (Fig. 3.3b, 3.5d) and POC (Fig. 3.3c, 3.5e) concentration are comprised of higher proportions of organic-core particles, especially leaf detritus, particles compared to the rest of the year. While leaf detritus filtered out all particles contributing to an overall decrease in seston concentration, leaf detritus particles are generated at a higher rate during the autumn than the rest of the year.

In late winter and early spring, the open canopy, increasing hours of daylight and warming temperatures are conducive to the growth of long algal filaments on rocks and sediments (Kaplan and Bott 1982; Hill et al. 2001) resulting in peaks of algal POC (Fig. 3.3c). However, the early spring peak did not occur in the organic-core particles in the historical data (Fig. 3.5d, e). In the historical data, carbon content of seston averaged approx. 11% for March and April averaged over the 14 year period, but carbon content was 16 and 14% for March 2006 and April 2006 from the historical data, respectively, which matches spring peaks in carbon content from WCC 2005-2006 data (Chapter 1). The high algal content of seston during the spring of 2006 may have resulted from exceptionally high primary production during the spring because of warmer than average temperatures and fewer storms (e.g. Fisher et al. 1982; Power and Stewart 1987).

Validation of the model – The mixing model is a valid tool for characterization of seston composition and sources; however there are some limitations and inherent

assumptions made when using the model. The three end members seemed to be an appropriate description of the seston data because all sample points were encompassed by the end member space (Fig. 3.2) indicating that they could all be separated by these end members. Microscopic analyses match with the results derived from the model. Most particles are small (<20 μm) clays and silts with high mineral content (Meybeck 1982; Chapter 1). Less frequently, single diatom cells or leaf fragments can be recognized but few particles are clearly identifiable as insect parts, macroinvertebrate frass, or large flocs under the microscope.

The algal composition was derived using elemental compositions of cultures of marine or lake diatoms in phytoplankton (Table 3.1) because I could primarily identify individual algal cells (diatoms) as the only algal derived particles. I had to make this assumption, because, in this study, I was unable to get an adequate end member sample from rock scrapings during the watershed habitat sampling due to the thin coating of periphyton on rocks, possibly because of macroinvertebrate grazing or low primary production with the closed summer canopy. Furthermore, I was unable to easily separate algal from sediment or allochthonous matter in periphyton by hand for previous sampling (Chapter 1). Future work will take advantage of centrifugation techniques to isolate algal cells from epilithon (e.g. McCutchan and Lewis 2002). From this study, the best example of algal particles was pond seston, which was similar to literature derived algal end members with high carbon and nitrogen content (Fig. 3.8), low C:N ratios (<9) and $\delta^{13}C$ that was closest to periphyton stable carbon isotopes (Fig. 3.8; Chapter 1; Walters et al. 2007). Furthermore, algal carbon was

positively correlated with pigment concentration (Fig. 3.4) and C:pigment ratios were within realistic values calculated in other studies (Angradi 1993), indicating the validity of the algal end member. However, while individual diatom cells can be clearly identified using a microscope, some suspended POC in the stream could come from extracellular polymeric substances produced by primary production on rocks and sediments (Decho and Lopez 1993; Sutherland et al. 1998) and was not accounted for using this model.

For most temperate deciduous forests with developed riparian zones, including WCC, litterfall is an important source of particulate organic matter to stream ecosystems (Fisher and Likens 1973; Benfield 1997). In WCC, most litterfall enters the stream as deciduous leaf detritus during mid to late October (J. D. Newbold unpubl.). Leaf detritus has low variation in carbon content (44%) and consistent nitrogen composition despite small variations in C:N ratio among different tree species (Ostrofsky 1997; Swan and Palmer 2004). The leaf detritus end member was assumed to have elemental composition similar to the senesced litterfall entering the stream. However, as leaf material is colonized by microorganisms in the stream the nitrogen content increases as the leaves decay because attached microorganisms will use inorganic nitrogen from the water column for cell growth (Suberkropp et al. 1976). This effect might lower the C:N ratio of the fragmented leaf material causing the leaf detritus end member to shift towards the algal end member (Fig. 3.2). This idea is supported by the CPOM sampled for the watershed habitat sampling. The

CPOM was collected from leaf packs within the stream and has higher nitrogen contents (Fig. 3.7, 1-2%) than the end member from the model (Table 3.1, 1%).

In this study, seston is divided into three distinct particle types. However, seston composition can include a number of other particle types including small particles (0.7 to 1 μm) such as bacterial cells, DOC aggregates, and clay particles with adsorbed inorganic nitrogen. Bacterial cells and DOC aggregates contain lipids, polysaccharides and proteins resulting in C:N ratios (5) lower than any of the end members for this study (Vallino et al. 1996; Kerner et al. 2003; Table 3.1). However, both bacterial cell and DOC aggregate carbon only contribute a small fraction of POC concentration in streams (Kaplan and Newbold 1995; Kerner et al. 2003). Mineral-core particles could include ammonium adsorbed to clays or silts through ionic bonding (Mayer et al. 1998). Theoretically, this could add a fourth particle end member with high nitrogen content with low carbon content. Streambed sediments have very low nitrogen content (0.03%; D. C. Richardson, unpubl.) indicating this fourth end member does not comprise a large proportion of seston in WCC.

Stable isotope analyses offer an interesting tool to elucidate seasonal trends of seston transport (Kendall et al. 2001) and to differentiate terrestrial organic matter sources from instream production (Finlay et al. 2002) since these sources have differing $\delta^{13}C$ values (Fry 2007). However, separation of POC sources in streams using end members such as periphyton, terrestrial vegetation, and soil OM is challenging because the ranges of $\delta^{13}C$ are large, variable and overlapping and seston

$\delta^{13}C$ was occasionally outside the range of measured isotope end members (Kendall et al. 2001; Chapter 1). Mixing models are ineffective without clear separation between end members and end members that encompass the samples (Fry 2007). Furthermore, algal $\delta^{13}C$ is known to vary spatially as a function of the concentration of dissolved free CO_2, the $\delta^{13}C$ of dissolved inorganic carbon, water-velocity effects on cell-wall CO_2 gradients, and primary production rates, creating a range of $\delta^{13}C$ that depends on position within the watershed and local reach morphology (Finlay et al. 1999; Walters et al. 2007). The proportion of seston existing as organic-core and mineral-core particles may be more easily obtained using carbon content because of the distinct separation between the two end members (Meybeck 1982). Because algal carbon and detrital organic matter from terrestrial leaves have differing C:N ratios (Table 3.1), further separation of seston into three particle types (mineral-core, algal cells, and leaf detritus fragments) was possible using both carbon and nitrogen content. Also, in the watershed sampling, the separation by elemental composition was significantly clearer than stable isotopes (Fig. 3.7 vs. Fig. 3.8).

Watershed habitat sampling – The watershed habitat sampling served to identify potential sources of seston to the 3rd-order WCC. Seston could come from litter of riparian vegetation entering by direct litterfall or lateral movements (Benfield 1997), slumping riparian herbaceous vegetation (Menninger and Palmer 2007), bank, soil or streambed sediments (Golladay et al. 2000), autochthonous production of periphyton (Angradi 1993; Sutherland et al. 1998), or from upstream ecosystems. Upstream ecosystems can include habitats such as zero order streams (seeps) or pond outlets

that are connected directly to stream networks. The transport and downstream influence of seston from seeps and ponds to a stream network depends on the magnitude of discharge coming from the habitat and the transport distance of the particle (Vadeboncoeur 1994; Thomas et al. 2001).

Seeps are fed by groundwater resulting in low disturbance environments with accumulations of decomposing CPOM (Burns et al. 1998; Duval and Hill 2006). Therefore, seeps contribute high concentrations of fragmented and egested leaf litter to the stream network; this idea is supported by the similarity of seep seston composition to CPOM (Fig. 3.7). Each individual seep has little flow to add fluxes of these particles to the stream network, but the cumulative total flux from all seeps across a forested stream network could contribute significantly to leaf detritus in the stream network.

Fluvial discontinuities such as lakes (natural and manmade) and ponds (e.g. natural, beaver, mill, farm, recreational) are common features of most landscapes (Winter 2001). Ponds are open, slowly moving or stagnant water that result in high primary productivity, and can export high quality autochthonous material to streams (Fig. 3.6, 3.7; Richardson and Mackay 1991). In this study, seston content at pond outflows could account for up to 50% of total instream seston fluxes (3 L s^{-1} x concentration from Table 3.2), although the pond seston likely only travel short distances downstream at low flow (Vadeboncoeur 1994; Thomas et al. 2001).

Riparian herbs and grasses can contribute significant quantities of detritus to streams through live or senesced overhanging vegetation or material entering the water column during floods (Gurnell 2007; Menninger and Palmer 2007). This can be especially important in deforested riparian zones where streamside herbs and grasses are the dominant terrestrial riparian vegetation. Within the WCC watershed, a portion of streams run through agricultural fields and residential property where grasses and herbs are the dominant riparian vegetation. Also, along the forested 3rd-order reach and in locations such as seeps and ponds, herbs and grasses grow abundantly alongside and within the stream channel. Live herbs and grasses, because of high nitrogen content, are a better quality resource (Fig. 3.7; MacKay et al. 1992; Menninger and Palmer 2007) and decompose more rapidly than leaf litter (Menninger and Palmer 2007). Decomposition of herbs and grasses could provide a source of high quality particles with a unique stable isotope signature (Fig. 3.8) to the stream ecosystem, but would require quantification of the mass of herbs and grasses entering WCC and whether the plants enter the stream channel live or senesced.

Importance of headwater stream seston – Within a 3rd-order piedmont stream, seston transport is dominated by mineral-core particles, while POC transport is dominated by organic-core particles including algal cells and leaf detritus. Seasonal patterns of seston transport and composition reflect seasonal cycles of litterfall, stream organism activity such as feeding and egestion of leaf litter, bioturbation and grazing, and, during some years, increased algal carbon from vernal algal production. Even in headwater streams, where historically, algal carbon was not considered an important

component of downstream carbon flux (Fisher and Likens 1973; Vannote et al. 1980), algal cells are a key component of POC transport throughout the year and their high lability could link carbon from headwater streams to immediate downstream reaches. More refractory leaf detritus and terrestrially-derived mineral-associated organic material may link headwaters to mid-order streams, rivers and estuaries (Cole and Caraco 2001). Furthermore, connections of ponds and seeps to streams within the watershed provide a local link of high carbon content particles to the stream network. Future work is needed to link particle composition to organic matter quality and transport to better understand the role of stream ecosystems in controlling the transformations of allochthonous and autochthonous POC transported from inland waters to the oceans (Thorp et al. 2006; Cole et al. 2007).

Tables

Table 3.1. End members (means ± 1 SE) from literature values and this study used for 3-compartment mixing model to separate seston carbon (C_x) and nitrogen content (N_x) in leaf detritus (d), algal cells (a), and mineral-core (m) particles.

	End members		
	Detrital (C_d or N_d)	Algal (C_a or N_a)	Mineral-core (C_m or N_m)
Carbon content (%)	42.1% ± 3.1	26% ± 4.9	1.5% ± 0.2
Nitrogen content (%)	0.98% ± 0.36	4.33% ± 1.41	0.22% ± 0.04
C:N	50 ± 17.9	7 ± 1.9	9 ± 1
Citations	3,4,7,10	3,4,5,6,8,9,11,12	1,2,7

[1]Aufdenkampe et al. 2001; [2]Aufdenkampe et al. 2007; [3]Cross et al. 2005; [4]Elser et al. 2000; [5]Geider and La Roche 2002; [6]Hedges 2002; [7]Hedges et al. 1986; [8]Hillerbrand and Sommer 1999; [9]Leonardos and Geider 2004; [10]Chapter 1; [11]This study (pond seston); [12]Ho et al. 2003

Table 3.2. Seston concentration (conc.) and composition (means ± 1 SE) from watershed habitat survey in June 2007. One-way ANOVAs for each seston variable test differences across habitats (riffles, runs, pond outflows, and seeps). Within each variable, habitats with different alphabetic superscripts are significantly different (Tukey's test).

	Seston variables					
Habitat	Conc. (mg L^{-1})	Carbon content (%)	Nitrogen content (%)	$\delta^{13}C$	$\delta^{15}N$	C:N
Riffle	4.2 ± 1.1 [a]	7.9 ± 0.9 [a]	0.9 ± 0.1 [a]	-27.7 ± 0.2 [a]	5.1 ± 0.3	10.0 ± 0.2 [a]
Run	3.0 ± 0.4 [a]	8.6 ± 0.7 [a]	1.0 ± 0.1 [a]	-29.3 ± 0.1 [a]	5.7 ± 0.5	9.6 ± 0.1 [a]
Pond	21.7 ± 4.2 [b]	26.9 ± 4.3 [b]	3.4 ± 0.5 [b]	-24.8 ± 1.1 [b]	5.7 ± 0.5	9.2 ± 0.1 [a]
Seep	20.2 ± 3.2 [b]	23.2 ± 4.4 [b]	1.6 ± 0.3 [a]	-27.8 ± 0.6 [a]	4.6 ± 0.6	17.0 ± 0.4 [b]
F-stat	$F_{3,7}$=17.7	$F_{3,7}$=10.8	$F_{3,7}$=15.9	$F_{3,7}$=11.2	$F_{3,7}$=1.1	$F_{3,7}$=2.3
p-value	0.001	0.005	0.002	0.005	0.42	<0.001

Figure Captions

Figure 3.1. Map of White Clay Creek, SE Pennsylvania where P1-P4 are ponds, S1-S4 are seeps, R1-R3 are riffles and N1-N3 are runs sampled for the watershed habitat sampling. The watershed boundary is indicated by the red line. Streams (lines) and ponds (circles) within the watershed indicated in white.

Figure 3.2. Hypothetical partitioning of source contributions from three selected end members (leaf detritus, algal cells and mineral associated particles for mixtures collected as seston. Open circles represent individual data points from WCC 05-06 sampling, while the boxes represent ±1 SD for end members collected from literature values.

Figure 3.3. Seasonal changes in (a) seston carbon:nitrogen (C:N) ratios and modeling results for (b) seston concentration, (c) suspended POC concentration, and (d) suspended particulate nitrogen concentration by particle type for WCC 2005-2006 samples. Data is plotted by julian day with sampling months are identified in panel a; error bars represent ± 1 SE.

Figure 3.4. Least squares linear regression between pigment concentration ($\mu g\ L^{-1}$) and algal POC ($mg\ L^{-1}$) from WCC 2005-2006 baseflow modeling (algal POC = 0.09 + 0.01 pigments, r^2=0.36, $p<0.001$).

Figure 3.5. WCC historic baseflow seston characteristics averaged over 14 years (1993-2006). Panels show seasonal changes in (a) seston concentration, (b) suspended POC concentration, and (c) carbon content. Modeling results of seston composition for seston concentration and suspended POC concentration are shown in panels (d) and (e). Error bars represent ± 1 SE.

Figure 3.6. Comparisons between pond outflow and seep (a) seston concentration, (b) carbon content, (c) nitrogen content, and (d) carbon:nitrogen ratios for watershed habitat samples collected in 2004. Error bars represent ± 1 SE and * indicates significantly different means ($p<0.05$).

Figure 3.7. Elemental composition by habitat (pond, riffle, run, seep) and sample type (seston, herbs, CPOM, soil sediments, bank sediments) for watershed habitat samples collected in 2007.

Figure 3.8. Stable isotope ratios by habitat (pond, riffle, run, seep) and sample type (seston, herbs, CPOM, soil sediments, bank sediments) for watershed habitat samples collected in 2007.

Figures

Figure 3.1

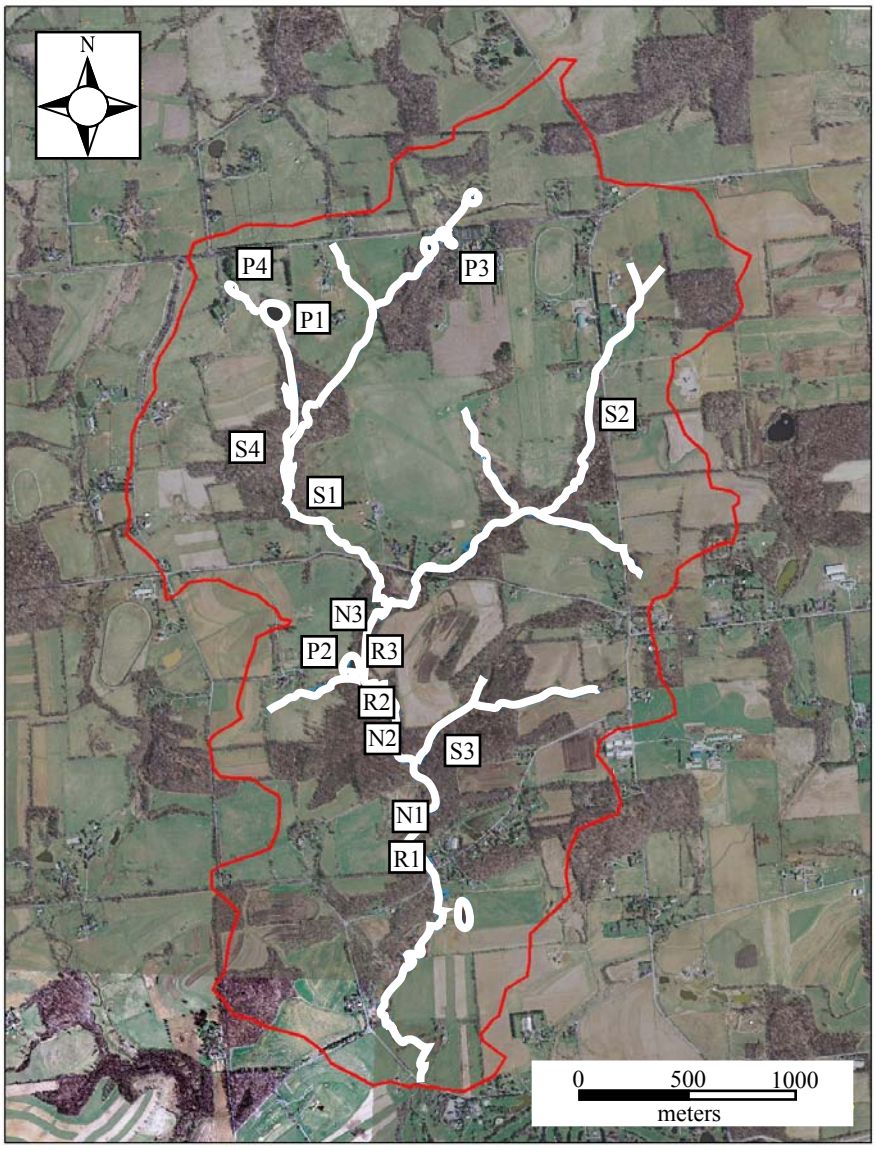


Figure 3.2

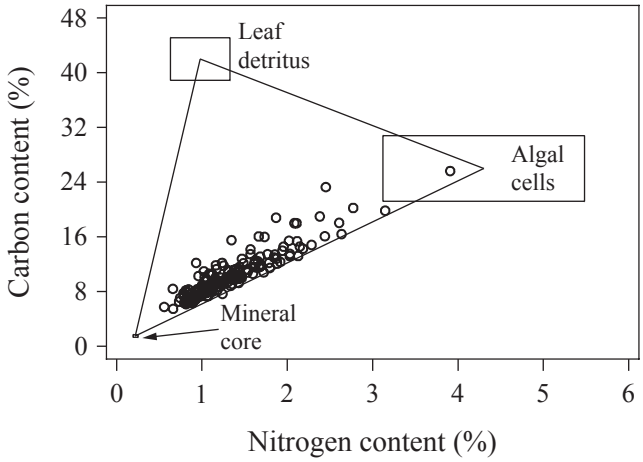

Leaf
detritus
Algal
cells
Mineral
core
Carbon content (%)
Nitrogen content (%)
0
8
16
24
32
40
48
0
1
2
3
4
5
6

Figure 3.3

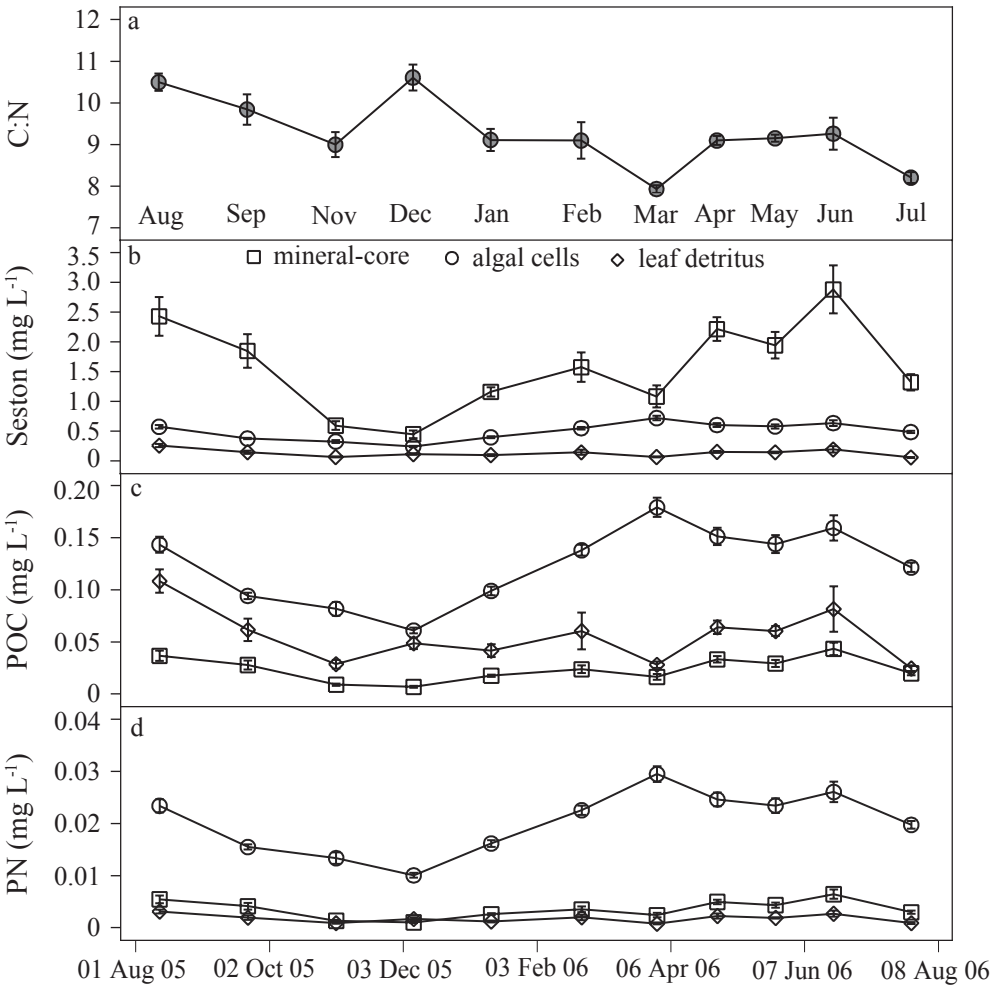

a
C:N
12
11
10
9
8
7
Aug
Sep
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
b
mineral-core
algal cells
leaf detritus
Seston (mg L-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
c
POC (mg L-1)
0.20
0.15
0.10
0.05
0
d
PN (mg L-1)
0.04
0.03
0.02
0.01
0
01 Aug 05
02 Oct 05
03 Dec 05
03 Feb 06
06 Apr 06
07 Jun 06
08 Aug 06

Figure 3.4

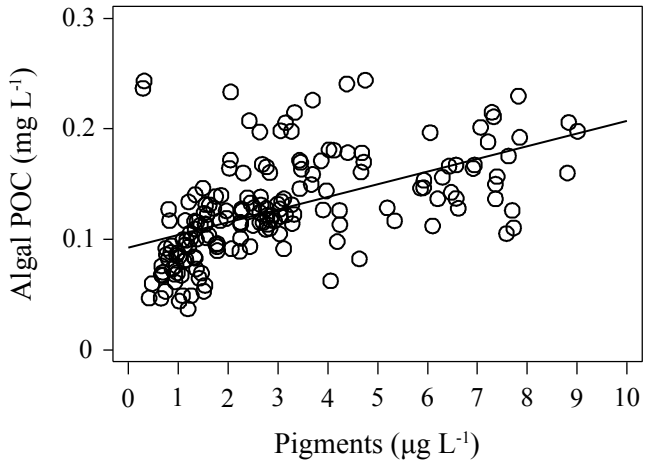


Figure 3.5

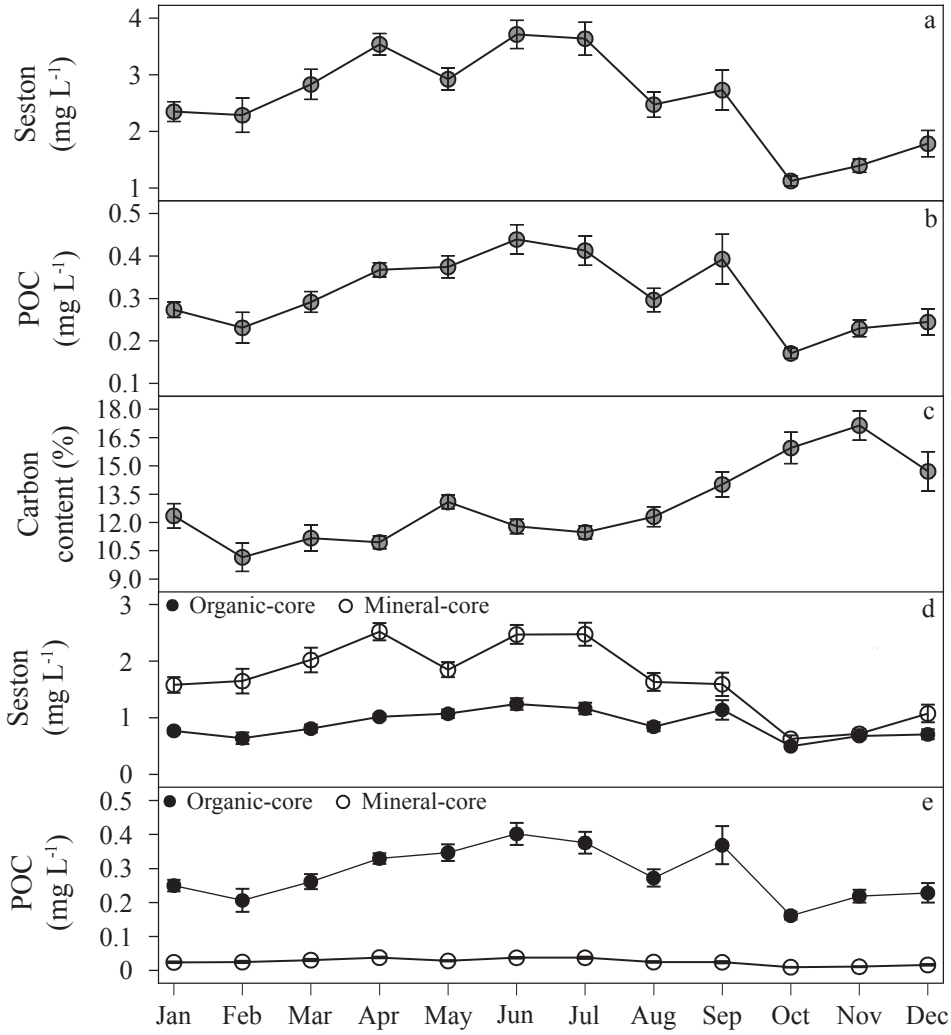

Seston (mg L-1)
POC (mg L-1)
Carbon content (%)
Seston (mg L-1)
POC (mg L-1)
a
b
c
d
e
Organic-core
Mineral-core
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

Figure 3.6

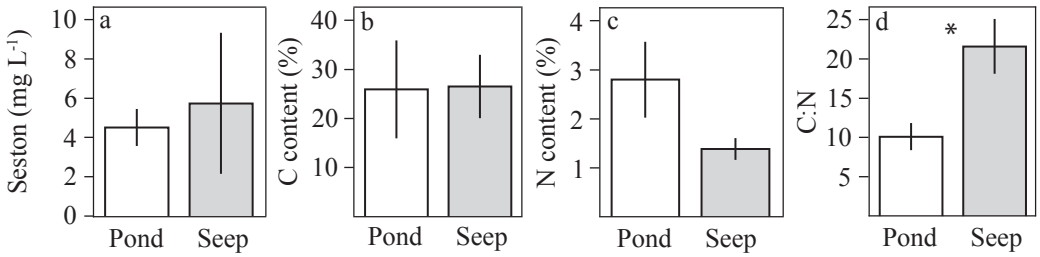

a
Seston (mg L-1)
10
8
6
4
2
0
Pond
Seep
b
C content (%)
40
30
20
10
Pond
Seep
c
N content (%)
4
3
2
1
Pond
Seep
d
*
C:N
25
20
15
10
5
Pond
Seep

Figure 3.7

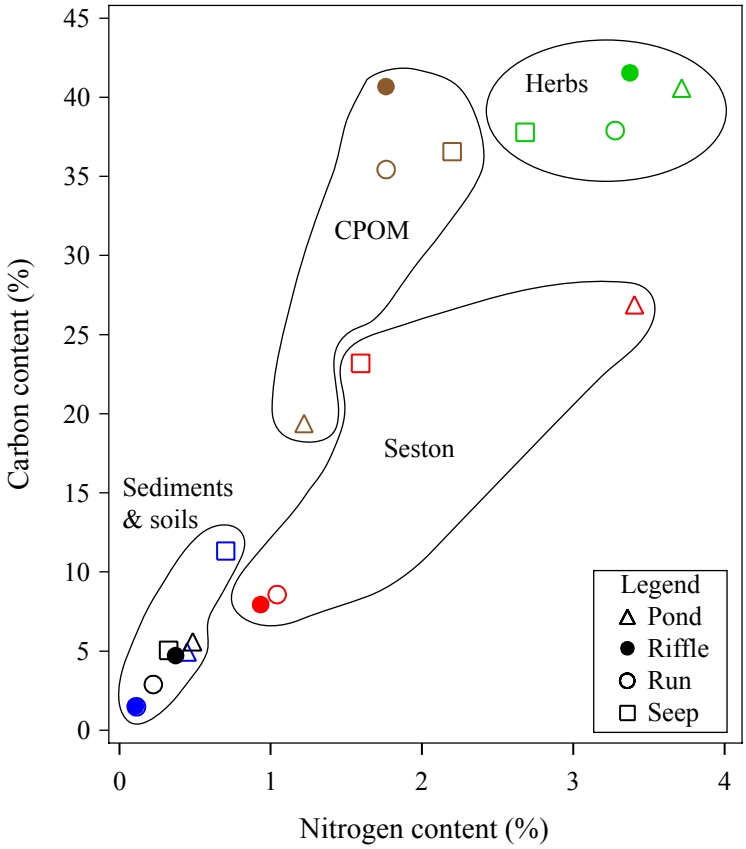
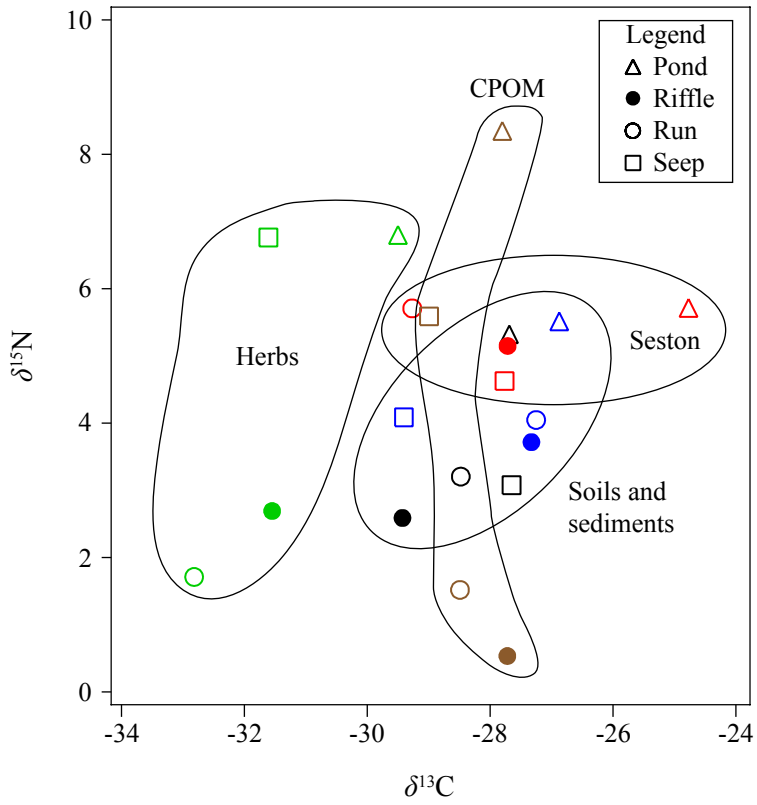

Carbon content (%)
Nitrogen content (%)
0
5
10
15
20
25
30
35
40
45
0
1
2
3
4
Herbs
CPOM
Seston
Sediments
& soils
Legend
Pond
Riffle
Run
Seep

Figure 3.8


Legend
Pond
Riffle
Run
Seep
CPOM
Herbs
Seston
Soils and
sediments
10
8
6
4
2
0
-34
-32
-30
-28
-26
-24
δ15N
δ13C

Chapter 4: A method for measuring bacterial mineralization rates of suspended particulate organic carbon in stream ecosystems

Introduction

Each year rivers export approximately 0.2 Pg of particulate organic carbon (POC) to the oceans (Spitzy and Ittekot 1991; Battin et al. 2008). Streams and rivers not only transport POC, but also play an active role in the processing of POC through mineralization of allochthonous and autochthonous organic matter. For example, CO_2 out-gassing from large rivers indicates that POC is metabolically important and at least a fraction of heterotrophic metabolism in the water column is supported by POC exported from upstream reaches (Cole and Caraco 2001; Richey et al. 2002; Mayorga et al. 2005; Battin et al. 2008). Yet, despite the ecological importance of POC, little is known about respiration rates of suspended POC in streams and, therefore, the quality of small (<1 mm) particles in transport (but see Naiman and Sedell 1979).

Most measurements of POC quality use benthic POC, which supports local ecosystems and is relatively easy to collect from the streambed. Webster et al. (1999) summarized results of respiration rates on benthic POC from several streams and determined that POC quality in small streams is low. In those studies, the biological turnover time, defined as the average time before organic carbon is respired as inorganic CO_2, was 2.6 years, implying that POC would travel tens to hundreds of

kilometers into downstream rivers, estuaries, and oceans before it is used by the heterotrophic community (Webster et al. 1999).

POC transport would therefore appear to be a weak longitudinal link between upstream production, both terrestrial and aquatic, and downstream rivers. However, the strength of the link is controlled by the quality (lability) of the transported POC (Mayorga et al. 2005). Labile POC should be metabolized in upstream reaches within short transport distances, while refractory (non-labile) POC should travel greater distances downstream with minimal biological processing (e.g. Kaplan and Newbold 2003). A continuous array of labilities exists within the suspended load (seston), with POC ranging from labile (e.g. fresh algal cells) to refractory (e.g. humified soil organic matter). Understanding the diversity of POC quality is critical in determining the fraction of ecosystem respiration supported by POC transported from upstream and the resulting degree of longitudinal energy transfer.

Microbial respiration rates are used to estimate POC lability and link energy use with organic matter quality (Benner et al. 1995; Opsahl 2005). The rate of dissolved oxygen (O_2) consumption under dark conditions is a simple and direct assessment of organic carbon oxidation resulting from heterotrophic metabolic activity and therefore an appropriate measure of carbon quality.

However, there are several methodological concerns regarding the ability to measure POC quality: (1) oxygen measurements need to be precise, (2) dissolved

organic carbon (DOC) respiration may mask POC respiration in small streams, and (3) present methods do not replicate instream conditions or measure the range of suspended POC lability. In most small forested streams, concentrations of suspended POC are quite low, and heterotrophic respiration yields small changes in oxygen. Without precise measurements of oxygen concentrations, the quality of suspended POC is difficult to assess. For example, an average suspended POC concentration in a piedmont 3rd-order stream is 0.15 mg POC L^{-1} (Chapter 1). Heterotrophic respiration of 0.03 mg POC L^{-1}, i.e. 20% of the total concentration, requires oxygen measurements with <1% coefficient of variation to detect the associated consumption of oxygen, assuming a respiratory quotient of 1. Furthermore, DOC concentrations often exceed POC concentrations in northeastern Piedmont streams (DOC:POC>1; Kaplan et al. 2006) and respiration of the larger DOC pool could mask oxygen consumption from POC respiration. The incubation of POC is also critical to measurements of heterotrophic oxygen consumption, and therefore measurements of POC quality. Even though seston is typically in a turbulent environment and constantly bouncing off the stream bed (McNair and Newbold 2001), most methods for measuring respiration require the samples to be shaken lightly, if at all (Benner et al. 1995; Webster et al. 1999; Bonin et al. 2000). Replicating suspension under laboratory conditions could be important for accurately measuring respiration rates, because suspension may maximize oxygen diffusion to the microorganisms attached to individual particles (Ploug and Grossart 1999). Also, most respiration rates are measured after short-term incubations (hours: Webster et al. 1999; Benner et al. 1995), yet, POC in streams and rivers could be comprised of a small, rapidly-used,

labile fraction mixed with a larger, gradually-consumed, refractory fraction. The idea that the carbon pool is comprised of a range of labilities has been similarly proposed for stream DOC (Kaplan and Newbold 2003), soil organic matter (Trumbore 2000) and marine sedimentary organic matter (Berner 1995). Short-term measurements do not sufficiently represent the continuum of POC lability, but long-term measurements (weeks to months) begin to distinguish labile from refractory or recalcitrant fractions.

In this chapter, I (1) report a method for measuring the quality of suspended POC from aquatic environments using heterotrophic respiration as a metric for lability, (2) test different components of the method, and (3) compare respiration rates of suspended POC from this study to reported respiration rates of POC. The method includes concentrating POC relative to DOC, keeping particles in suspension, making precise oxygen measurements over a period of 35-40 days, and correcting for respiration of DOC. I measured suspended POC quality from a 3rd-order SE Pennsylvania stream on two different dates and compared treatments where POC was allowed to settle versus those where POC was kept continually in suspension. I also tested the effect of adding beads, which disperse POC and break up flocs. Finally, I tested the effect that concentration of POC had on DOC lability. Using this method, initial respiration rates for suspended POC were an order of magnitude higher than those reported in the literature for benthic POC (Webster et al. 1999).

Methods

Site description – Three experiments were conducted to assess a new method for measuring heterotrophic respiration of suspended particulate and dissolved organic carbon. The three experiments started on 17 January 2006 (*Jan 2006*), 04 April 2007 (*Apr 2007*) and 15 August 2007 (*DOC lability*). Stream water was collected for all three experiments from the upper east branch of White Clay Creek (WCC: N39°51'33.9", W75°46'59.6"), a 3rd-order watershed draining 7.3 km^2 of eastern piedmont land. The watershed has 23% of total area as temperate deciduous forest and 74% as agricultural land, but has a largely intact riparian forest and livestock have minimal access to the stream (Newbold et al. 1997). At baseflow, WCC averages 122 L s^{-1} discharge, has high nutrient concentrations (3-5 mg NO_3-N L^{-1}; 4-33 μg PO_4-P L^{-1}) from agricultural land use, moderate dissolved organic carbon concentrations (1.5 mg DOC L^{-1}; Newbold et al. 1997) and between 0.1 and 0.4 mg suspended POC L^{-1} (Chapter 1).

Sample collection and concentration – For each experiment, stream water was collected from WCC at baseflow using a submersible battery operated pump (Whale pump) suspended at 2/3 depth in the water column (approximately 15 cm deep). Water was pumped through a 53 μm sieve to remove any large particles, while still collecting ~90% of baseflow seston particles (Kaplan et al. 2002; Kaplan et al. 2006). I concentrated the particles using tangential flow filtration (TFF; Millipore Pellicon) to increase POC concentrations. TFF applies flow across the surface of a membrane (0.65 μm pore-size Millipore Pellicon 2 Cassette Filter), driven by a peristaltic pump.

Particles that are larger than the membrane pore size are kept in suspension to minimize accumulation and clogging on the membrane while pressure forces a portion of the water through the membrane as filtrate. Approximately 200 L was concentrated to 20 L of sample for Jan 2006 and Apr 2007 experiments and 100 L of stream water to 10 L of sample for the DOC lability experiment (Aug 2007). However, the TFF process may involve loss of particle mass because of adsorption to the membrane (Minor and Nallathamby 2004).

Experimental design – For the four treatments tested in each experiment (Jan 2006 and Apr 2007), the final volume of concentrated water (20 L) was divided into four 5-L aliquots (Table 4.1). I measured heterotrophic respiration on DOC alone as a control and to correct for respiration of DOC in bottles containing both DOC and POC. I also tested the effects of (1) keeping particles suspended using a rotating axle (see *incubations* section), (2) dispersing particles and aggregates using glass beads, and (3) allowing the particles to settle in static treatments. For the DOC lability experiment, there were only two treatments and both were particle-free (preTFF and postTFF) to test the effect of TFF on DOC quantity and quality.

To remove particles for the DOC control treatments, I filtered 5 L of concentrated particles through glass fiber filters (Millipore AP40 with nominal pore-size of 0.7 μm) using vacuum filtration. All 5 L subsamples, with and without POC, were left in a water bath (<8 hrs) to equilibrate with the incubation temperature (18°C) and then were shaken vigorously by hand for 5 minutes to saturate the water

with oxygen. Sample water from each 5L bottle was divided into 21 muffled borosilicate glass 60-mL BOD bottles (Wheaton) using a 5 L churn splitter (USGS 2002) to ensure homogenous distribution of POC into each BOD bottle. Each bottle was filled at a slow but steady rate to overflow to avoid adding air bubbles to the bottle. The bottle was capped with a glass stopper and another plastic cap was placed over the stopper while the bottle was submerged in a water bath to eliminate any potential for atmospheric exchange with gases in the sample water. To test the effect of dispersing particle and bacterial flocs in both POC (Jan 2006) and DOC alone (Apr 2007) treatments (Table 4.1), I added 20 acid washed (1N HCl) and muffled (6 hours, 480°C) glass beads (4 mm diameter) to the 60-mL BOD bottles prior to adding the sample water. The beads provided additional surface area (50 mm^2 $bead^{-1}$) for bacterial growth. However, adding 20 beads only increased the internal surface area of the BOD bottle (11,000 mm^2) by 9%.

Carbon measurements – Replicate aliquots of sample water were taken from the churn splitter at the beginning, middle and end of each sample distribution to analyze for initial POC and DOC concentrations. Sample water for all treatments was filtered through stacked pairs of identical pre-weighed and pre-ashed (480ºC for 4.5 hours) glass fiber filters (Millipore AP40 with nominal pore-size of 0.7 μm). The filters were dried for 12-18 hours at 60ºC and re-weighed to calculate seston concentration (mg L^{-1}). Subsamples of each seston filter were analyzed for carbon content using an Elemental Analyzer (Carlo Erba) (Chapter 1). DOC concentration was measured from the filtrate water (<0.7 μm) using UV catalyzed persulfate oxidation detected by conductivity (Sievers 800 or 900 TOC analyzers; Kaplan 1992).

Incubations – Each 60-mL BOD bottle was incubated in the dark in a large temperature controlled water bath. For the settled particle treatment, BOD bottles were placed on the water bath floor and particles settled to the bottom of the bottle within 1 hour. The remaining bottles (suspended treatments) were strapped to the incubation axle to keep particles in suspension (Fig. 4.1). The incubation axle was a 2 m PVC axle with maximum capacity of 108 sixty-mL BOD bottles. The axle, connected to a motor by a plastic gear and chain, rotated at 5 revolutions per minute to keep particles in constant suspension. Temperature in the water bath was maintained at 18°C for all experiments by circulating water between the water bath and a thermostatically regulated reservoir.

Dissolved oxygen measurements – Three randomly selected replicate bottles were removed at 7 time periods for Jan 2006 and Apr 2007 experiments (approximate sampling times after initialization of the experiment: 0 hours, 5-7 hours, 24-36 hours, 7, 14, 28, and 35 days). Only the first 5 time periods were sampled for the DOC lability experiment. I quantified bacterial respiration by measuring changes in oxygen concentration over time using the Winkler technique with auto-titration (Bryan et al. 1976; Graneli and Graneli 1991). Each bottle was treated with 0.4 mL of manganous sulfate and 0.4 mL of alkaline iodide solutions followed by 0.8 mL of hydrosulfuric acid (18 N) (Bryan et al. 1976). A 25-mL aliquot was drawn from the acidified solution with a volumetric pipette and was titrated using sodium thiosulfate (0.0125 N). Titration equivalence points were determined potentiometrically using an

auto-titrator (Mettler DL-21) equipped with a platinum combination electrode (Mettler DM 140-SC) (Graneli and Graneli 1991; Furuya and Harada 1995). Oxygen concentrations can be routinely measured with 0.25% coefficient of variation using careful handling procedures and automated titrations (A.K. Aufdenkampe unpubl.). As a further check, each time I ran sequences of samples on the auto-titrator, one duplicate sample was measured immediately after the first. Despite known issues with volatilization of iodine, analytical uncertainty was 0.6%, measured as percent coefficient of variation.

Model fitting and treatment comparisons – For each treatment in each experiment, I fit the loss of oxygen concentration over time (oxygen consumption curve) with a first order loss rate curve (eq. 4.1) using nonlinear least squares analysis (proc NLIN, SAS 8.1; SAS Institute Inc., Cary, NC, U.S.A.)

Equation 4.1 $O_2 = c_1 e^{-k * t} + c_0$

where t is time (hours). I fit three constant parameters to eq. 4.1: the first parameter, k, is the temporal loss rate ($hours^{-1}$); the reciprocal of the loss rate is the observed biological turnover time ($T_{b\text{-}observed}$) of POC. The second parameter, c_1 (mg O_2 L^{-1}), is the total possible oxygen consumed from heterotrophic respiration. The third parameter, c_0 (mg O_2 L^{-1}), is the oxygen concentration that is unconsumed by respiration; $c_1 + c_0$ is the total initial oxygen concentration in each bottle at the start of the experiment.

To derive oxygen consumption for POC alone, oxygen consumption for the POC + DOC treatments was corrected for heterotrophic respiration of DOC (eq. 4.2, see Appendix 1 for derivation).

Equation 4.2 $O_{2\ corrected} = A_{exp} - (c_{1doc}\ e^{-kdoc\ *\ t} + c_{0doc}) + (c_1\ e^{-k\ *\ t} + c_0)$

For each experiment, the oxygen concentration consumed by DOC respiration was calculated by subtracting the DOC consumption curve, using k_{doc}, c_{1doc}, and c_{0doc} parameters for the DOC control, from A_{exp}, the initial oxygen concentration for each experiment (8.3 mg L^{-1} for Jan 2006 and 8.05 mg L^{-1} for Apr 2007). Oxygen consumption from DOC respiration was added to the oxygen consumption curve from total carbon (POC + DOC) using k, c_1, and c_0, parameters from the POC + DOC consumption curve of interest. The resulting oxygen consumption curve, for POC alone, was fit with a new curve with parameters k, c_1, and c_0 (eq. 4.1).

Model fits (r^2, *p*-values from *F*-statistics) were calculated for each curve using the extra sums of squares principal (Draper and Smith 1998). I report approximate 95% confidence limits for all model parameters (c_0, c_1, k) as calculated by the least squares analysis (proc NLIN). Comparisons between treatments (t-tests) were made using estimates of the rate coefficients and standard errors from nonlinear least squares analysis. The absolute value of the first derivative (instantaneous slope) of eq. 4.1 was derived (eq. 4.3) to calculate change in oxygen per unit time (eq. 4.3).

Equation 4.3 $dO_2\ dt^{-1} = c_1\ k\ e^{-k * t}$

To calculate respiration rates, eq. 4.3 was scaled to the change in oxygen mass per initial mass of carbon (mg O_2 initial g C^{-1} day^{-1}) using the BOD bottle volume (60 mL) and the initial mass of DOC or POC in each bottle. The instantaneous biological turnover time ($T_{b\text{-instant}}$) was calculated by taking the reciprocal of the decomposition rates calculated from respiration rates assuming a respiratory quotient of 1 (Webster et al. 1999).

Results

Jan 2006 and Apr 2007 experiments – POC concentrations were elevated 5-fold above stream POC concentrations after tangential flow filtration for both experiments. POC, as the % of total organic carbon (POC + DOC), was increased from 11% to 44% for Jan 2006 and 9% to 33% for Apr 2007 (Table 4.2); however TFF concentration of particles resulted in ~50% mass recovery in both experiments. For the Jan 2006 experiment, heterotrophic oxygen consumption was highest in the POC + DOC treatments (dispersed, suspended particles>suspended particles>settled particles) and lowest in the DOC control treatment (Fig. 4.2a). For the Apr 2007 experiment, heterotrophic oxygen consumption was highest in the POC + DOC treatments (dispersed, suspended particle and settled particle) and lowest in the DOC control treatments (DOC control and dispersed DOC control) (Fig. 4.3a). The first order decay model (eq. 4.1) was an excellent fit for all curves in both experiments

(Table 4.3, r^2>0.97). After correcting for DOC driven oxygen consumption, at the onset of the experiment, respiration rates for POC were 180 mg O_2 initial g C^{-1} day^{-1} for the Jan 2006 experiment and 300 mg O_2 initial g C^{-1} day^{-1} for the Apr 2007 experiment. After 30-35 days, respiration rates slowed to approximately 10 mg O_2 initial g C^{-1} day^{-1}, or 5% and 3% of the initial rates, respectively (Fig. 4.2b, 4.3b). For the Jan 2006 experiment, the largest difference among POC treatments occurred within the first week, with respiration rates reduced by 33% from the dispersed, suspended particle to the suspended particle treatments and 50% from suspended particle to settled particle treatments (Fig. 4.2b). For the Apr 2007 experiment, the largest difference among POC treatments also occurred within the first week, with respiration rates reduced by 26% from dispersed, suspended particle to settled particle treatments (Fig. 4.3b). The respiration rates for dispersed, suspended particles were, at first, 4- to 6-times greater than the respiration on DOC alone, although after 3+ weeks, the respiration rates approached the same asymptote at approximately 10 mg O_2 initial g C^{-1} day^{-1} (Fig. 4.2b, 4.3b).

Using the temporal loss rate coefficient (k) for the Jan 2006 experiment, I calculated that the biological turnover time for carbon ($T_{b\text{-observed}}$) was shortest in the dispersed, suspended particle treatment, followed by the suspended particle treatment, settled particle treatment, and lastly in the DOC control (Table 4.4). For the Apr 2007 experiment, $T_{b\text{-observed}}$ was shortest in the dispersed, suspended particle and settled particle treatments, and those turnover times were not significantly different from each other (Table 4.4; t=-2.17, df=8, p=0.06). The two DOC control treatments had slower turnover times, and those rates were not significantly different from each

other (Table 4.4; t=-0.88, df=36, p=0.38). The dispersed, particle treatment turnover time was 3x faster than the DOC control (Table 4.4; t=9.12, df=22, p<0.001). The turnover time for DOC control was not statistically different between the Jan 2006 and Apr 2007 experiments (Table 4.4; t=0.14, df=36, p=0.88). However, when comparing dispersed, suspended particle treatments from both experiments, the turnover time for Apr 2007 was 40% shorter than for the Jan 2006 experiment (Table 4.4; t=3.2, df=8; p=0.01).

DOC lability experiment – Tangential flow filtration elevated DOC concentrations by 60% from 1.3 mg DOC L^{-1} to 2.1 mg DOC L^{-1}. Heterotrophic consumption of oxygen was higher in the postTFF than preTFF treatment (Fig. 4.4a) and the first order decay model (eq. 4.1) was an adequate fit for both treatments (r^2>0.92; Table 4.3). $T_{b\text{-observed}}$ for preTFF DOC (4.6 days) and postTFF (12.8 days) were not significantly different (Fig. 4.4b, Table 4.4; t=1.27, df=24, p=0.21).

Discussion

I found that TFF elevated POC concentration enough to measure respiration over short incubation times with small changes in oxygen, especially when using the Winkler method with auto-titration for oxygen measurements. Since respiration of DOC is a significant fraction of oxygen consumption, correction for respiration of DOC was necessary in treatments with both POC and DOC to estimate the heterotrophic respiration rate of POC alone. Keeping particles suspended and dispersed maximized oxygen delivery to the particles and provided the most realistic

conditions for incubations. Finally, long-term incubations provided insight to the continuum of POC lability. I discuss each of these components of the method below.

Small changes in oxygen concentration due to heterotrophic respiration of suspended POC are difficult to measure due to high DOC:POC ratios and low concentrations of POC. I was unable to find any other studies of POC lability that corrected for respiration of DOC during incubations. This correction is necessary because DOC consumption was a significant portion of total oxygen consumption in POC + DOC treatments (Fig. 4.2a, 4.3a). Without correction for DOC respiration, oxygen consumption due to POC would have been overestimated by at least 25% at the beginning of the study and by 50% the end of the incubation.

Although benthic POC with a mean particle size >125 μm has been used for most studies of POC quality in streams (Fuss and Smock 1996; Webster et al. 1999; Bonin et al. 2000), most suspended POC in headwater streams is between 0.7 and 20 μm in diameter (Kaplan et al. 2006). Methods to collect POC with nets or sieves only concentrates particles ≥15-20 μm and recovery of gravity-filtered particles off glass fiber or membrane filters has proved to be challenging and ineffective (D. C. Richardson pers. obs.). Using TFF, I was able to concentrate small particles well above stream concentrations and relative to DOC, causing greater changes in oxygen concentration due to heterotrophic consumption of POC than would be typically measured. TFF might not be necessary to measure POC quality when POC concentrations are high such as in samples from large rivers like the Amazon or

Mississippi, anthropogenically impacted areas, pond and reservoir outflows, or those collected during storms (Whiles and Dodds 2002; Dodds and Whiles 2004). However, in forested headwater streams with high DOC:POC ratios and low POC concentrations, concentration of suspended POC is necessary to adequately measure POC respiration rates.

Although TFF was advantageous in elevating POC concentrations, recovery of POC from TFF (50-60%) was less than optimal. This inefficiency led to more processing time and extended the length of time before the incubation could start. Poor recovery may be due to adsorption of particles to the membrane (Minor et al. 1999), and I am currently investigating its causes and modifying the TFF procedure to maximize POC return. Additionally, electrostatic gradients repel DOC molecules (Benner et al. 1997) and cause an unintended concentration of DOC by 60% (Aug 2007; Table 4.2). I am uncertain if TFF modified DOC quality because I could not detect significant differences in turnover times between pre- and post-TFF DOC in the DOC lability experiment (Table 4.4). If TFF concentrates DOC without discrimination between DOC molecules of differing lability, then the respiration rates, corrected for initial DOC concentration, and turnover times should be equal between pre- and post-TFF DOC. In this study, I accounted for this potential change in DOC quality by using post-TFF DOC as the DOC correction for all POC treatments.

Rotation and mixing of BOD bottles using the incubation axle increased oxygen consumption and respiration rates over static bottles for both experiments, especially during the initial week (Fig. 4.2, 4.3). Diffusion of oxygen to particle-attached microorganisms and POC is faster for suspended rather than settled particles since layering in settled treatments will limit solute exchange to lower layers of particles (Ploug and Grossart 1999). For example, Ploug and Grossart (1999, 2000) found that bacterial production on particles increased 5- to 10-fold when seston was kept in suspension during incubations compared to those under static conditions. In addition to rotating samples, I added beads to some bottles to generate turbulence and break up flocs. In initial experiments using the incubation axle, flocs, visible to the naked eye, formed in the BOD bottles while rotating on the incubation axle (D. C. Richardson pers. obs.). These flocs likely form because of binding bacterial extracellular polymeric substances and charge attractions in quiescent water (Droppo 2004), but I did not see them in any microscopy of WCC seston. The beads increase internal mixing and break up flocs, but do not artificially increase substratum for bacterial growth and use of DOC (Fig. 4.3b). Conditions of mass transfer and chemical microenvironments for particles are likely more similar to natural stream conditions using the incubation axle and beads due to suspension and mixing of particles.

When measuring the oxygen concentrations in each bottle, I used the Winkler method with auto-titration. This is an efficient, accurate and precise method to measure oxygen concentration, especially when attempting to detect small amounts

of oxygen consumption (Furuya and Harada 1995). Auto-titration was accurate within ±0.06 mg O_2 L^{-1} for Winkler measurements (Ploug et al. 2002), which is advantageous over previously used oxygen concentration measurements such as the Gilson respirometer (Webster et al.1999) or using polargraphic measurements of oxygen using probes, which can lack the precision and accuracy necessary to measure the small changes in oxygen concentration (CWT 2004). This precision is especially important during the initial 24 hours of oxygen measurements when concentrations changed by <0.5 mg L^{-1} (Fig. 4.2a, 4.3a).

Incubations and measurements over 30+ days enable separation of high quality, labile organic matter from refractory organic matter (Fig. 4.2b, 4.3b). Short-term measurements are adequate for measuring initial rates but do not allow for separation of a continuous range of labilities including labile, resistant, and refractory classes (Berner 1995; Trumbore 2000; Kaplan and Newbold 2003). Combining the range of lability classes with differential transport of suspended POC produces upstream-downstream linkages of varying scales. Labile POC, with turnover times of less than 10 days (Table 4.4), transported under base-flow conditions could support metabolism in local downstream reaches. Labile POC could also be transported long distances by storms and would support heterotrophic respiration in mid-order streams. Less labile material could be transported by storms and baseflow until the POC is used for metabolism in rivers far downstream (e.g. Cole and Caraco 2001; Mayorga et al. 2005) or is exported from the river network.

The observed difference in oxygen consumption between experiments (Jan 2006 and Apr 2007) was most evident in the initial respiration rates (Fig. 4.5). POC lability is likely linked to seasonal differences in the composition of seston, specifically increases in algal derived carbon. In early April, the stream canopy is open, light levels are higher, and periphyton growth is at a maximum on the streambed leading to high chlorophyll *a* and carbon content of seston (Chapter 1; Pozo et al. 1994). In this study, I saw a 2-fold increase in initial respiration rates from the January to the April experiment (Fig. 4.5). This lability could be linked to an increase in high nutrient content organic matter (algal cells) (Chapter 3; Pozo et al. 1994). Similarly, in a eutrophic lake, suspended POC quality dramatically increased during warm season algal blooms (Parparov et al. 1998) and increasing nitrogen content of benthic POC was correlated with increasing heterotrophic respiration rates (Fuss and Smock 1996).

Respiration rate comparisons – I compared the respiration rates from both dispersed, suspended particle treatments to a range of POC respiration rates measured in a variety of small streams and organic matter from different aquatic environments (Table 4.5). The respiration rates for POC measured using the method reported here were initially 15-fold greater than those reported by Webster et al. (1999) for benthic POC from a range of locations including Michigan, Oregon, Idaho, Virginia, and North Carolina streams (Fig. 4.5). However, the initial rates from the current experiment were comparable to respiration rates for marine DOM from shallow water (Biddanda et al. 1994), eutrophic lake seston (Parparov et al. 1998), and diatom

formed aggregates (Ploug and Grossart 2000). After 25-27 days, the respiration rates had decreased to the range of literature reported rates for stream POC (Fig. 4.5; Fuss and Smock 1996), leaves and wood veneers incubated in the stream (Stelzer et al. 2003), and wetland DOM (Opsahl 2005). These comparisons support the hypothesis that high initial rates of respiration in this study were driven by algal sources with rapid biological turnover times ($T_{b\text{-}instant}$<1 week, Fig. 4.5). Respiration rates after one month were possibly driven by remaining semi-labile and refractory POC including mineral-organic complexes (i.e. clay particles with adsorbed organic carbon) and leaf fragments ($T_{b\text{-}instant}$ >4 months, Fig. 4.5).

The differences between measurements of suspended POC from this study and benthic POC from other studies (Table 4.5) appear to be related to both the ability of the method reported here to measure rapid initial respiration rates and to the greater lability of suspended POC relative to benthic POC. This difference in lability may be related to particle size, i.e. the majority of benthic POC is >50 μm, while the majority of suspended POC is <20 μm (Minshall et al. 1983; Kaplan et al. 2002). At baseflow, suspended POC likely represents a subset of small and labile or semi-labile benthic particles that is easily suspended and transported downstream including individual diatom cells, bacterial cells, leaf fragments and small clays with adsorbed organic matter. Anthropogenic point sources, such as farm ponds, may also contribute labile POC during baseflow (Chapter 3).

Directions for future research – Over a 35 day period, I measured a range of respiration rates for suspended POC, from 10 to 300 mg O_2 initial g C^{-1} day^{-1} with observed biological turnover times of approximately 10 days (Table 4.4). The results from this study showed the continuous range of lability of POC and indicated that suspended POC has the potential to influence metabolism in reaches immediately downstream as well as rivers (Cole and Caraco 2001; Mayorga et al. 2005). At low flows, small particles move downstream at 150 m day^{-1} (Newbold et al. 2005) and with a turnover time of 10 days (this study), the associated POC would be metabolized after traveling only 1.5 km downstream. This travel distance is far shorter than the 42 km of travel distance estimated by Webster et al. (1999). Using this method to measure suspended POC quality from a range of stream ecosystems, including streams with high primary production (e.g. meadow streams) or streams dominated by fluxes of terrestrially-derived organic matter (e.g. small forested headwaters), will help determine ranges of POC lability in stream ecosystems and quality of organic matter exported from headwaters. Application of the method presented here to other stream ecosystems will help advance the understanding of the role of river networks in controlling the transformations of allochthonous and autochthonous POC transported from inland waters to the oceans (Cole et al. 2007).

Tables

Table 4.1. Experimental Design for January 2006 and April 2007 experiments. ● indicates the treatment combination was used in that experiment; X indicates that there were particles in the bottle (POC), particles were kept in suspension (Incubation axle) and the bottle was mixed and particles were dispersed (Beads).

Treatment	Jan 2006	Apr 2007	POC	Incubation axle	Beads
Dispersed, suspended particles	●	●	X	X	X
Suspended particles	●		X	X	
Settled particles	●	●	X		
DOC control	●	●		X	
Dispersed DOC control		●		X	X

Table 4.2. Initial particulate and dissolved organic carbon concentrations (mean ± SD) for all experiments. N.A. indicates that POC concentration was not measured.

Experiment	Treatment	POC (mg L^{-1})	DOC (mg L^{-1})
Jan 2006	DOC control	<0.03	1.5 ± 0.05
	Settled particles	0.98 ± 0.04	1.5 ± 0.02
	Suspended particles	0.92 ± 0.10	1.5 ± 0.04
	Dispersed, suspended particles	0.99 ± 0.06	1.5 ± 0.02
Apr 2007	DOC control	<0.03	2.1 ± 0.08
	Dispersed DOC control	<0.03	2.2 ± 0.09
	Settled particles	1.12 ± 0.08	2.1 ± 0.08
	Dispersed, suspended particles	0.97 ± 0.07	2.1 ± 0.05
DOC lability	preTFF	N.A.	1.3 ± 0.02
	postTFF	N.A.	2.1 ± 0.08

Table 4.3. Oxygen consumption curve ($O_2 = c_1 e^{-k X t} + c_0$) parameter fits for each treatment and experiment. Estimates (95% confidence intervals) for parameters k, c_0, and c_1 included with model fits (r^2). All models were significant fits ($p<0.001$).

Exp.	Treatment	k ($hour^{-1}$)	c_1 (mg O_2 L^{-1})	c_0 (mg O_2 L^{-1})	r^2
Jan 2006	DOC control	0.0013 (0.0003-0.0023)	2.0 (1.1-2.8)	6.3 (5.4-7.2)	0.96
	Settled particles	0.0016 (0.0013-0.0019)	3.1 (2.8-3.5)	5.3 (4.9-5.7)	0.99
	Suspended particles	0.0023 (0.0013-0.0033)	3.3 (2.7-3.8)	5.0 (4.4-5.6)	0.97
	Dispersed, suspended particles	0.0028 (0.0020-0.0036)	3.6 (3.3-4.0)	4.8 (4.4-5.2)	0.98
Apr 2007	DOC control	0.0014 (0.0008-0.0021)	3.6 (2.8-4.5)	4.5 (3.6-5.5)	0.98
	Dispersed DOC control	0.0018 (0.0012-0.0023)	4.0 (3.4-4.6)	4.2 (3.6-4.9)	0.98
	Settled particles	0.0030 (0.0027-0.0034)	5.1 (4.9-5.3)	3.4 (3.2-3.6)	0.99
	Dispersed, suspended particles	0.0030 (0.0024-0.0036)	6.1 (5.7-6.5)	2.5 (2.1-2.9)	0.99
DOC lability	preTFF	0.0090 (0.0011-0.0168)	0.6 (0.5-0.8)	7.8 (7.6-7.9)	0.96
	postTFF	0.0033 (-0.0026-0.0091)	1.5 (-0.1-3.1)	6.9 (5.2-8.6)	0.92

Table 4.4. Temporal loss rate coefficients (k) and biological turnover times ($T_{b\text{-}observed}$) for all treatments with either POC corrected for DOC respiration or DOC alone. Loss rate coefficients of treatments with the same letter were not significantly different when analyzed using all pairwise t-tests within the experiment where $p<0.05$. T-tests were completed using estimates of k and standard errors generated from nonlinear least squares analysis.

Exp.	Treatment	Carbon Source	k ($hour^{-1}$)	Biological Turnover Time ($T_{b\text{-}observed}$ = 1/k, days)	Within Experiment Grouping
Jan 2006	DOC control	DOC	0.0013	31.6	a
	Settled particles	POC	0.0020	21.3	a
	Suspended particles	POC	0.0032	12.9	b
	Dispersed, suspended particles	POC	0.0040	10.5	c
Apr 2007	DOC control	DOC	0.0014	29.8	a
	Dispersed DOC control	DOC	0.0018	23.7	a
	Settled particles	POC	0.0056	7.5	b
	Dispersed, suspended particles	POC	0.0046	9.0	b
DOC lability	preTFF	DOC	0.0090	12.8	a
	postTFF	DOC	0.0033	4.6	a

Table 4.5. Ranges of respiration rates for different carbon sources. Biological turnover time ($T_{b\text{-}instant}$) was calculated from respiration rates assuming a respiratory quotient of 1.

Carbon Source	Respiration rate (mg O_2 g C^{-1} day^{-1})	Biological turnover time ($T_{b\text{-}instant}$, days)	Citation
Stream suspended POC	10 - 300	9-270	This study
Benthic POC	1 - 21	130-2660	Webster et al. 1999[a]
Benthic POC	5 - 20	130-530	Bonin et al. 2000
Benthic POC, Leaves, Wood	3 - 7	380-890	Fuss and Smock 1996
Leaves and Wood	2 - 22	120-1330	Stelzer et al. 2003
Wetland DOC	1 - 19	140-2660	Opsahl 2005
Marine DOC	101 - 143	19-26	Biddanda et al. 1994
Lake suspended POC	69 - 187	14-39	Parparov et al. 1998[a]

[a]See citation for literature summary of respiration rates

Figure Captions

Figure 4.1. The left side of the incubation axle in the temperature controlled water bath.

Figure 4.2. (a) Oxygen consumption, where lines indicates model fit ($O_2 = c_1 e^{-k * t} + c_0$), and (b) respiration rates for Jan 2006 experiment. POC respiration rates are corrected for DOC respiration. Treatments are DOC control (DOC), settled particles (Pset), suspended particles (Psus), and dispersed, suspended particles (Psus,dis). Error bars are standard errors.

Figure 4.3. (a) Oxygen consumption, where lines indicates model fit ($O_2 = c_1 e^{-k * t} + c_0$), and (b) respiration rates for Apr 2007 experiment. POC respiration rates are corrected for DOC respiration. Treatments are DOC control (DOC), dispersed DOC control (DOCdis), settled particles (Psus), and dispersed, suspended particles (Psus,dis). Error bars are standard errors.

Figure 4.4. (a) Oxygen consumption, where line indicates model fit ($O_2 = c_1 e^{-k * t} + c_0$), and (b) respiration rates for DOC lability experiment. Treatments are pre-tangential flow filtration (preTFF) and post-tangential flow filtration (postTFF). Error bars are standard errors.

Figure 4.5. Respiration rates for the dispersed, suspended particle treatments from Jan 2006 and Apr 2007 experiments. The grey box represents the range of measured respiration rates from Webster et al. (1999). Right y-axis values indicate biological turnover time ($T_{b\text{-}instant}$) assuming respiratory quotient of 1 (Webster et al. 1999).

Figures

Figure 4.1

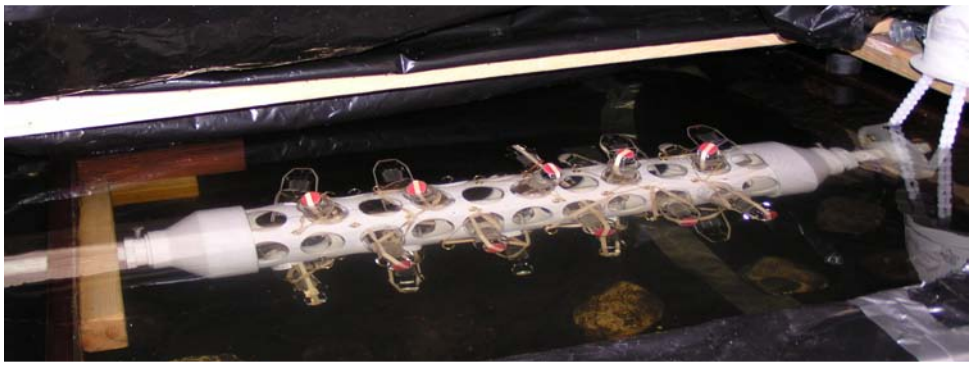


Figure 4.2

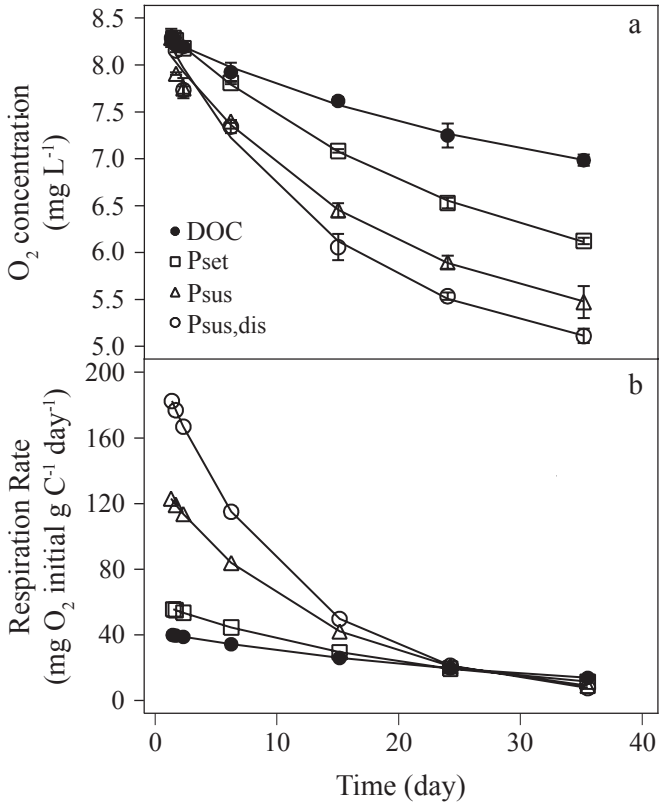

a
b
O_2 concentration (mg L^{-1})
Respiration Rate (mg O_2 initial g C^{-1} day^{-1})
DOC
Pset
Psus
Psus,dis
Time (day)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
200
160
120
80
40
0
0
10
20
30
40

Figure 4.3

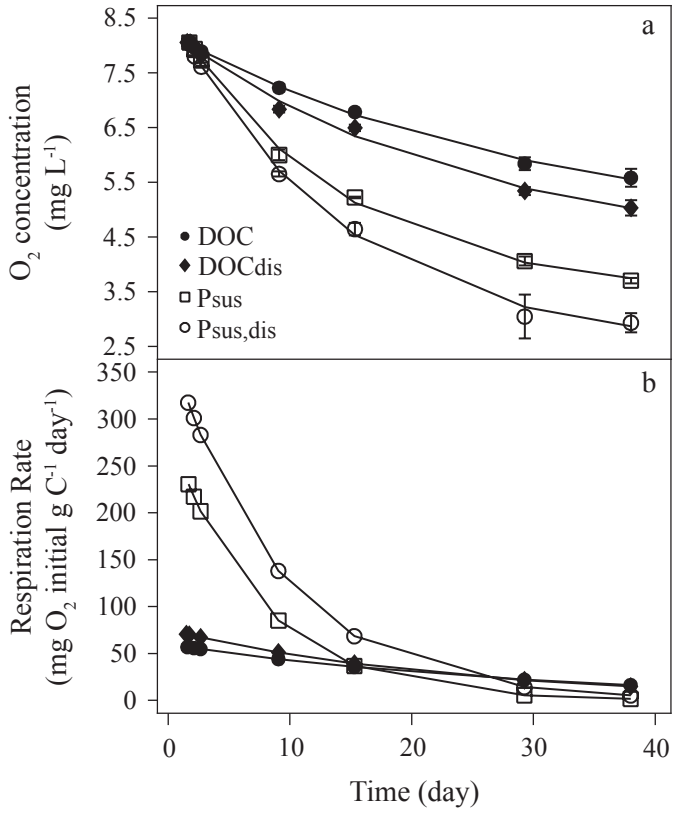

a
b
O_2 concentration (mg L^{-1})
Respiration Rate (mg O_2 initial g C^{-1} day^{-1})
DOC
DOCdis
Psus
Psus,dis
Time (day)
8.5
7.5
6.5
5.5
4.5
3.5
2.5
350
300
250
200
150
100
50
0
0
10
20
30
40

Figure 4.4

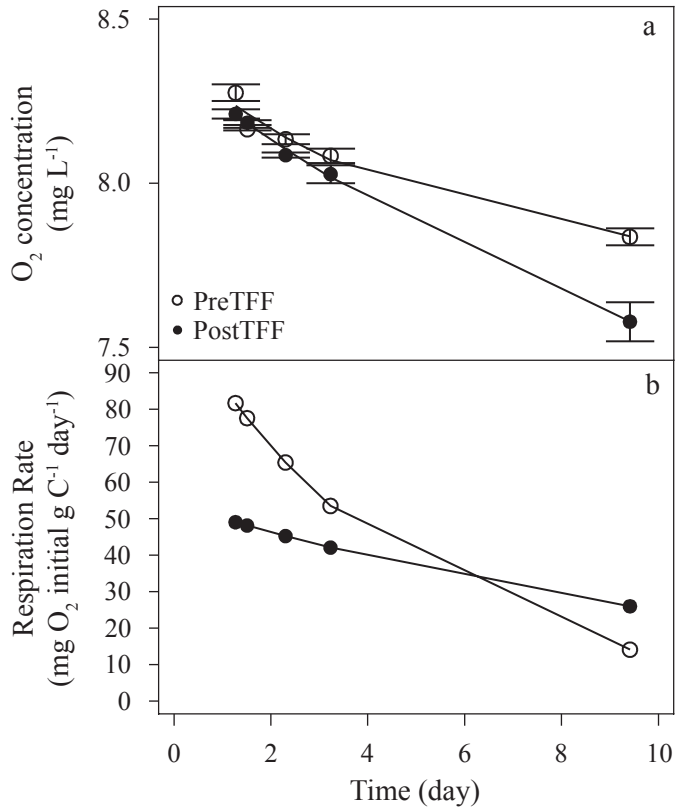
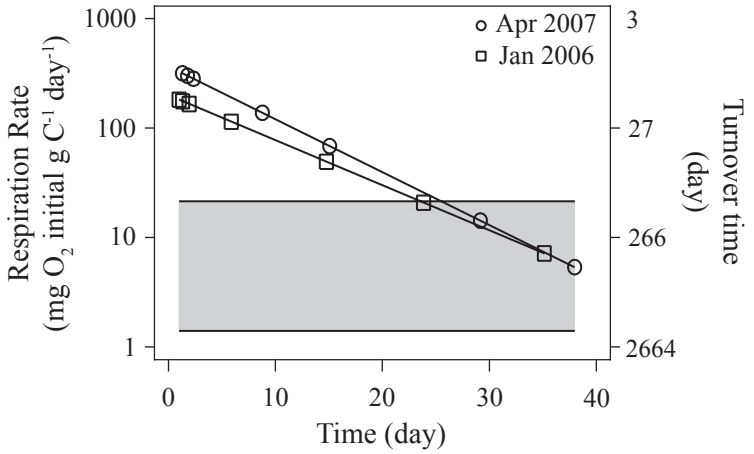

a
b
8.5
8.0
7.5
O_2 concentration
(mg L^{-1})
PreTFF
PostTFF
90
80
70
60
50
40
30
20
10
0
Respiration Rate
(mg O_2 initial g C^{-1} day^{-1})
0
2
4
6
8
10
Time (day)

Figure 4.5


Apr 2007
Jan 2006
Respiration Rate
(mg O_2 initial g C^{-1} day^{-1})
1000
100
10
1
3
27
266
2664
Turnover time
(day)
0
10
20
30
40
Time (day)

Appendices

Appendix 1

Derivation of Equation 4.2: $O_{2\ corrected} = A_{exp} - (c_{1doc}\ e^{-kdoc\ *\ t} + c_{0doc}) + (c_1\ e^{-k\ *\ t} + c_0)$

$P = c_1\ e^{-k\ *\ t} + c_0$

P is the equation for oxygen consumption curve from POC + DOC with parameters estimated from least squares analysis (Figure A1).

$D = c_{1doc}\ e^{-kdoc\ *\ t} + c_{0doc}$

D is the equation for oxygen consumption curve from DOC alone treatments for each experiment with parameters estimated from least squares analysis (Fig. A1).

$X(t) = A_{exp} - P$

X(t) is the total oxygen consumed by respiration of both POC and DOC at time (t) and A_{exp} is the initial oxygen concentration for each experiment (Fig. A1).

$Y(t) = A_{exp} - D$

Y(t) is the total oxygen consumed by respiration of only DOC at time (t) (Fig. A1).

X(t) – Y(t) = the oxygen consumed by POC alone at time (t).

N(t) is the oxygen consumption curve for POC alone (Fig. A1).

$$N(t) = A_{exp} - \{X(t) - Y(t)\}$$

Simplify and rearrange N(t):

$$N(t) = A_{exp} - \{ A_{exp} - P - (A_{exp} - D)\}$$

$$N(t) = A_{exp} - \{ A_{exp} - P - A_{exp} + D \}$$

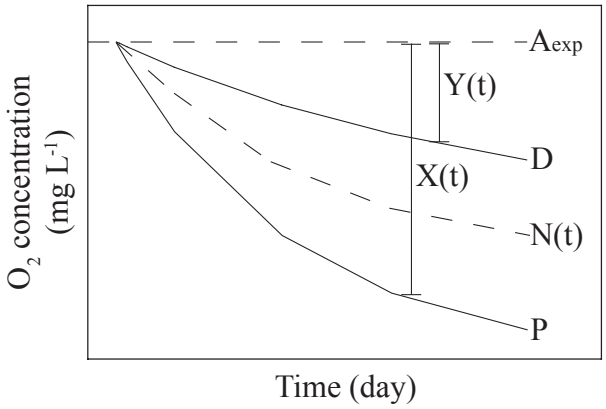
$$N(t) = A_{exp} - A_{exp} + P + A_{exp} - D$$

$$N(t) = A_{exp} - D + P$$

Hence eq. 4.2: $O_{2\ corrected} = A_{exp} - (c_{1doc}\ e^{-kdoc\ *\ t} + c_{0doc}) + (c_1\ e^{-k\ *\ t} + c_0)$

Appendix 1 Figure

Figure A1. Theoretical oxygen consumption curves for DOC alone (D), POC + DOC (P) and estimation of the DO consumption curve for POC alone (N(t)). A_{exp} is the initial oxygen concentration and Y(t) and X(t) are the total oxygen consumed by respiration for each curve.



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