FOOD RESOURCE QUALITY AND ITS UTILIZTION BY A NATIVE AND AN INVASIVE BIVALVE IN A GULF COASTAL PLAIN STREAM

by

CARLA L. ATKINSON

(Under the Direction of Alan Covich and Stephen Opsahl)

ABSTRACT

Food quality in low-gradient streams of the southeastern coastal plain is controlled by streamflow, connectivity to the floodplain, and the position within the watershed. These important abiotic factors structure streams along with co-occurring biotic interactions. Freshwater mussels are a dominant invertebrate in these streams and have the ability to alter the availability and quality of basal food resources through their filtration and excretion activities. *Corbicula fluminea*, an invasive bivalve species, has become established throughout North America and has the potential to alter available food resources. This study examined the functional roles of *Elliptio crassidens* (native) and *C. fluminea*. Both species selected for organic, living materials over inorganic, non-living materials, however these species assimilated different resources into their tissues as reflected by their stable isotopic signatures. Additionally, *C. fluminea* released more nitrogen back into the sediments through biodeposits. This research indicates the need to understand more about invasive species' functional roles.

INDEX WORDS: Seston, unionid, *Corbicula fluminea*, stable isotope, stoichiometry, carbon, nitrogen, stream, low-gradient, lateral connectivity, floodplain

FOOD RESOURCE QUALITY AND ITS UTILIZATION BY A NATIVE AND AN INVASIVE BIVALVE IN A GULF COASTAL PLAIN STREAM

by

CARLA L. ATKINSON

B.S., Missouri State University, 2006

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment
of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2008

FOOD RESOURCE QUALITY AND ITS UTILIZATION BY A NATIVE AND AN INVASIVE BIVALVE IN A GULF COASTAL PLAIN STREAM

by

CARLA L. ATKINSON

Major Professors:	Alan P. Covich Stephen P. Opsahl
Committee:	Stephen W. Golladay Amy D. Rosemond

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2008

ACKNOWLEDGEMENTS

I would like to thank my co-advisors and committee: Alan Covich, Steve Opsahl, Steve Gollday, and Amy Rosemond. Without them I would not have gotten through this. I would especially like to thank Alan P. Covich for reminding me when I began to “keep it simple.” I would like to thank Stephen P. Opsahl for all his help with stable isotopes and chemistry. Steve Golladay helped me locate mussel beds, kept me caffeinated with fabulous coffee, and connected me with other people fascinated with mussels. I would like to also thank Amy D. Rosemond for her thoughtful comments on my chapters and advice throughout this process. I am grateful to all of you for engaging in this work, putting in hours of effort, and caring about the outcome of this research. Dr. Mike Conner was a great resource for both statistics and coffee. Tom Maddox analyzed my isotope samples efficiently and was always ready to answer any questions. Liz Cox was amazing and always willing to help me find obscure books and documents. Jean Brock is a GIS master and helped me get through all my mapping issues. Many other professors at University of Georgia in both Ecology and the Warnell School of Forestry helped shape my education and career.

I had an amazing group of fellow graduate students and field technicians that I ran ideas by and assisted me in the field: Tara Muenz, Lindsey Sargent, Brian Cloninger, Scott Wiggers, Sean Sterrett, Julie McEntire, Stephanie Allums, and Greg Knothe. You all are the best! Tara Muenz was a great resource helping me learn the local mussel fauna and a wonderful friend. Lindsey Sargent helped me in the field and was a great lab mate and friend through everything. M. Scott Wiggers was always there to help me with field work, GIS, and statistics when I needed

him. Sean Sterrett got me out in the field when I was tired of writing and taught me about riverine turtles. Cindy Tant's bed and breakfast always provided me a place to crash when I was in Athens. Also, to housemates and friends, thank you for everything. You have all contributed to pulling me through my little challenges, encouraged me, and have helped make this experience truly great. I would also like to thank all the helpful and friendly people at the Jones Ecological Research Center at Ichauway for their southern hospitality and helping me through all the non-academic training I received. Last and not least, I would like to thank my family for supporting and encouraging me through everything. Even though I moved away from them, they have still been here for me. Thank you everybody!

TABLE OF CONTENTS

Page

CHAPTER 1

LITERATURE REVIEW AND SUMMARY OF OBJECTIVES

Introduction

Understanding how streams are structured and function due to both abiotic and biotic factors have been investigated by stream ecologists for decades. In low gradient Coastal Plain streams, such as Ichawaynochaway Creek in the Flint River drainage of southwest Georgia, water clarity varies with discharge, which is typically linked to the season (Golladay and Battle 2002). Other researchers have worked in Coastal Plain streams and have investigated primarily how abiotic factors affect the biotic communities of these streams (Benke 2000, Benke 2001, Martin and Paller 2008). Spatial and temporal variability causes differences in basal resources that are available to primary consumers; however consumers can also alter the availability and composition of these resources.

Freshwater mussels may be the most dominant invertebrate, in terms of biomass, in many eastern North American streams (Parmalee and Bogan 1998). Bivalves affect the ecosystem through their biofiltration (Vaughn et al. 2008 and references therein). Bivalves play an important functional role in stream ecosystems by filtering suspended particulates out of the water column and depositing material in the benthic environment (Vaughn and Hakenkamp 2001; Spooner and Vaughn 2006; Vaughn et al. 2007). These processes affect resource availability and are known to alter composition and dominance of species in aquatic communities (Vaughn and Hakenkamp 2001; Howard and Cuffey 2006; Spooner and Vaughn 2006; Vaughn and Spooner 2006). Unfortunately, land use changes, hydrologic alterations, and declines in native fish species have adversely affected native mussel abundance and diversity in the United States. Understanding the role these species play in terms of food-web structure and nutrient cycling is essential for knowing how these streams may be altered if these species continue to decline.

Corbicula fluminea, the invasive Asian clam is widespread and has become “naturalized” in many river basins across the United States, including the Flint River Basin. Its occurrence in the Lower Flint River Basin was first recorded in 1970 from a collection made in Chickasawhatchee Creek near Newton, GA (USGS). Like native mussels, *C. fluminea,* is primarily a filter-feeding consumer, and depends heavily upon microbes and detrital material available in the water column and benthic environment. *C. fluminea* feeds on particles of a narrow size class (10 μm to ≤ 1 μm), typically smaller than what most native mussel species can utilize (McMahon and Bogan 2001). *C. fluminea* can filter feed greater amounts of material more rapidly (Leff et al. 1990) and filter bacteria at a higher rate (Silverman et al. 1995) than native unionid mussels. Vaughn and Spooner (2006) found that *C. fluminea* abundance was negatively correlated to native mussel abundance at small spatial scales. They hypothesized several mechanisms that could drive this pattern, including lack of space due to locally reduced food resources and physical disturbance, and inability of *C. fluminea* to withstand environmental stresses as readily as unionids. As unionid numbers have decreased, *C. fluminea* has become a dominant filtering bivalve in many systems. This change in dominance could lead to changes in the structure and function of stream ecosystems. We need a better understanding of the differing functional roles of native mussels and *C. fluminea* within streams.

I investigated how flow and drainage-network position within the Ichawaynochaway basin can cause variation in the quality of basal resources. A related question was how a native freshwater mussel, *Elliptio crassidens*, and an invasive bivalve *Corbicula fluminea*, utilize and assimilate these suspended materials. I investigated the two bivalve’s selection of materials through filtration and then determined their isotopic signatures to determine what they assimilated. I also determined the tissue C:N of these two species and compared it to the materials they biodeposit in the stream. This study had 3 objectives:

Objective 1: Assessment of the spatial and temporal factors that affect basal resource quality in the Ichawaynochaway Creek watershed.

The connection of a stream to its surrounding floodplain is an important ecological linkage that affects spatial and temporal dynamics of the basal resources available to primary consumers in streams. Seasonal flooding is a dynamic process that drives essential ecological interactions between a river channel and its floodplain (Junk et al 1989, Ward 1989, Bayley 1995, Lindholm 2007, Martin and Paller 2008). Seasonally flooded low gradient streams with extensive riparian forests are a common feature of the Coastal Plain of the southeastern United States (Osterkamp and Hupp 1984, Hupp and Osterkamp 1996, Benke et al. 2000, Hupp 2000). During floods, riparian forests contribute significant quantities of organic material to streams (e.g. Cuffney 1988, Meyer and Edwards 1990, Benke et al. 2000), and cause spatial and temporal variation in food resources available to consumers. Suspended organic material and associated microorganisms (seston) vary in quality both seasonally and inter-annually within streams because of changing inputs from floodplain sources. Although researchers have investigated the quality of different size fractions of material, previous studies have not assessed these differences based on hydrology and the geomorphic structure of streams. The purpose of this portion of the study was to examine the temporal and spatial variation of seston quality (C:N) and stable isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) in relation to flow regime, particle size, and connection of the stream to the surrounding floodplain.

Objective 2: Evaluate what suspended materials a native mussel, *Elliptio crassidens*, and an invasive bivalve, *Corbicula fluminea,* filter out of the water column.

The North American freshwater mussels (Superfamily Unionoidea) are the most diverse in the world, with approximately 308 native species (Turgeon et al. 1998), and are known for their ability to filter large amounts of water. Historically, filter-feeding bivalves were the dominant invertebrate, in terms of biomass, in many eastern North American streams (Parmalee and Bogan 1998). They are also one of the most endangered groups of freshwater invertebrates in the world (Dudgeon et al. 2006, Bogan 2008). Thus, there is a need to understand more about the impacts of both uptake and release of materials during bivalve feeding. While the success of invasive, non-native bivalve species is evident from their increasing abundance, their functional roles in comparison with native species are poorly understood. *Corbicula fluminea*, the Asiatic clam, is widespread across the United States and is common throughout the southeastern United States (McMahon and Bogan 2001). Despite their abundance and wide distribution, their feeding ecology is not completely understood. Both native and invasive bivalve species co-occur within habitats in the Ichawaynochaway Creek, and *C. fluminea* has become a dominant filter feeder in this ecosystem. The purpose of this study was to determine if a native mussel species and *C. fluminea* select for the same materials from the water column. This study combined two different methods to determine the suspended materials left in the water column after bivalve filtration. I combined a gravimetric approach and flow cytometry to determine which materials and how much suspended particulates these two species utilized.

Objective 3: Identify whether *Elliptio crassidens,* a native mussel and *Corbicula fluminea,* a non-native species, feed and cycle nutrients similarly within the ecosystem.

Freshwater mussels play an important role in the stream community through their biofiltration activities. Through filtration, mussels transfer suspended materials and associated nutrients from the water column to the surrounding benthic area, stimulating primary and secondary production (Howard and Cuffney 2006, Spooner and Vaughn 2006, Vaughn et al. 2007). Particles in unionid guts resemble the suspended seston found in the habitats they occur (Coker et al. 1921, Nichols and Garling 2000). However, little is understood about which materials are actually assimilated. If an introduced species can profoundly alter ecosystem-level processes such as primary or secondary productivity, nutrient cycling, or disturbance frequency, then the properties of individual species can potentially control the functioning of whole ecosystems (Vitousek 1990). Native and invasive species may utilize different materials and cycle materials and nutrients differently. Small functional differences can lead to larger ecosystem scale variability. As unionid numbers have decreased, *C. fluminea* has become a dominant filtering bivalve in the system. This change could lead to alterations in the structure and function within the stream ecosystem despite their similar functional role during some environmental conditions. Trophic fidelity describes the ability of an organism or group of organisms to adhere to specific feeding habits through space and time. Given the invasive properties of *C. fluminea*, this species may have lower trophic fidelity giving it the ability to take advantage of a wider variety of food resources. Our goal was to understand the trophic niche dynamics of these two species within the same functional group, to compare their trophic fidelity, and to determine retention and cycling of nutrients.

Prospectus:

The following chapters present the methods and results of 1) determination of the baseline quality and isotopic signatures of suspended organic matter, 2) determination of what a native mussel and *C. fluminea* filter and assimilate from these materials, and 3) an analysis of how these species utilize and cycle nutrients. Chapter 2 is presented in manuscript format, as prepared for submission to the Journal of the North American Benthological Society. Chapter 3 is presented in manuscript format, as prepared for submission to Freshwater Biology. Chapter 4 presents stable isotope and nutrient cycling data for *Elliptio crassidens* and *C. fluminea*. Chapter 5 presents a summary and conclusions of these studies.

References:

Bayley, P.B. 1995. Understanding large river-floodplain ecosystems. BioScience 45:153-158.

Benke, A. C. 2001. Importance of flood regime to invertebrate habitat in an unregulated river-floodplain ecosystem. Journal of the North American Benthological Society 20:225-240.

Benke, A.C., I. Chaubey, G.M. Ward, and E.L. Dunn. 2000. Flood pulse dynamics of an unregulated river floodplain in the southeastern U.S. coastal plain. Ecology 81:2730-2741.

Bogan, A.E. 2008. Global diversity of freshwater mussels (Mollusca, Bivalvia)in freshwater. Hydrobiologia 595:139-147.

Coker, R.E., A.F. Shira, H.W. Clark, and A.D. Howard. 1921. Natural history and propagation of freshwater-mussels. Bulletin of the Bureau of Fisheries 37:77-181.

Cuffney, T.F. 1988. Input, movement and exchange of organic matter within a subtropical coastal blackwater river-floodplain system. Freshwater Biology 19:305-320.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L.J., and Sullivan, C.A. 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Review 81:163-182.

Golladay, S.W. and J. Battle. 2002. Effects of flooding and drought on water quality in gulf coastal plain streams in Georgia. Journal of Environmental Quality 31:1266-1272.

Howard, J.K. and K.M. Cuffey. 2006. The functional role of native freshwater mussels in the fluvial benthic environment. Freshwater Biology 51:460-474.

Hupp, C.R. 2000. Hydrology, geomorphology, and vegetation of Coastal Plain rivers in the south-eastern USA. Hydrological Processes 14:2991-3010.

Hupp, C.R. and W.R. Osterkamp. 1996. Riparian vegetation and fluvial geomorphic processes. Geomorphology 14:277-295.

Junk, W.J., P.B. Bayley, and R.E. Sparks. 1989. The flood pulse concept in river-floodplain systems. In: D.P. Dodge. Proceedings of the International Large Rivers Symposium. Canadian Special Publication in Fisheries and Aquatic Sciences 106:110-127.

Leff, L.G., J.L. Burch, and J.V. McArthur. 1990. Spatial distribution, seston removal, and potential competitive interactions of the bivalves *Corbicula fluminea* and *Ellipto complanata*, in a coastal plain stream. Freshwater Biology 24:409-416.

Lindholm, M., D.O. Hessen, K. Mosepele, and P. Wolski. 2007. Food webs and energy fluxes on a seasonal floodplain: the influence of flood size. Wetlands 27:775-784.

Martin, F. D., and M. H. Paller. 2008. Ichthyoplankton transport in relation to floodplain width and inundation and tributary creek discharge in the lower Savannah River of Georgia and South Carolina. Hydrobiologia 598:139-148.

McMahon, R.F. and A.E. Bogan. 2001. Mollusca: Bivalvia: 331-416. In: J.H. Thorp and A.P. Covich. Ecology and Classification of North American Freshwater Invertebrates, 2nd Edition. Academic Press, San Diego.

Meyer, J.L. and R.T. Edwards. 1990. Ecosystem metabolism and turnover of organic carbon along a blackwater river continuum. Ecology 71:668-677.

Osterkamp, W.R. and C.R. Hupp. 1984. Geomorphic and vegetative characteristics along three northern Virginia streams. Geological Society of America Bulletin 95:1093-1101.

Parmalee, P.W. and A.E. Bogan. 1998. The freshwater mussels of Tennessee. University of Tennessee, Knoxville, TN.

Silverman, H.S., E.E. Archberger, J.W. Lynn, and T.H. Dietz. 1995. Filtration and utilization of laboratory-cultured bacteria by *Dreissena polymorpha, Corbicula fluminea* and *Carunculina texasensis*. Biological Bulletin 189:308-319.

Spooner, D.E. and C.C. Vaughn. 2006. Context-dependent effects of freshwater mussels on the benthic community. Freshwater Biology 51:1016-1024.

Vaughn, C.C. and C.C. Hakenkamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46:1431-1446.

Vaughn, C.C., D.E. Spooner, and H.S. Galbraith. 2007. Context-dependent species identity effects within a functional group of filter-feeding bivalves. Ecology 88:1664-1662.

Vaughn, C.C. and D.E. Spooner. 2006. Scale-dependent associations between native freshwater mussels and invasive *Corbicula*. Hydrobiologia 568:331-339.

Vaughn, C.C., S.J. Nichols, and D.E. Spooner. 2008. Community and foodweb ecology of freshwater mussels. Journal of the North American Benthological Society 27:409-423.

Vitousek, P.M. 1990. Biological invasions and ecosystem processes – towards an integration of population biology and ecosystem studies. Oikos 57:7-13.

Ward, J.V. 1989. The four-dimensional nature of lotic ecosystems. Journal of the North American Benthological Society 8:2-8.

CHAPTER 2

STREAM DISCHARGE AND FLOODPLAIN CONNECTIONS AFFECT SESTON QUALITY AND STABLE ISOTOPIC SIGNATURES IN A COASTAL PLAIN STREAM[1]

---

[1] Atkinson, C.L., S.W. Golladay, S.P. Opsahl, and A.P. Covich. Submitted to the *Journal of the North American Benthological Society*, 7/25/08.

Abstract

Connections of a stream to its surrounding floodplain are important ecological linkages that affect spatial and temporal dynamics of the basal resources available to primary consumers in streams. Suspended organic material and associated microorganisms (seston) vary in quality both seasonally and inter-annually within streams because of changing inputs from riparian and floodplain sources. Although researchers have investigated the quality of different size fractions of material, previous studies have not assessed these differences based on hydrology and the geomorphic structure of streams. We investigated how quality, represented by the amount of carbon and nitrogen, and stable isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) of 3 seston size fractions varied in Ichawaynochaway Creek, a 5th order tributary of the lower Flint River in the Coastal Plain of southwestern Georgia. Samples were collected throughout the watershed during varying flow regimes to determine quality and source of materials available over different temporal and spatial scales. Our results indicate significant differences in quality and stable isotopic signatures based on particle size, discharge, and geomorphic structure of the stream and floodplain (constrained vs. unconstrained reaches). During low flow periods there was higher quality seston with more enriched $\delta^{13}C$ and $\delta^{15}N$ signatures in the constrained portions of the stream, which occurred in the lower section of the watershed. However, high flow periods transferred higher quality seston into the stream from the adjacent floodplain into all portions of the watershed. Insights gained from this study indicated how terrestrial and aquatic linkages and the natural flow regime affected the dynamics of basal resources and their availability to primary consumers in streams.

Introduction:

Seasonal flooding is a dynamic process that drives essential ecological interactions between a river channel and its floodplain (Junk et al 1989, Ward 1989, Martin and Paller 2008). Frequently flooded low gradient streams with extensive riparian forests are a common feature of the Coastal Plain of the southeastern United States. Riparian forests are seasonally flooded contributing significant quantities of organic material to southeastern Coastal Plain streams (Cuffney 1988, Meyer and Edwards 1990, Benke et al. 2000). Pulsed floods create spatial and temporal variation in food resources available to consumers. The flood pulse concept is generally illustrative of low gradient streams with a broad floodplain (Junk et al. 1989) and is applicable to many southeastern streams (Benke et al. 2000). The flood pulse concept considers high flows to be typically predictable events that fuel seasonal productivity in these streams. Organic matter originating in floodplain forests tends to accumulate and is partially processed in the soils. During flood pulses, substantial quantities of organic materials are transported to stream channels (Meyer and Edwards 1990, Golladay et al. 2000). Understanding variability in food resources is necessary for recognizing patterns of community composition and life history strategies. However, few studies have addressed how the quality of material entering the stream varies with cycles of flooding. This seasonal variability is especially important to study as extremes in rainfall (floods and droughts) are predicted to increase (IPPC 2007). These predictions can be placed in perspective by examining past variability in long-term data (such as the effects of Tropical Storm Alberto in 1994) on detrital sources and transport.

Understanding how food quality, expressed as the amount of C and N in available food sources, and the sources of detrital resources vary in response to seasonal flooding is generally important for improving understanding of the basis of in-stream productivity. Ecological stoichiometry has been used to integrate energy, available food materials, and trophic linkages in many ecosystems by focusing on the imbalance between the elemental requirements of organisms and the composition of their food sources (Sterner and Elser 2002, Elser et al. 2007). Analysis of stable isotopes has been useful in studies of aquatic food webs and trophic structure (Vander Zanden and Rasmussen 1999, Post 2002, Zueg and Winemiller 2008). Carbon isotopic signatures vary due to source material, with C_3 plants typically having a signature of -28‰ (Fry 2006). Biogeochemical transformations can cause systematic variation in $\delta^{15}N$ (Robinson 2001). Influences such as microbial colonization have been implicated in altering $\delta^{15}N$ signatures (Macko and Estep 1984). In streams, systematic differences in the isotopic signature of food resources, which indicates the source of material, and quality, can occur due to size of material, stream discharge, and other factors.

Prior studies have measured lateral exchange processes in large rivers or coastal plain streams (Jones and Smock 1991, Golladay et al. 2000), but rarely has the quality or source of these materials been assessed. This study focused on the lateral and temporal dimensions of hydrological connectivity, defined herein as the permanent or episodic links between the main channel of a river and the various water bodies of its alluvial floodplain (e.g., Ward 1989). We hypothesized that episodic flooding would result in suspension of high quality materials of floodplain origin. Three size fractions of seston were collected to determine variation in quality and isotopic signature across a range of suspended particle sizes that include materials in different states of biogeochemical breakdown. We also investigated differences in food quality and isotopic signature in areas with varying connectivity to the floodplain (constrained vs. unconstrained reaches) and throughout a wide range of flow regimes in an entire watershed. The aim of this study was to examine the temporal and spatial variation of seston quality (C:N) and stable isotopic signature (δ^{13}C and δ^{15}N) in relation to flow regime, particle size, and connection of the stream to the surrounding floodplain.

Methods:

Study sites:

Ichawaynochaway Creek (IC) is a 5th order, low gradient tributary to the lower Flint River on the Gulf Coastal Plain of southwestern Georgia, U.S.A (Golladay et al. 2000). Agriculture and forested lands dominate the upland landscape. Nine sampling sites were established in the IC drainage basin including two sites on tributaries, Pachitla and Chickasawhatchee Creeks (Fig. 2.1 and Table 2.1). Chickasawhatchee Creek (CC) flows through Chickasawhatchee Swamp, the second largest tract of wetlands in Georgia (Golladay and Battle 2002). Riparian areas in the region are composed of maturing second growth hardwood forest (Golladay et al. 2000). These areas vary greatly in the degree of floodplain widths (55-980 m). About 10 km downstream from the confluence with CC, the IC discharges into the Flint River.

The IC basin is situated in the Dougherty Plain physiogeographic district where the mantled karst physiography controls the hydrology. The bedrock is primarily Ocala limestone, an extensively fractured and porous rock layer that has high hydraulic transmissivity, and is the principal water-bearing stratum for the Upper Floridian Aquifer (Hicks et al. 1981). The streams originate as springs and seeps from the Claiborne and Clayton aquifers in the Fall Line Hills physiographic district before flowing across the Dougherty Plain where base flows are supported by the Upper Floridian aquifer (Golladay and Battle 2002). Patterns of evapotranspiration and temperature variations are strongly seasonal and produce flooding in the winter and spring in the southeastern USA (Benke 2000). Streamflow varies seasonally, with periods of low flow occurring during summer and fall, and winter and spring being periods of higher flow. Inundation of the floodplain tends to occur between October and April, and has the greatest extent in Chickasawhatchee Swamp. This basin has swampy wetlands in the mid-reaches, causing the stream channel to be very wide and unconfined, thus leading to a wider floodplain (Fig. 1). Bottomland hardwood forests adjacent to the stream serve as storage areas for detritus during low flow periods and are locations of export during high flows. Downstream, the streams become confined, leading to a smaller floodplain and greater flow.

Field Collection and Sample Analysis:

Seston samples were collected at 9 study sites six times between June 1994 and October 1996 and archived as part of a long-term study to determine effects of agricultural development on seston availability and water quality. Samples were collected seasonally during a variety of flow regimes. Material was fractionated into a series of 3 size categories using different sized plankton nets and sieves: large (>250 μm), medium (250-45 μm), and small (45 -10 μm). Samples were then dried and ground. Total carbon and total nitrogen composition as well as the nitrogen and carbon stable isotopic signatures were determined using a Finnigan Delta Plus mass spectrometer in the University of Georgia's Ecology Analytical Laboratory. Isotope ratios were expressed in the delta (δ) format: $\delta^{13}C$ or $\delta^{15}N$ (units of ‰) = $([R_{sample} / R_{standard}]-1) \times 1000$, where R is the $^{13}C:^{12}C$ ratio or $^{15}N:^{14}N$ ratio. A bovine protein (peptone) lab standard was referenced against an international standard, and precision averaged 0.1‰ or less.

Historical discharge data were obtained for three (USGS 02353500, USGS 02353400, USGS 02354500) sampling sites from the US Geological Survey Water Watch website (http://water.usgs.gov/waterwatch/ ?m=real&r=ga). Relative floodplain width and channel width were determined using the methods of Golladay et al. (2000) for the IC basin and was used as a measure of floodplain connectivity and channel constraint (constrained and unconstrained). The one-year flood recurrence interval discharge was calculated using the annual maximum series (uses the single largest discharge for each year of record) data from USGS 02353500, which is at site 6 (Fig. 1). The recurrence interval (T in years) was calculated:

$$T = (n + 1) / N$$

where n is the number of years of record and N is the rank of the particular event (Knighton 1998). If the average discharge during the sampling date was greater than the one year flood recurrence interval discharge, that date was considered a high flow period, and if the flow was less during the date that period was considered a low flow period.

Data Analysis:

We were interested in determining how much seston quality and stable isotopic signature were affected by particle size, hydrology, floodplain width, and position within the watershed. Seston quality was quantified using C:N, assuming a lower value indicating higher quality. Prior to running a two-way ANOVA, C:N values were log transformed to meet the assumptions of normality and homogeneity of variance implicit in parametric analyses. The $\delta^{13}C$ and $\delta^{15}N$ met the assumptions of normality, so those values were not transformed. Two-way ANOVAs were performed using PROC GLM to quantify the magnitude and how C:N, $\delta^{13}C$, and $\delta^{15}N$ of material differed with particle size, stream discharge (high versus low flow), and if there was an interaction between these two variables. Significant ANOVAs were followed by Tukey's HSD multiple comparisons to determine if significant differences existed between size classes and flow conditions ($\alpha = 0.05$; Littell et al. 2002). Linear regressions were performed using PROC REG to assess relationships between the C:N, $\delta^{13}C$, and $\delta^{15}N$ of seston and stream discharge. Discharge data from three sites with USGS gauging stations were used in the analyses (Fig. 1;

sites 3, 6, and 7). To determine if floodplain width / channel width affected seston quality and stable isotopic signature, linear regression analyses were done to determine trends in C:N, $\delta^{13}C$, and $\delta^{15}N$ due to floodplain connectivity during high flow versus low flow periods. In order to isolate temporal variation in floodplain connectivity, we analyzed high and low flow periods separately (constrained versus unconstrained times). Linear regression analyses was also performed to determine if there was a trend in the mean C:N, $\delta^{13}C$, and $\delta^{15}N$ across all dates during high versus low flow periods due to the distance from the headwaters. All statistical analyses were performed using SAS v9.1 (SAS Institute, Cary, NC).

Results:

Hydrology:

This study included diverse hydrologic conditions in the IC basin (Fig. 2.2). Tropical Storm Alberto and other tropical storms resulted in near record precipitation (192 cm total, 60 cm above average) during the summer and fall of 1994 (Golladay et al. 2000). Thus, IC experienced above average discharges during the summer 1994 until the spring of 1995 including the greatest flow on record. Using the annual duration series, the 1-year recurrence interval flood discharge at the USGS gauging station at site 6 (USGS 02353500) was 27.00 m^3s^{-1} (Table 2.2). Low flow periods included the dates of: 6/22/94, 8/31/95, 10/23/96, and 2/29/96, and high flow periods included the dates of 7/25/94 and 3/22/95 (Table 2.2).

Particle Size:

There was a significant difference (ANOVA, $p < 0.0001$) in the C:N between the three size fractions collected during this study (Table 2.3, Fig. 2.3A). C:N was lowest in the smallest size class and increased significantly with increasing particle size (Tukey HSD, $p < 0.0001$). The

C:N within size classes also varied significantly due to flow regime (ANOVA, $p = 0.018$); the small and medium size classes had a significantly lower C:N during high flows (Tukey HSD, $p = 0.0015$, $p = 0.0072$). No significant differences were seen in the large size class due to flow regime (Tukey HSD, $p = 0.6634$; Fig. 2.3A). The interaction of size class and flow condition on C:N of seston was not significant (ANOVA, $p = 0.055$). There was a significant difference (ANOVA, $p = 0.0002$) in the $\delta^{13}C$ between the three size fractions (Table 2.3, Fig. 2.3B), with the small and medium size fractions being less depleted in $\delta^{13}C$ than the large size fraction (Tukey HSD, $p < 0.001$). The $\delta^{13}C$ within size classes also varied significantly due to flow regime (ANOVA, $p < 0.04$). The small and medium size classes were more enriched in $\delta^{13}C$ than the large size class during low flow periods (Tukey HSD, $p = 0.0005$), while there were no significant differences among the size classes during high flow periods (Tukey HSD, $p = 0.1142$). The interaction between size class and flow was not found to be significant (ANOVA, $p = 0.6446$). There was a significant difference (ANOVA, $p < 0.0001$) in the $\delta^{15}N$ between the three size fractions collected (Table 2.3, Fig. 2.3C). The $\delta^{15}N$ was the most enriched in the small and medium size classes than the large size class (Tukey HSD, $p < 0.0001$). The $\delta^{15}N$ within size classes also varied significantly due to flow regime (ANOVA, $p < 0.0001$). The small, medium, and large size classes were significantly different from high and low flows (Tukey HSD, $p = 0.0006$, $p = 0.0008$, $p = 0.0014$; Table 2.3, Fig. 2.3C). During both low and high flows, the large size class was significantly more $\delta^{15}N$ depleted than the small and medium size classes, and the small and medium size class did not vary significantly (Tukey HSD, $p < 0.0001$, $p < 0.001$).

Hydrological Effects:

The C:N of the smallest seston size fraction significantly decreased with increasing discharge (r^2=0.56; p = 0.001; see Table 2.3, Fig. 2.4A). The C:N of the medium and large size classes did not have a significant relationship with discharge (p = 0.313, p = 0.334). The δ^{13}C signature of the small size class of seston was positively related to discharge (r^2 = 0.44; p < 0.007; Fig 2.4B) becoming lighter as the discharge increased. The δ^{13}C signature of the medium and large size classes showed the same trends but was not significantly related to discharge (p = 0.16, p = 0.48). Discharge did not significantly affect the δ^{15}N signature of the small (p = 0.985), medium (p = 0.83), or large (p = 0.42) size classes.

Floodplain Geomorphology:

During low flow periods, higher floodplain/channel width was positively related to the C:N of the small size class (r^2 = 0.66; p = 0.008; Fig. 2.5A). However, there was no significant trend evident during high flow periods (r^2 = 0.12; p = 0.353). Yet, the range of C:N tended to be lower for high flow periods (7.62-16.07) than low flow periods (12.50-25.34). The C:N of the medium size class showed a similar trend, however, it was not significant during low flow (r^2 = 0.36; p = 0.122) or high flow periods (r^2 = 0.03; p = 0.65). There was no trend with the C:N in the large class during low (p = 0.82) or high flow conditions (p = 0.93). There was also a significant negative relation with the distance from the headwaters and C:N of the small size class during low flow periods (r^2 = 0.84; p < 0.001; 2.6A). No significant trend was noted during high flow periods in C:N due to the distance from the headwaters (r^2 = 0.14, p = 0.29; Fig. 2.6A). However, the C:N was lower at all sampling sites during high flow periods. The C:N of the medium size class showed the same trends with the distance from the headwaters as the small size class did. However, these trends were not significant during low flow (r^2 = 0.36; p = 0.09) or high flow (r^2 = 0.06; p = 0.53) periods. The δ^{13}C of the small (Fig. 2.5B) and large size classes was not significantly related with the floodplain to channel ratio during low ($p <$ 0.10, $p <$ 0.10) or high flow periods ($p <$ 0.10, p = 0.34). The medium size class however, had a significant negative relation with the δ^{13}C being less enriched with greater floodplain/channel width during both low flow (r^2 = 0.61; p = 0.01) and high flow (r^2 = 0.72; p = 0.004) conditions. The δ^{13}C of the small and medium size classes had a significant positive relation (Fig. 2.6B) to the distance from the headwaters during both low flow periods (r^2 = 0.45; p = 0.05, r^2 = 0.56; p = 0.02) and high flow periods (r^2 = 0.46; $p <$ 0.05, r^2 = 0.55; p = 0.02). The δ^{13}C of the large size class was significantly more enriched with greater distance from the headwaters only during high flow (r^2 = 0.49; p = 0.04) and not low flow (r^2 = 0.07; p = 0.51) conditions. The δ^{15}N of the small size class was positively related to the floodplain to channel ratio during high flow periods (r^2 = 0.50; p = 0.03), but not during low flow periods (p = 0.17) and the flow periods were nearly equal with greater connectivity to the floodplain (Fig. 2.5C). The medium and large size classes showed the same trends with δ^{15}N and floodplain to channel ratio as the small size class. However, neither the medium or large size classes were significantly related to floodplain to channel ratio during low flow (p = 0.81, p = 0.54) or high flow (p = 0.06, p = 0.07) periods. Additionally, δ^{15}N of the small size class was positively correlated to the distance from the headwaters during low flow periods (r^2 = 0.48; $p <$ 0.04), but not during high flow periods (r^2 = 0.22; p = 0.20), and the flow periods were nearly equal in δ^{15}N in the headwaters (Fig. 2.6C). The same trends were apparent in the medium and large size classes, however these trends were not significant during low (p = 0.38, p = 0.89) or high (p = 0.84, p = 0.11) flows.

Discussion:

Particle Size:

In the IC basin, the quality of seston, as indicated by a low C:N, was the greatest in the smaller size classes. Lower C:N ratios in small seston size fractions have been found by other investigators (e.g., Sinsabaugh and Linkins 1990). Smaller materials generally have higher nutrient content and lower C: nutrient ratios in comparison with larger particles (Sinsabaugh and Linkins 1990, Bonin et al. 2000, Cross et al. 2003). The trend of higher quality has been associated with a greater proportion of bacterial biomass attached to smaller particles (Kondratieff and Simmons 1985, Edwards 1987, Kamauchi 2005). Because bacteria are nutrient-rich, their consumption by filter feeders and other consumers is an important food-web connection (Meyer 1994, Sterner and Elser 2002, Makino et al. 2003). The trend of smaller particles being higher quality may be linked to patterns in $\delta^{15}N$; higher microbial biomass may impart a more enriched $\delta^{15}N$ signature in seston as found for some bacteria (Macko and Estep 1984). Angradi (1994) also noted a trend of enriched $\delta^{15}N$ in smaller seston particles in the Colorado River and hypothesized that microbes were dominant contributors. Not only is the small material of the highest quality, smaller particles are the most abundant materials transported in streams (Wallace et al. 1982, Golladay et al. 2000, Colón-Gaud et al. 2008). The large size class of seston was more depleted in $\delta^{13}C$ than the other two classes, which likely reflects the predominant contribution of C_3 riparian vegetation. Particles in the small and medium size classes have a less depleted $\delta^{13}C$ which indicates possible modification by microbial degradation and contributions from other sources. However, during high flow events, quality is higher and $\delta^{15}N$ is lower. The lower $\delta^{15}N$ value may be attributed to high discharge events carrying fresher, less decomposed materials. In the IC basin, small particles dominate seston concentration (Golladay et al. 2000); thus, there is a large quantity of high quality material during high discharge periods.

Seasonal Flooding:

Seasonal flood pulses are a controlling factor of basal resource quality in the IC basin and possibly other low-gradient southeastern streams. High flow periods are also responsible for transporting greater quantities of seston into the channel (Golladay et al. 2000). Seston quality in the IC basin was influenced by discharge with higher quality seston being transported during floods. High quality materials in suspension during high discharge events are most likely coming from the floodplain in combination with fine particles being resuspended from the benthic zone. Food quality of seston has been suggested to affect the distribution and production of filter-feeding insects (Wallace and Merritt 1980). The low C:N of the seston during higher discharge periods can reduce elemental imbalances of food resources and promote growth of consumers at the base of food webs (Frost et al. 2002). Inadequate supplies of nutrients can slow the growth of organisms and alter their life histories and behavior (Sterner and Elser 2002). Tuchman et al. (2002) found that *Tipula* larvae had lower growth and consumption rates when fed conditioned leaves with high C:N. Gutseit et al. (2007) attributed higher growth and reproductive rates of *Daphnia* in clear lakes (mean C:N was 8) than humic lakes (mean C:N was 10.5) to lower C:N ratios; this was also related to materials with lower C:N having higher fatty acid quality. Studies have also attributed greater abundance, biomass, and secondary production of aquatic insects due to reduced C: nutrient ratios from stream nutrient enrichment (Cross et al. 2006, Greenwood et al. 2007).

By supplying consumers with high quality resources, the timing of floods is important for growth and reproduction. During high flows the stream has a greater connection to the riparian forests, especially in areas where the floodplain is broad. Many studies have demonstrated that streams rely on inputs of particulate and dissolved organic matter of riparian origin for much of the energy that drives stream food webs (e.g., Cuffney 1988, Wallace et al. 1997). Determining if these resources vary in quality over spatial and temporal scales is essential to understanding stream food webs. For example, in mayflies, growth and development is sensitive to C:N ratios in detrital food (Söderström 1988). High biomass and production of aquatic insects are supported by abundant microbially enriched detritus from floodplains in other coastal plain streams (Edwards and Meyer 1990). Seasonally flooded forests have been shown to contribute significant quantities of organic material to streams (Meyer and Edwards 1990, Golladay et al. 2000), and our results indicate that these periods of inundation are also important in transporting high quality material into the channel.

In the IC and other similar streams, unconstrained reaches, with their broad floodplains, act as important source areas during high flows exporting high quality material into the stream drainage network. While a significant source of suspended material originates from riparian forests, high flow periods are important in that they bring fresh materials into streams. Our data demonstrate that floods are responsible for supplying a large amount of high quality seston which is potentially an important food resource throughout the basin. In IC, potential consumers include filter-feeding bivalves (native unionids and introduced *Corbicula fluminea*) and net-spinning caddisflies (Hydropsychidae). If frequency and duration of flooding were decreased this could reduce the availability of abundant high quality food materials, potentially altering energy flow to consumers.

Floodplain Geomorphology:

Connectivity of the stream (constrained versus unconstrained) to the floodplain is another controlling factor of food quality and isotopic signatures in the IC basin. During low flows, increased floodplain connectivity resulted in lower quality seston as indicated by the higher C:N composition of seston. Lower quality seston in unconfined reaches of IC during low flow periods is likely a result of the physical characteristics of this stream. The upper portion of the basin contains many of the unconfined sections of the stream, and during low flow, material transport from upstream and the floodplain is reduced. Hoffman (2005) showed that the seston quality decreased with the residence time of the material in a stream, so that quality declined when export from the floodplain was negligible. During low flow periods in summer, the upper portion of the IC basin is more shaded leading to low primary production and receives fewer inputs from upstream and the floodplain. This leads to the quality of the materials being lower and having a more depleted $\delta^{13}C$ signature indicative of terrestrial C_3 sources (Fry 2006). It has also been shown that biofilm typically exhibits a downstream enrichment in $\delta^{13}C$ which is related to a downstream reduction in dissolved CO_2 concentration and photosynthetic fractionation rates (Finlay 2001, 2004), and seston in IC exhibits a similar pattern. The lower areas of the watershed, which are more confined, receive more flow and less shading allowing primary production in those reaches. This production is evident because the quality of seston increases, $\delta^{13}C$ is less depleted, and $\delta^{15}N$ increase in a downstream direction during low flow periods, suggesting that higher quality materials in the downstream reaches are likely due to greater primary production.

During high flows, there is no correlation between the quality of the material in the stream and floodplain connectivity, which could be due to the material in the stream being more homogenized. Yet, seston was of greater quality in all reaches during high flow periods when the stream was connected to the floodplain and materials were in transport. As connectivity to the floodplain increased $\delta^{15}N$ also increased, which may be indicative of microbially enriched materials (Macko and Estep 1984, Angradi 1994, Goedkoop et al. 2006) originating from the floodplain. There was also no significant relationship between C:N or $\delta^{15}N$ and the distance from the headwaters during high flow periods. Other studies have shown that greater water velocity and stream flow increases the transport distance of particles in streams, which influences the turnover length of the material (Meyer and Edwards 1990, Paul and Hall 2002). In IC greater transport length during high flow periods appeared to cause a greater degree of homogenization throughout the watershed. The combination of these factors leads to higher quality materials in suspension in all reaches during high flow periods. During low flow periods, there are higher quality materials in the confined reaches because of greater flow in those sections which allows export to persist and the lack of shading allows benthic primary production. Multiple scales, including spatial and temporal variability, are influencing material transport and productivity in the IC basin.

Altered floodplains and potential impacts on food quality:

The inundation of the floodplain is a natural process that supports productivity and maintains biological diversity in large rivers and low-gradient streams (Junk et al. 1989, Amoros and Bornette 2002). Our results indicate that floods may be particularly important for supplying the stream with high quality food resources. Additionally, the information provided insights into past variability (such as the effects of Tropical Storm Alberto in 1994) on the transport of seston. Reductions in active (natural) floodplain and wetland surface area may have significant implications for floodplain-river food webs (Edwards 1987, Zueg and Winemiller 2008). Inundated floodplains provide an important habitat for young fish and export organic material and organisms returning to the main channel during high flows (Junk et al, 1989, Poff et al, 1997). In addition, unconstrained stream reaches appear to be important source areas for material scavenged during floods (see also Golladay et al. 2000). Riparian forests, particularly those on reaches with broad floodplains, need to be preserved or restored in order to support in-stream food webs. Our results suggest that the spatial and temporal complexity of stream ecosystems including flooding regimes and distribution of floodplain forests interact to determine the input and quality of basal food resources to stream consumers.

Acknowledgments:

M. Drew, F. Hudson, T. Chapal, P. Houhoulis, M. Kizer, J. Ott, M. Lauck, C. Peeler, T. Muenz, and B. Taylor assisted with the laboratory and fieldwork. Tom Maddox performed the stable isotope analyses. Jean Brock provided GIS support. Liz Cox helped locate key references. We thank J.B. Wallace, A.D. Rosemond, R.O. Hall, K.A. Medley, M.S. Wiggers, and two anonymous reviewers for helpful comments on this manuscript. We would like to thank the R.W. Woodruff Foundation, J.W. Jones Ecological Research Center, and the University of Georgia Graduate School for funding and support.

Literature Cited:

Amoros, C. and G. Bornette. 2002. Connectivity and biocomplexity in waterbodies of riverine floodplains. Freshwater Biology 47:761-776.

Angradi, T.R. 1994. Trophic linkages in the lower Colorado River: multiple stable isotope evidence. Journal of the North American Benthological Society 13:479-495.

Benke, A.C., I. Chaubey, G.M. Ward, and E.L. Dunn. 2000. Flood pulse dynamics of an unregulated river floodplain in the southeastern U.S. coastal plain. Ecology 81:2730-2741.

Bonin, H.L., Griffiths R.P., and Caldwell B.A. 2000. Nutrient and microbial characteristics of fine benthic organic matter in mountain streams. Journal of the North American Benthological Society 19:235–249.

Carlough, L.A. 1994. Origins, structure, and trophic significance of amorphous seston in a blackwater river. Freshwater Biology 31:227-237.

Colón-Gaud, C., S. Peterson, M.R. Whiles, S.S. Kilham, K.R. Lips, and C.M. Pringle. 2008. Allochthonous litter inputs, organic matter standing stocks, and organic seston dynamics in upland Panamanian streams: potential effects of larval amphibians on organic matter dynamics. Hydrobiologia 603:301-312.

Cross, W.F., J.P. Benstead, A.D. Rosemond, and J.B. Wallace. 2003. Consumer-resource stoichiometry in detritus-based streams. Ecology Letters 6:721-732.

Cross, W.F., J.B. Wallace, A.D. Rosemond, and S.L. Eggert. 2006. Whole-system nutrient enrichment increases secondary production in a detritus-based ecosystem. Ecology 87:1556-1565.

Cuffney, T.F. 1988. Input, movement and exchange of organic matter within a subtropical coastal blackwater river-floodplain system. Freshwater Biology 19:305-320.

Edwards, R.T. 1987. Sestonic bacteria as a food source for filtering invertebrates in two southeastern blackwater rivers. Limnology and Oceanography 32:221-234.

Edwards, R.T. and J.L. Meyer. 1990. Bacterivory by deposit-feeding mayfly (*Stenonema* spp.). Freshwater Biology 24:453-462.

Elser J.J., M.E.S. Bracken, E.E. Cleland, D.S. Gruner, W.S. Harpole, H. Hillebrand, J.T. Ngai, E.W. Seabloom, J.B. Shurin, and J.E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine, and terrestrial ecosystems. Ecology Letters 10:1135–1142

Finlay, J.C. 2001. Stable-carbon-isotope ratios of river biota: implications for energy flow in lotic food webs. Ecology 82:1052-1064.

Finlay, J.C. 2004. Patterns and controls of lotic algal stable carbon isotope ratios. Limnology and Oceanography 49:850-861.

Frost, P.C., R.S. Stelzer, G.A. Lamberti, and J.J. Elser. 2002. Ecological stoichiometry of trophic interactions in the benthos: Understanding the role of C:N:P ratios in lentic and lotic habitats. Journal of the North American Benthological Society 21:515-528.

Fry, B. 2006. Stable isotope ecology. Springer, New York, NY.

Golladay, S.W. and J. Battle. 2002. Effects of flooding and drought on water quality in Gulf Coastal Plain streams in Georgia. Journal of Environmental Quality 31:1266-1272.

Golladay, S.W., K. Watt, S. Entrekin, and J. Battle. 2000. Hydrologic and geomorphic controls on suspended particulate organic matter concentration and transport in Ichawaynochaway Creek, Georgia, USA. Archiv. für Hydrobiologia 149:655-678.

Goedkoop, W, N. Akerblom, and M.H. Demandt. 2006. Trophic fractionation of carbon and nitrogen stable isotopes in *Chironomus riparius* reared on food of aquatic and terrestrial origin. Freshwater Biology 51:878-886.

Greenwood, J.L., A.D. Rosemond, J.B. Wallace, W.F. Cross, and H.S. Weyers. 2007. Nutrients stimulate leaf breakdown rates and detritivore biomass: bottom-up effects via heterotrophy. Oecologia 151:637-649.

Gutseit, K., O. Berglund, and W. Graneli. 2007. Food quality for *Daphnia* in humic and clear water lakes. Freshwater Biology 52:344-356.

Hicks, D.W., R.E. Krause, and J.S. Clarke. 1981. Geohydrology of the Albany Area, Georgia. U.S. Geological Survey, Atlanta.

Hoffman, A. 2005. Dynamics of fine particulate organic matter (FPOM) and macroinvertebrates in natural and artificial leaf packs. Hydrobiologia 549:167-178.

IPPC. 2007. Climate change 2007: Impacts, adaptation and vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on

Climate Change, M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden and C.E. Hanson (Eds.), Cambridge University Press, Cambridge, UK.

Jones, J.B. and L.A. Smock. 1991. Transport and retention of particulate organic matter in two low-gradient headwater streams. Journal of the North American Benthological Society 10:115-126.

Junk, W.J., P.B. Bayley, and R.E. Sparks. 1989. The flood pulse concept in river-floodplain systems. In: D.P. Dodge. Proceedings of the International Large Rivers Symposium. Canadian Special Publication in Fisheries and Aquatic Sciences 106:110-127.

Kamauchi, H. 2005. Bacterial biomass on fine detritus particles in a woodland stream of Hokkaido, Japan. Ecological Research 20:109-114.

Knighton, D. 1998. Fluvial forms and processes: a new perspective. Oxford University Press, New York, NY.

Kondratieff, P.F. and G.M. Simmons. 1984. Nutritive quality and size fractions of natural seston in an impounded river. Archiv. für Hydrobiologia 101:401-412.

Littell, R.C. W.W. Stroup, and R.J. Freund. 2002. SAS for linear models, 4th ed. SAS Institute, Cary, NC.

Makino, W., Cotner J.B., Sterner R.W., and Elser J.J. 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P. Functional Ecology 17:121–130.

Macko, S.A. and M.L.F. Estep. 1984. Microbial alteration of stable nitrogen and carbon isotopic compositions. Organic Geochemistry 6:787-790.

Martin, F.D. and M.H. Paller. 2008. Ichthyoplankton transport in relation to floodplain width and inundation and tributary creek discharge in the lower Savannah River of Georgia and South Carolina. Hydrobiologia 598:139-148.

Meyer, J.L. 1994. The microbial loop in flowing waters. Microbial Ecology 28: 195-199.

Meyer, J.L. and R.T. Edwards. 1990. Ecosystem metabolism and turnover of organic carbon along a blackwater river continuum. Ecology 71:668-677.

Paul, M.J. and R.O. Hall. 2002. Particle transport and transient storage along a stream-size gradient in the Hubbard Brook Experimental Forest. Journal of the North American Benthological Society 21:195-205.

Poff, N.L., J.D. Allan, M.B. Bain, J.R. Karr, K.L. Prestegaard, B.D. Richter, R.E. Sparks, and J.C. Stromberg. 1997. The natural flow regime: a paradigm for river conservation and restoration. BioScience 47:769-784.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83:703-718.

Sinsabaugh R.L. and A.E. Linkins. 1990. Enzymatic and chemical analysis of particulate organic-matter from a boreal river. Freshwater Biology 23:301-309.

Söderström, O. 1988. Effects of temperature and food quality on life-history parameters in *Parameletus chelifer* and *P. minor* (Ephemeroptera): a laboratory study. Freshwater Biology 20:295-303.

Sterner, R.W. and J.J. Elser. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, U.S.A.

Tuchman, N.C., R.G. Wetzl, S.T. Rier, K.A. Wahtera, and J.A. Teeri. 2002. Elevated atmospheric CO_2 lowers leaf litter nutritional quality for stream ecosystem food webs. Global Change Biology 8:1-8.

U.S. Department of Agriculture (USDA), Soil Conservation Service. 1994. State soil geographic (STATSGO) data base for Georgia. U.S. Department of Agriculture, Ft. Worth, Texas.

Vander Zaden, M.J. and J. B. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology 80:1395–1404.

Wallace, J.B., S.L. Eggert, J.L. Meyer, and J.R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277:102-104.

Wallace, J.B. and R.W. Merritt. 1980. Filter-feeding ecology of aquatic insects. Annual Review of Entomology 25:103-132.

Wallace, J.B., D. H. Ross, and J.L. Meyer. 1982. Seston and dissolved organic carbon dynamics in a southern Appalachian stream. Ecology 63:824-838.

Ward, J.V. 1989. The four-dimensional nature of lotic ecosystems. Journal of the North American Benthological Society 8:2-8.

Zueg, S.C. and K.O. Winemiller. 2008. Evidence supporting the importance of terrestrial carbon in a large-river food web. Ecology 89:1733-1743.

Table 2.1: Summary of physical characteristics of the nine sampling sites in the Ichawaynochaway Creek basin, Georgia, U.S.A. Location of study sites can be found in Fig. 1.

Site	Distance from Headwaters (km)	Floodplain Width (m)	Channel Width (m)	Floodplain / Channel Width
1	10.00	328	13	51.7
2	22.50	609	21	40.6
3	39.86	476	21	22.3
4	51.25	869	14	38.6
5	57.50	981	22	32.7
6	68.75	281	31	20.8
7	71.86	314	18	17.6
8	81.25	74	40	2.5
9	96.25	57	35	1.3

Table 2.2: Summary to indicate what dates were classified as high flow or low flow based on the 1-year flood recurrence interval. The 1-year flood recurrence interval discharge is based on a previous record of 78 years of discharge data.

Hydrology	**Date**	**Discharge at USGS 02353500 (m^3s^{-1})**
1 year recurrence flood		27.00
Low Flow	June 22, 1994	20.78
High Flow	July 25, 1994	48.14
High Flow	March 22, 1995	43.89
Low Flow	August 31, 1995	7.48
Low Flow	February 29, 1996	25.34
Low Flow	October 23, 1996	9.63

Table 2.3: The mean values of C:N, δ^{13}C, and δ^{15}N (*n* = 150) with ± SE indicated during all flow conditions (*n* = 150), low flow conditions (*n* = 102), and high flow conditions (*n* = 48). Letters denote significant differences within size classes, and the asterisks denote differences within size classes due to different flow regimes at α = 0.05 (Tukey's HSD, Littell et al. 2002).

Flow Condition	**Size Class**	**C:N**	**δ^{13}C (‰)**	**δ^{15}N (‰)**
All Flow	Small	15.74 + / - 0.34^{A}	-27.57 + / - 0.06^{A}	5.45 + / - 0.15^{A}
	Medium	17.75 + / - 0.44^{B}	-27.53 + / - 0.05^{A}	5.02 + / - 0.17^{A}
	Large	27.65 + / - 0.86^{C}	-27.87 + / - 0.07^{B}	3.85 + / - 0.16^{B}
Low Flow	Small	16.43 + / - 0.43^{A,**}	-27.59 + / - 0.14^{A}	5.78 + / - 0.02^{A,**}
	Medium	18.47 + / - 0.60^{A,**}	-27.58 + / - 0.19^{A}	5.40 + / - 0.02^{A,**}
	Large	27.53 + / - 1.15^{B}	-27.94 + / - 0.19^{B}	4.22 + / - 0.02^{B,**}
High Flow	Small	14.27 + / - 0.35^{A,**}	-27.52 + / - 0.09^{A}	4.75 + / - 0.29^{A,**}
	Medium	16.30 + / - 0.35^{A,**}	-27.43 + / - 0.10^{A}	4.28 + / - 0.24^{A,**}
	Large	27.87 + / - 1.20^{B}	-27.71 + / - 0.10^{A}	3.15 + / - 0.25^{B,**}

Figure 2.1: Ichawaynochaway Creek basin, which flows into the Flint River, is located in southwest Georgia, USA. Sample areas with gauge station information available were sites 3, 6, and 7. The Ichawaynochaway Basin has a large areal extent that floods in a typical year between October and April. The area delineated around the stream channel represents an estimate of the area of land that is flooded in any typical year (USDA 1994).

Figure 2.2: Discharge at USGS 02353500 (site 6) on Ichawaynochaway Creek from 1/1/94 to 12/31/96 is indicated by the solid line. The dashed line indicates the mean daily discharge. Arrows indicate dates when samples were collected.

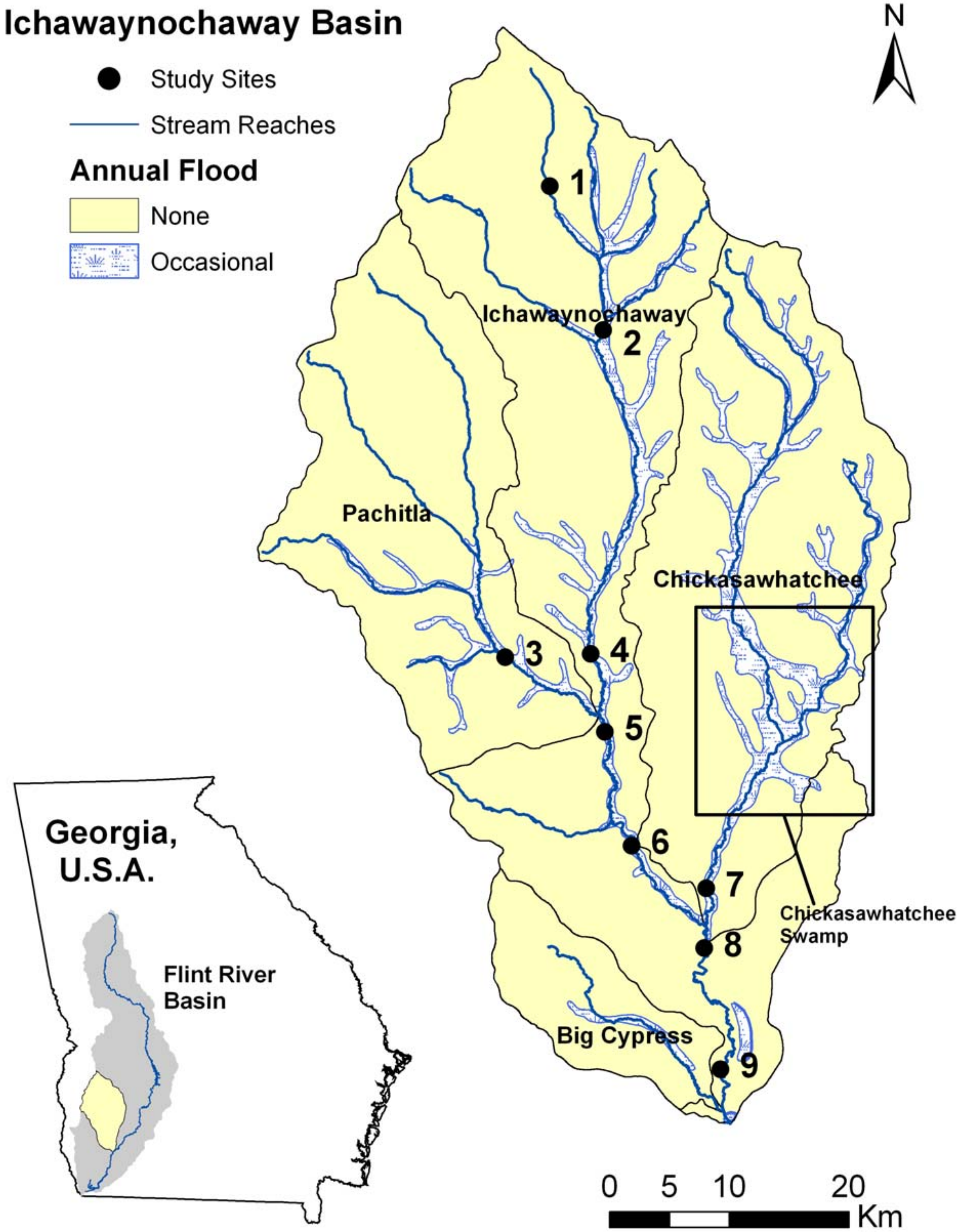
Figure 2.3: (A) Mean C:N by seston size class ($n = 150$) with the error bars indicating the standard error. (B) Mean $\delta^{13}C$ (‰) by seston size class broken out due to flow regime ($n = 150$) with the error bars indicating the standard error. (C) Mean $\delta^{15}N$ (‰) by seston size class broken out due to flow regime ($n = 150$) with the error bars indicating the standard error. Size fractions: small (45-10 μm), medium (250-45 μm), and large (>250 μm). Letter denote differences due to size class and asterisks denote differences within a size class due to varying flow regimes at $\alpha = 0.05$ (Tukey's HSD, Littell et al. 2002).

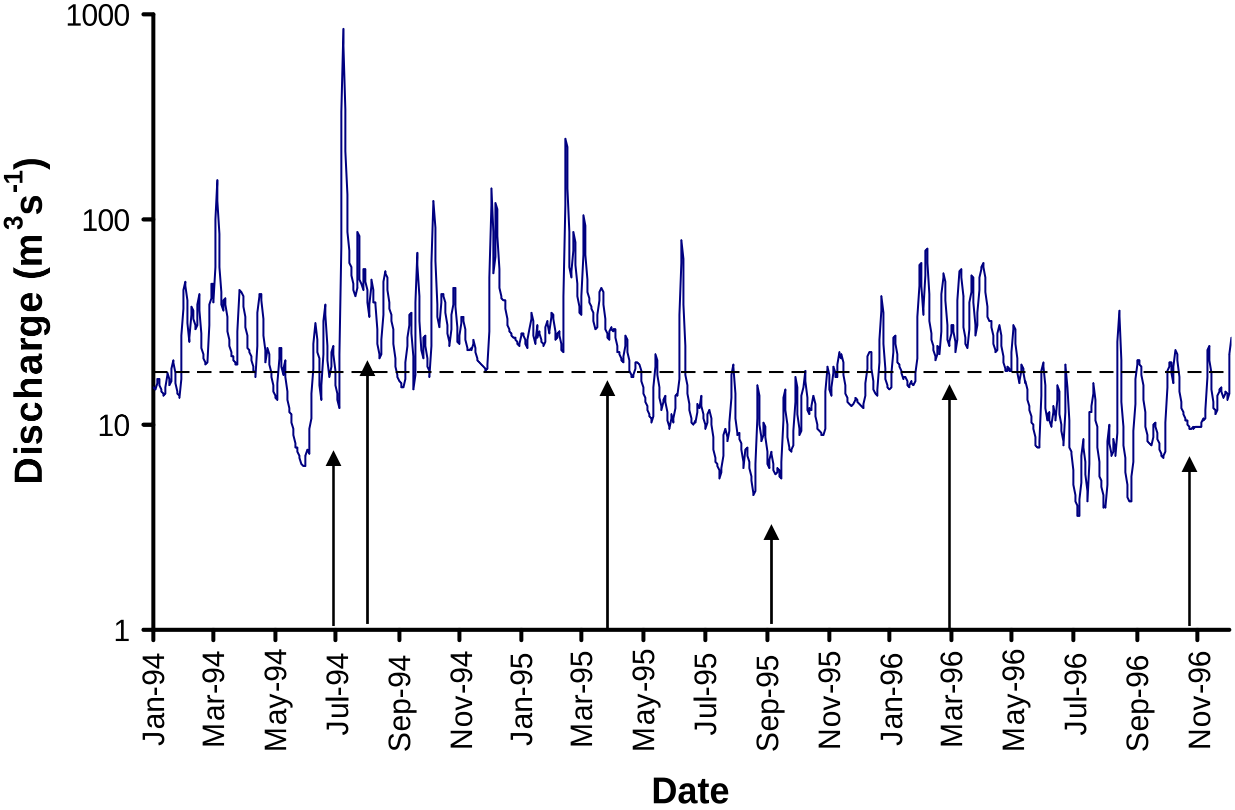
Figure 2.4: (A) Linear regression for C:N of the small size class and average discharge the day the sample was taken ($y = -0.095x + 16.97$; $r^2 = 0.56$; $p = 0.001$; $n = 15$). (B) Linear regression

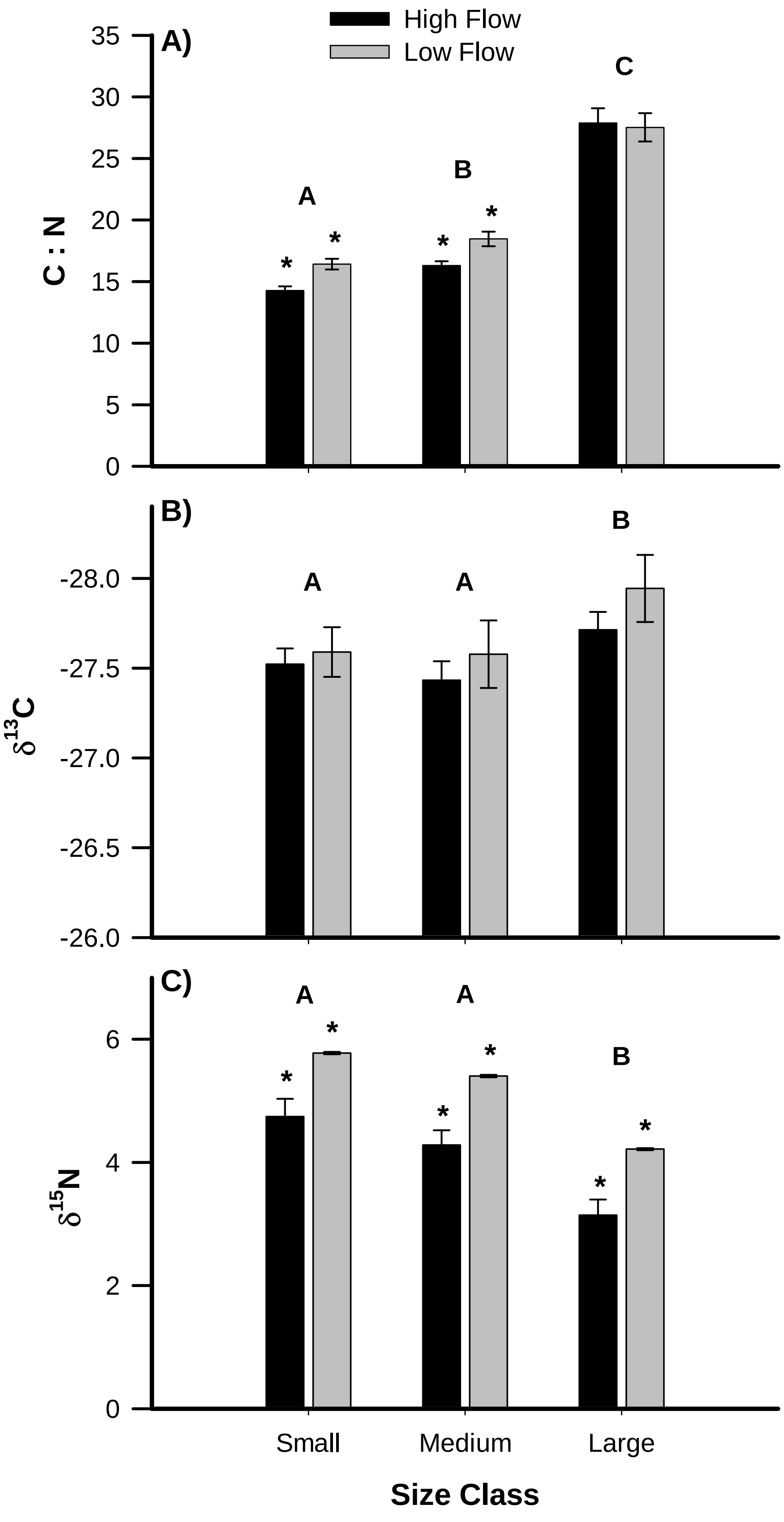
for $\delta^{13}C$ (‰) of the small size class and average discharge the day the sample was taken ($y = 0.018x - 27.882$; $r^2 = 0.44$; $p < 0.007$; $n = 15$). Discharge data was available for sites 3, 6, and 7.

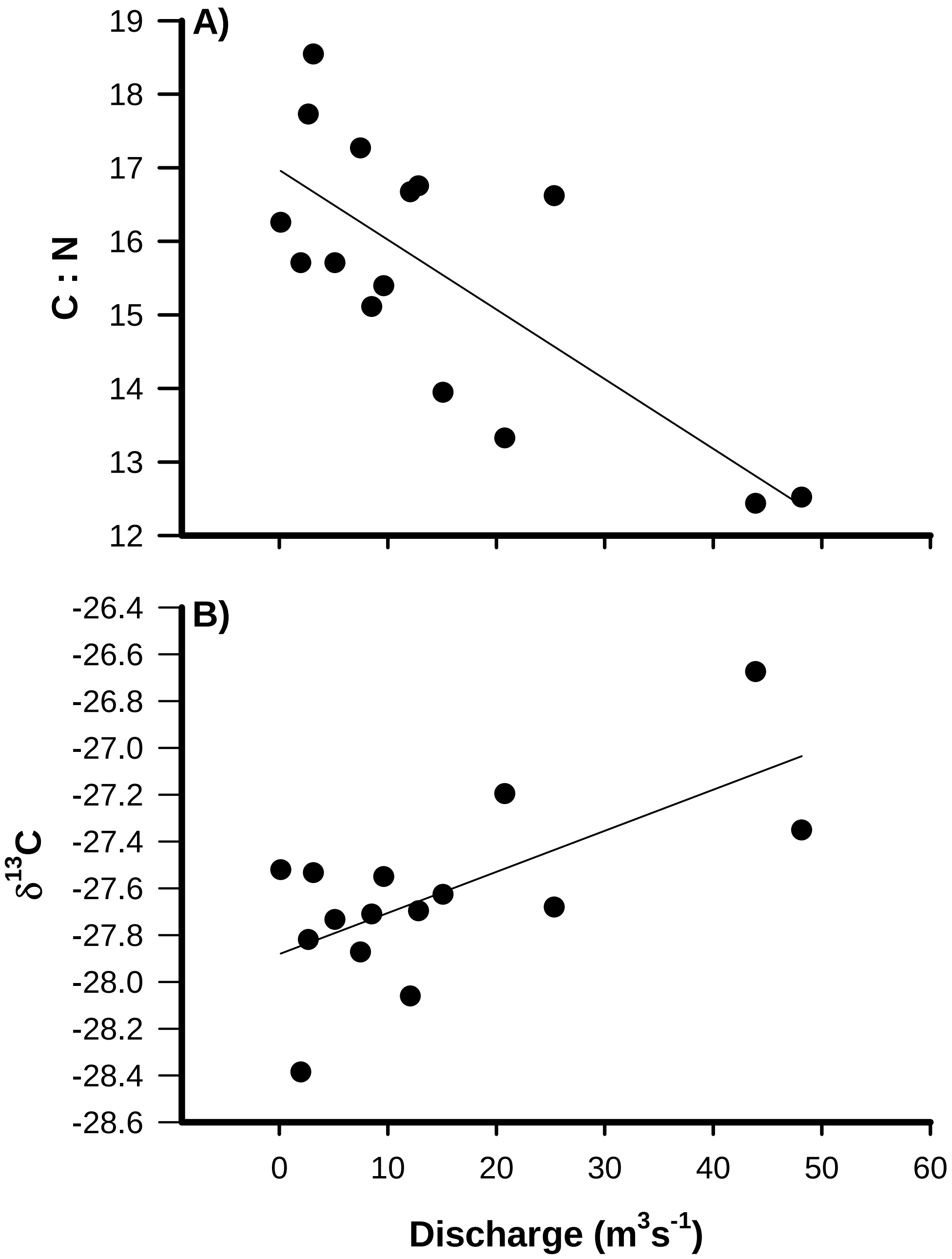
Figure 2.5: Linear regression of the small seston size class and the floodplain width (m)/bankfull width (m) of the mean (A) C:N during low ($y = 0.083x + 14.506$; $r^2 = 0.66$; $p < 0.01$; $n = 9$) and high ($y = 0.028x + 13.016$; $r^2 = 0.12$; $p = 0.37$, N.S.; $n = 9$) flow periods, (B) $\delta^{13}C$ (‰) during low ($y = -0.011x - 27.324$; $r^2 = 0.34$; $p < 0.10$; $n = 9$) and high ($y = -0.009x - 27.319$; $r^2 = 0.29$; $p = 0.14$, N.S.; $n = 9$) flow periods, (C) $\delta^{15}N$ (‰) during low ($y = -0.012x + 6.065$; $r^2 = 0.25$; $p = 0.17$, N.S.; $n = 9$) and high ($y = 0.037x + 3.884$; $r^2 = 0.50$; $p = 0.03$; $n = 9$) flow periods at each sample location.

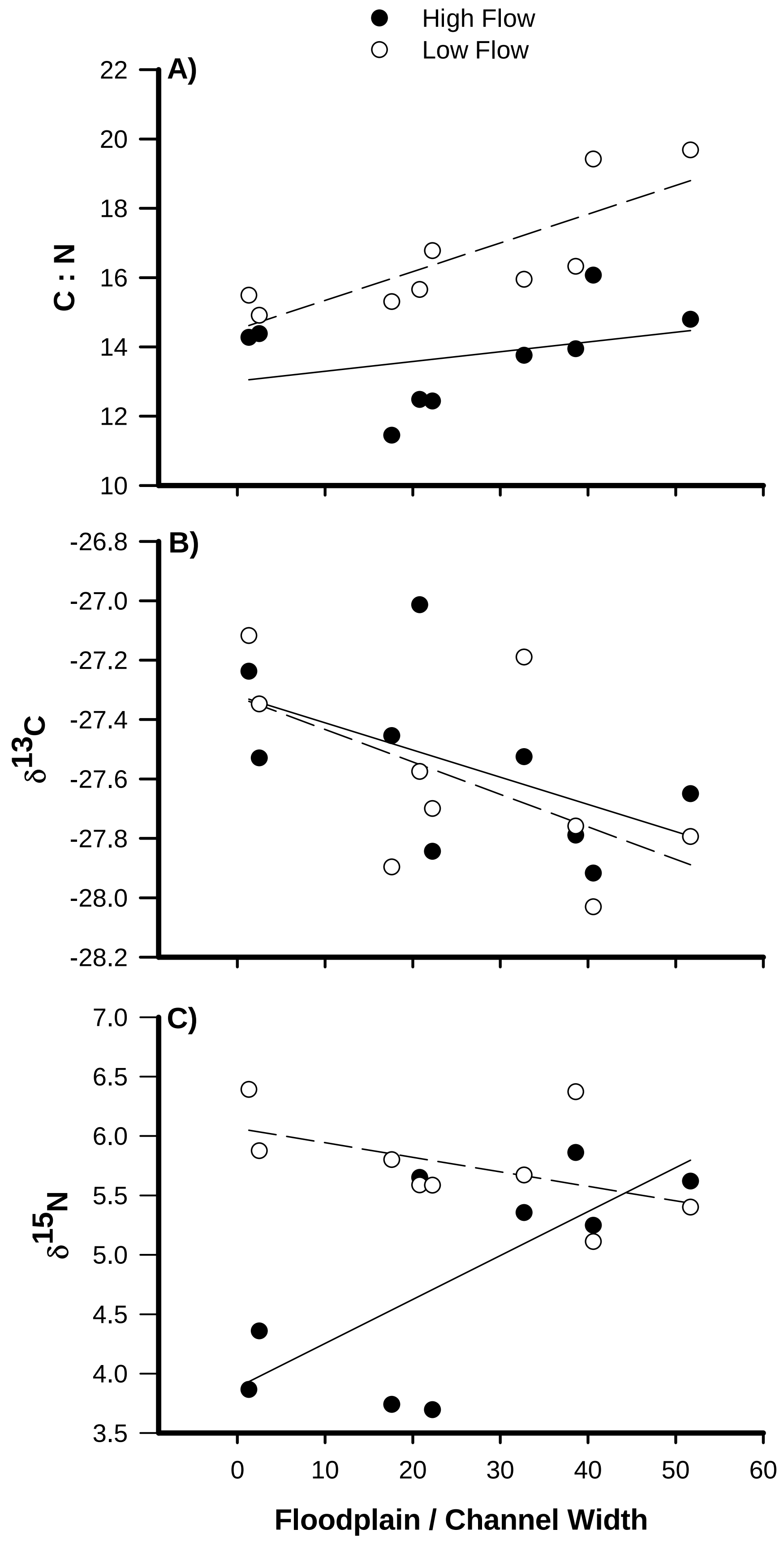
Figure 2.6: Linear regression of the small size class of seston plotted against the distance from the headwaters of the mean (A) C:N during low ($y = -0.0578x + 19.822$; $r^2 = 0.84$; $p < 0.001$; $n = 9$) and high ($y = -0.020x + 14.814$; $r^2 = 0.15$; $p > 0.30$, N.S.; $n = 9$) flow periods, (B) $\delta^{13}C$ (‰) during low ($y = 0.008x - 28.029$; $r^2 = 0.45$; $p < 0.05$; $n = 9$) and high ($y = 0.007x - 27.948$; $r^2 = 0.46$; $p < 0.05$; $n = 9$) flow periods, and (C) $\delta^{15}N$ (‰) during low ($y = 0.011x + 5.175$; $r^2 = 0.48$; $p = 0.04$; $n = 9$) and high ($y = -0.015x + 5.661$; $r^2 = 0.22$; $p = 0.20$, N.S.; $n = 9$) flow periods at each sample location.

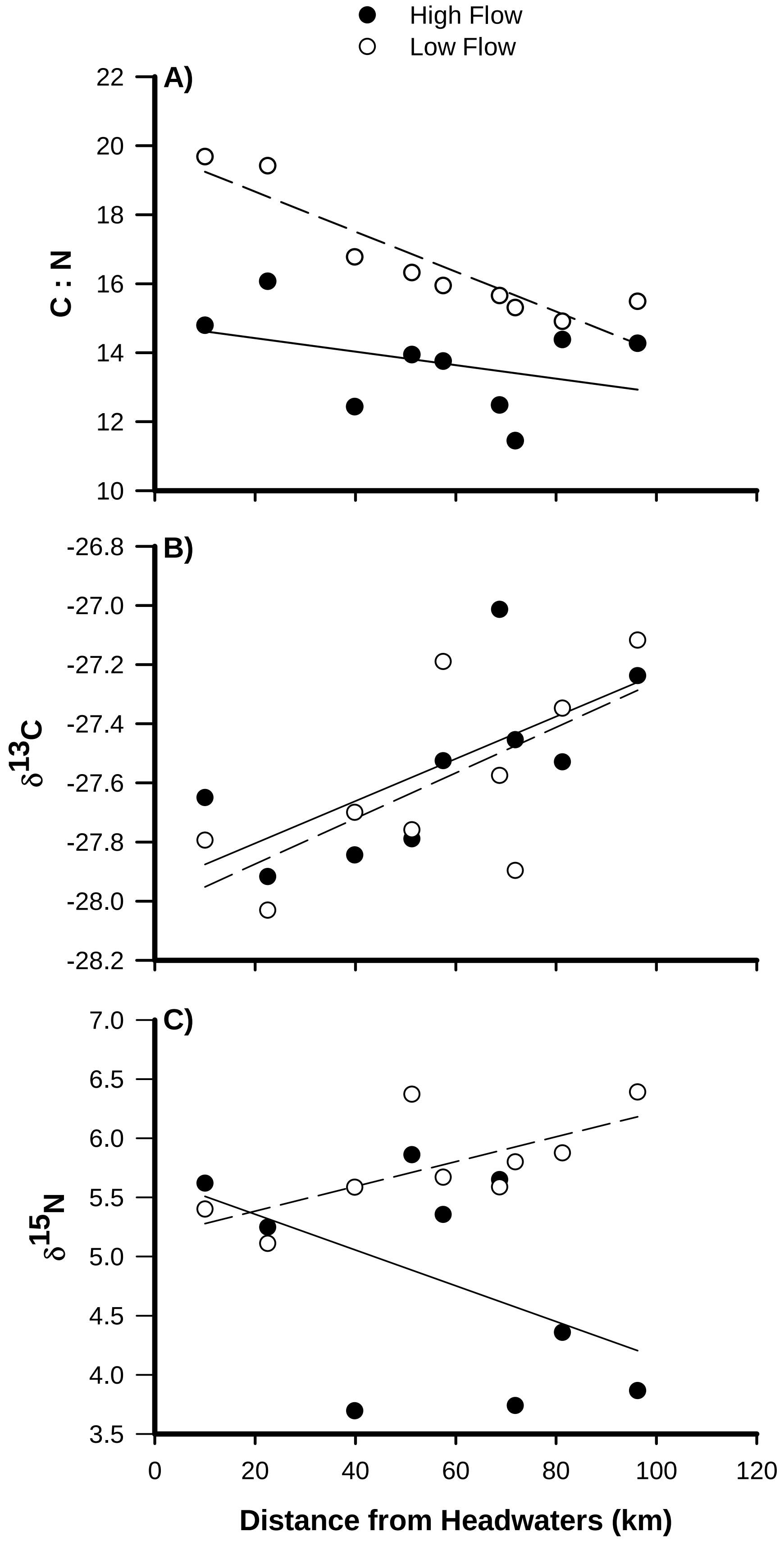

Ichawaynochaway Basin
Study Sites
Stream Reaches
Annual Flood
None
Occasional
N
1
Ichawaynochaway
2
Pachitla
Chickasawhatchee
3
4
5
6
7
8
9
Chickasawhatchee Swamp
Big Cypress
Georgia, U.S.A.
Flint River Basin
0
5
10
20
Km


1000
100
10
1
Discharge ($m^3 s^{-1}$)
Jan-94
Mar-94
May-94
Jul-94
Sep-94
Nov-94
Jan-95
Mar-95
May-95
Jul-95
Sep-95
Nov-95
Jan-96
Mar-96
May-96
Jul-96
Sep-96
Nov-96
Date


High Flow
Low Flow
A)
C : N
35
30
25
20
15
10
5
0
A
B
C
*
*
*
*
B)
$\delta^{13}C$
-28.0
-27.5
-27.0
-26.5
-26.0
A
A
B
C)
$\delta^{15}N$
6
4
2
0
A
A
B
*
*
*
*
*
*
Small
Medium
Large
Size Class


A)
B)
C : N
$\delta^{13}C$
19
18
17
16
15
14
13
12
-26.4
-26.6
-26.8
-27.0
-27.2
-27.4
-27.6
-27.8
-28.0
-28.2
-28.4
-28.6
0
10
20
30
40
50
60
Discharge (m^3s^{-1})


● High Flow
○ Low Flow
A)
C : N
22
20
18
16
14
12
10
B)
$\delta^{13}C$
-26.8
-27.0
-27.2
-27.4
-27.6
-27.8
-28.0
-28.2
C)
$\delta^{15}N$
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
0
10
20
30
40
50
60
Floodplain / Channel Width


● High Flow
○ Low Flow
A)
C : N
22
20
18
16
14
12
10
B)
$\delta^{13}C$
-26.8
-27.0
-27.2
-27.4
-27.6
-27.8
-28.0
-28.2
C)
$\delta^{15}N$
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
0
20
40
60
80
100
120
Distance from Headwaters (km)

CHAPTER 3

APPLICATION OF FLOW CYTOMETRY AND MASS LOSS TO UNDERSTAND PREFERENTIAL FILTRATION BY A NATIVE AND INVASIVE BIVALVE SPECIES[2]

[2] Atkinson, C.L., M.R. First, A.P. Covich, S.P. Opsahl, and S.W. Golladay. To be submitted to *Freshwater Biology*.

Abstract:

Unionid mussels, among the most threatened groups of freshwater organisms in the United States, are known for their ability to filter food particles from water. The ecology of filter feeding unionid mussels and the non-native Asian bivalve, *Corbicula fluminea* is incompletely understood. *C. fluminea* was introduced to North America and has become widespread in many freshwater systems. We hypothesized that *Elliptio crassidens* (native) and *C. fluminea* (invasive) would select for specific organic components present within seston over inorganic and dead materials. Secondly, we hypothesized that *C. fluminea* would be more efficient at capturing small particles than *E. crassidens*. We examined dry mass and ash-free dry mass loss over time due to bivalve feeding activity in three size classes of material that where isolated using filtration techniques. These replicates were also sub-sampled for flow cytometry, which separated the suspended materials in the stream water into 5 categories: detritus, bacteria, picoautotrophs, nanoautotrophs, and nanoautotrophs. Our results indicated that both species of bivalve showed preference for organic and living materials versus inorganic and non-living suspended materials, however they may have used different components of the particulate materials available. These results further demonstrated that *E. crassidens* were filtering primarily nanoeukaryotes. The decrease of nanoeukaryotes occurred simultaneously with an increase in bacteria. In contrast, *C. fluminea* preferred smaller materials through the filtration of picoautotrophs. Additionally, both species increased the concentration of large materials towards the end of the experiment due to their excretory activities. To our knowledge, this is the first study to examine grazing by bivalve species on natural stream particulate matter. In the future, studies incorporating flow cytometry will likely yield information on additional resource-specific dynamics related to differences in

filtration by these and other co-occurring species. Our results suggest that native and non-native mussels have different functional roles in streams and rivers.

Introduction:

The North American fauna of freshwater mussels (Superfamily Unionoidea) are the most diverse in the world, with approximately 308 native species (Turgeon *et al*., 1998). They are known for their ability to filter large amounts of water and are also one of the most endangered groups of freshwater invertebrates in the world (Dudgeon *et al*., 2006; Bogan, 2008). Historically, filter-feeding bivalves were the dominant invertebrate, in terms of biomass, in many eastern North American streams (Parmalee & Bogan, 1998). The ecological functions performed by mussels (e.g., filter-feeding, nutrient excretion, biodeposition, bioturbation) impact both primary producers and consumers through direct and indirect pathways (Howard & Cuffney, 2006; Spooner & Vaughn, 2006; Vaughn & Spooner, 2006; Vaughn *et al*., 2007). The well-documented abundance of bivalves and their high filtering rates suggest they can potentially structure the food web within streams and rivers (Vaughn *et al*., 2008). Adult mussels can clear and assimilate a wide range of suspended fine particulate organic matter (FPOM) ranging in size from 1 µm up to at least 40 µm (Brönmark & Malmqvist, 1982; Paterson, 1986; Vanderploeg *et al*., 1995). Thus, there is a need to understand the impacts of both uptake and release of materials during bivalve feeding.

The introduction of non-native bivalve species has been a threat to populations of native mussels. Because invasive species differ greatly from native species in resource use or trophic interactions, they may have the most potential to negatively affect an ecosystem (Vitousek, 1990). Invasive aquatic mussel species can alter community structure and ecosystem function due to rapid establishment and high filtering capacity. For example, the zebra mussel displaced native filter-feeders in North American lakes (Horgan & Mills, 1997; Idrisi *et al*., 2001; Naddfi *et al*., 2007) and caused changes in water clarity in rivers as a result of their abundance and efficient filtration (Caraco *et al*., 1997). They are also known to alter microbial and plantkonic community structure and ecosystem function in rivers due to rapid establishment and high filtering capacity (Weitere *et al*., 2008). While the success of invasive bivalve species is evident from their increasing abundance, their functional roles in comparison with native species are poorly understood. *Corbicula fluminea*, the introduced Asian clam, is widespread across the United States and is common throughout the southeastern United States (McMahon & Bogan, 2001). Despite their abundance and wide distribution, their feeding ecology is not well understood. Leff *et al.* (1990) showed that *C. fluminea* can filter feed at higher rates compared to the native mussel, *Ellipto complanata.* However, the size range of particles retained by native mussels and *C. fluminea* is not well known. Due to potential diet overlap, there is a need to understand the filtering preferences of *C. fluminea* compared to native mussels.

Flow cytometry (FC) is a technique that permits a detailed examination of suspended particle mixtures and allows for accurate and rapid enumeration of organic living and non-living particles (Moreira-Torcq & Martin, 1998). FC has numerous applications in the study of aquatic ecology, but the majority of studies that have applied FC are from marine systems (Marie *et al*., 1997; Li, 1997; Partensky *et al*., 1999; Legendre *et al*., 2001; Veldhuis & Timmermans, 2007). Despite the widespread use of FC it has been rarely used in the study of freshwater systems (exceptions include Crosbie *et al*., 2003; Dionisio Pires *et al*., 2004; Sarmento *et al*., 2008), and to our knowledge, FC has only been applied in freshwater to the study of lakes (del Giorgio *et al*., 1996; Crosbie *et al*., 2003; Dionisio Pires *et al*., 2004; Sarmento *et al*., 2008).

In the present study, we combined two different methods (gravimetric and FC) to determine the abundance and composition of suspended materials left in the water column after bivalve filtration and consequential excretory activities. Bivalves and water were collected from Ichawaynochaway Creek, a tributary to the lower Flint River on the Coastal Plain of the southeastern United States. We hypothesized that both species would select for organic and living materials over inorganic and dead materials and that *C. fluminea* would be more efficient at capturing smaller particles due to the close proximity of their cirri in comparison to unionid mussel species. The objectives of this study were to determine what materials were available in the water column, their quantity, and to compare the efficiency with which a native and invasive bivalve utilized the pool of available materials.

Methods:

Sample collection and processing:

Bivalve individuals, *E. crassidens* (*n* = 12) and *C. fluminea* (*n* = 12), were collected from Ichawaynochaway Creek (approximately 5.5 km upstream of USGS station, 02355350). The shells of all individuals were cleaned thoroughly with a brush and water. Total wet mass (g) was measured for all individuals and then converted to tissue wet mass using a regression equation that was generated using previous data on these species (Atkinson, unpublished data). Bivalves were placed in individually aerated containers with 1000 ml of filtered (GF-75, Sterlitech Corp., Kent, WA) water for 48 hours (water was changed every 12 hours) to purge gut contents and to acclimate the bivalves to laboratory conditions. The day of the experiment, water from Ichawaynochaway Creek was collected, filtered through a 45µm sieve, and 2 L was poured into each container. Our laboratory experiments used water from the stream, thus mussels were exposed to their typical particle assemblages. The experiment was set up as a 2-way completely randomized design with the two main effects being bivalve treatment and time. There were 3 bivalve treatments: control (no bivalve), *E. crassidens*, and *C. fluminea*. There were 5 time treatments in the experiment: 0, 1, 3, 6, and 12 hours. Each bivalve treatment × time was replicated 3 times for a total of $n = 45$. Time for each individual tank began once it was noted that a bivalve had begun to filter. The experiment took place in a temperature controlled incubator that was kept at 25˚ C (range: 24.7˚ – 25.5˚ C), which is typical summertime temperature in Ichawaynochaway Creek. At the end of each time treatment, a 10 ml subsample was collected and preserved in formalin (1% final concentration) for FC. These samples were immediately stored at 4˚ C and kept in the dark until analysis. The remaining water from the treatments was sieved into 3 size classes: 0.3 – 1.5 µm (S), 1.5 - 10 µm (M), and 10 – 45 µm (L) and each fraction filtered onto ashed glass fiber filters. Filtered samples were oven dried (24 h at 55˚ C), desiccated, weighed, ashed (20 min at 500˚ C), rewetted with deionized water, redried, desiccated, and weighed to the nearest milligram to determine total dry mass (DM), organic (ash-free dry mass, AFDM) and inorganic content (ash) of the three size classes of the various treatments and controls.

Samples for FC for the determination of bacteria, heterotrophic flagellates, and phytoplankton were collected throughout the incubation period. Samples were stained with the DNA stain, 4',6-diamidino-2-phenylindole (DAPI), to indicate intact cells. We used a Cyan (Dako) flow cytometer to analyze samples, with illumination at three wavelengths (405, 488, and 610 nm). Forward angle and side angle light scattering (FALS and SALS, respectively) were measured. FALS is a proximal measurement of object size. Several sizes of microspheres (1, 3, and 6 µm in diameter) were used to provide a relative scale of the sizes of objects detected on the

FALS scale. DAPI fluorescence was detected in a photomultiplier tube (PMT) with a 450 nm band pass filter; chlorophyll a (CHL) fluorescence was detected in a PMT with a 665 nm filter. Samples were run for 5 min, resulting in approximately 0.4 ml of each sample being analyzed (determined from the mean weight lost during analysis). All objects with a SALS signal in the top 99% of the data range were collected.

FC data were analyzed with FlowJo V8.7.1 (Treestar Inc., Ashland, OR). The total data set was gated first according to FALS. Objects with a FALS signal in the lowest 0.012 % of data range were considered to be noise and were removed from the analysis. The gate limits were set according to the size ranges of microspheres (Fig. 3.1). The secondary gating was based upon DAPI and CHL fluorescence signals. Objects with low DAPI and CHL fluorescence signals were considered to be non-living detrital particles (See Fig. 1). High DAPI, low CHL were defined as either heterotrophic bacteria (<3 μm) or nanoeukaryotes (>3 μm). High CHL populations were either determined to be picoautotrophs or nanoautotrophs (based upon the FALS signal).

Data analysis:

To determine if bivalves significantly affected the concentration and distribution of different types of particles in the water column, we compared treatments and time effects using ANCOVA. Analysis of covariance was performed with treatment as a main effect and time as a covariate using PROC GLM to determine if the DM and AFDM of the three size classes were significantly different among the various bivalve and time treatments and if there was an interaction between these two variables. An analysis of covariance was performed with bivalve treatment as the main affect and time as a covariate to determine differences in the total mass of organic and inorganic materials. Analysis of covariance was also performed with treatment as a main affect and time as a covariate using PROC GLM to determine whether differences exist in the concentration of detritus, heterotrophic bacteria, picoautotrophs, nanoautotrophs, and nanoeukayotes. We also categorized those variables into living and dead particles to determine if there was selectivity for living or dead particles. The concentration of living and dead particles did not meet the assumptions of normality implicit in parametric analyses, so these data were rank transformed because parametric transformations did not normalize data before running ANCOVA (Conover & Iman 1981). ANCOVA was run with treatment as a main effect and time as a covariate to determine if there were significant differences in the concentration of living and dead particles in the treatments over time. Significant ANCOVAs were followed by protected Least Square Difference multiple comparisons to determine if significant differences existed between bivalve treatment and time ($\alpha = 0.05$; Littell *et al*., 2002). This was followed by multiple comparison tests for one factor at each level using one-way ANOVAs. All statistical analyses were performed using SAS v9.1 (SAS Institute, Cary, NC).

Results:

Changes in concentration of particle size classes:

There were significant differences in both L-DM (ANCOVA, $p = 0.0001$; Table 3.1; Fig. 3.2A), which is the amount of bulk material, and AFDM (ANCOVA, $p = 0.028$; Table 3.2; Fig 3.3A), which represents the amount of organic materials, among the treatments. As time passed, both bivalve species significantly increased the amount of L-DM and L-AFDM in the containers, but the effect of the time treatment was dependent upon the bivalve treatment. The initial 3 hrs of the experiment were time periods of net filtration, while the last remaining 2 sampling periods were times of net excretion. There was an initial significant decrease in the amount of L-DM up to 3 hrs and then a significant increase in the latter time periods. The two bivalve treatments significantly increased the amount of L-DM and L-AFDM compared to the control treatments by t = 12 hrs. *C. fluminea*, through excretion, caused a net increase of 0.54 ± 0.10 mg l^{-1} L-DM and 0.09 ± 0.04 mg l^{-1} L-AFDM in the large size class. *E. crassidens* lead to a net increase of 0.90 ± 0.23 mg l^{-1} L-DM and 0.20 ± 0.08 mg l^{-1} L-AFDM by t = 12 hr. Bivalves, especially the native species, were increasing the amount of material in the larger size class.

There were also significant differences in M-DM among the treatments (Table 3.1; Fig 3.2B). However, no significant differences were noted in the amount of M-AFDM due to bivalve treatment, time, or the interaction between the two main effects (Table 3.2; Fig. 3.3B). M-DM was significantly lower in the t = 3 and t =12 hr treatments from t = 1 and t = 6 hrs for the *C. fluminea* treatments. The t = 0 and t = 6 hr treatments were greater in M-DM than all other time treatments, but not significantly different from each other within the *C. fluminea* treatments. *E. crassidens* treatments were significantly lower in the amount of M-DM than the control and *C. fluminea* treatments at t = 6 hrs. This indicated that *E. crassidens* may select heavily for this size class.

There were also significant differences in S-DM (ANCOVA, p = 0.0127; Table 3.1; Fig. 3.2C) and S-AFDM (ANOVA, p = 0.0265; Fig 3.3C) among the treatments. The *C. fluminea* treatment was found to be significantly different, with the t = 3 and t =12 hr treatments having significantly less S-DM than the t = 0 hr treatment. However, there were no differences in S-AFDM between the control and *C. fluminea* treatments across time. The bivalve treatments were not significantly different from each other during any of the time treatments. The *E. crassidens* treatments were significantly lower in S-DM than t = 0 hr at all other time treatments and t =12 hr was lower in S-AFDM than all other time treatments.

Changes in the concentration of total organic and inorganic matter:

There were significant differences in the total amount of organic material between bivalve treatment when all the size classes were summed (ANCOVA, $p < 0.0094$; Fig. 3.4). Bivalve treatment did not have a significant effect on the mass of organic materials ($p = 0.313$). However, time ($p = 0.006$) and the bivalve treatment × time interaction was significant ($p = 0.023$). The controls did not differ significantly from one another due to time ($p = 0.808$). The *C. fluminea* treatments differed significantly over time with t = 0, t = 3, and t =12 hrs being significantly different from all times; t = 3 hr had the least and t = 12 hr had the greatest amount of organic matter. The t = 1 and t =6 hr treatments were not different from one another within the *C. fluminea* treatments ($p = 0.0001$). The *E. crassidens* treatments also differed significantly in the mass of organic materials with t = 12 hr having greater amounts of organic matter than all times ($p = 0.044$). The time period from the beginning of the experiment to t = 3 hr was a period of net filtration, and the latter portions were periods of net excretion. There were no significant difference between the bivalve treatments at t = 0 ($p = 0.251$), t = 1 ($p = 0.098$), t = 3 ($p = 0.658$) and t = 6 hrs ($p = 0.906$). At t = 12 hr, the *E. crassidens* and *C. fluminea* treatments had significantly more organic matter than the controls ($p = 0.038$). However, no significant differences were noted in the mass of inorganic materials due to bivalve treatment ($p = 0.391$), time ($p = 0.375$), or the interaction between the two main effects ($p = 0.595$).

Flow cytometry:

Detritus was the most dominant particle type found using FC. However, the frequency of detritus did not change over time or due to the bivalve treatments (ANCOVA, $p = 0.365$; Table 3.3; Fig. 3.5). Heterotrophic bacteria were found to be the second most abundant particle in the water, and significant differences were found among the treatments and times. The bivalve treatments were significantly different at α = 0.10 and time had a significant effect at α = 0.05 on the frequency of bacteria. The concentration of bacteria was significantly different in the *C. fluminea* treatments over time; the t = 1 hr time treatment was significantly lower than all times. The concentration of bacteria was significantly lower in the *E. crassidens* treatments at t = 6 hrs. The concentration of bacteria in the *E. crassidens* treatments was significantly greater at t = 12 hr from all other time periods. The concentration of bacteria in the controls did not significantly differ at α = 0.05. There were no significant differences in the concentration of bacteria among the bivalve treatments at t = 0, t = 1, t = 3, and t = 12 hrs. Nanoautotrophs were common and were found to differ significantly at α = 0.1 (ANCOVA, *p* = 0.089), however the differences could not be attributed to the bivalve treatments, time, or the treatment × time interaction (see Table 3.3). The frequency of picoautotrophs were found to significantly differ among the treatments (ANCOVA, *p* = 0.029; Fig. 5). Time had a significant affect on the frequency of picoautotrophs (Table 3.3). The frequency of picoautotrophs differed significantly within the *C. fluminea* treatments, with t = 1 hr having fewer picoautotrophs and being significantly different than t = 6 hr. The concentration of picoautotrophs also differed significantly in the *E. crassidens* treatments, with t = 3 and t = 6 hr treatments having a greater concentration than t = 1 and t = 12 hr treatments. There were no statistically significant differences in the concentration of picoautrophs between the bivalve treatments at t = 0, t = 3, t = 6, and t = 12 hrs. However, the *C. fluminea* and *E. crassidens* treatments were significantly lower than the controls in the concentration of picoautotrophs at t = 1 hr. There were also significant differences among the treatments in the concentration of nanoeukaryotes such as flagellates (ANCOVA, *p* = 0.027). Time and the bivalve treatment × time interaction had a significant affect on the concentration of nanoeukaryotes (Table 3.3). The controls and the *C. fluminea* treatments did not differ in the concentration of nanoeukaryotes over time. However, the concentration of nanoeukaryotes in the *E. crassidens* treatments were significantly lower over time, with t = 0 and t = 3 being significantly different than t = 6 and t = 12 and t = 1 being significantly different than t =12 (Fig 3.5).

Overall, there were significant differences in the concentration of live particles found in the treatments (ANCOVA, *p* = 0.025, Fig. 3.6). The *E. crassidens* treatments were significantly different from both the controls and *C. fluminea* treatments (*p* = 0.016), with the *E. crassidens* treatments having fewer live particles at t = 3 and t = 6 hrs. The control approached having significantly different concentrations of living particles over time (*p* = 0.059). The *C. fluminea* treatments differed over time with t = 0 and t = 1 having significantly more living particles than the t = 3, t = 6, and t = 12 hr treatments had more living particles (*p* = 0.002). The *E. crassidens* treatments did not differ in the concentration of living particles over time (*p* = 0.906). No significant differences were seen in the treatments at t = 0 (*p* = 0.732), t = 1 (*p* = 0.420), t = 3 (*p* = 0.063), t = 6 (*p* = 0.190) and t = 12 (*p* = 0.691). No significant trends were seen in the concentration of dead particles (ANCOVA, *p* = 0.155). The treatments (*p* = 0.981), time (*p* = 0.131), and the bivalve treatment × time interaction (*p* = 0.326) did not have significant affects on the concentration of dead particles.

Discussion:

This experiment demonstrated the important processes bivalves perform in aquatic environments. During this experiment, both net filtration and net excretion occurred. In the initial 3 hrs of the experiment, both species caused a decrease in the amount of material within the experimental units due to net filtration. While during the latter portion of the experiment, where was an increase in the concentration of materials caused by net excretion by the 2 species.

Through filtration and excretion processes, bivalves transform materials into new forms through their production of feces and psuedofeces. This release of particulate organic matter by mussels into the stream has been shown to create food for other organisms (Nichols & Garling, 2000; Raikow & Hamilton, 2001; Howard & Cuffey, 2006). This is an important ecological service that bivalves provide to benthic invertebrates and vertebrates. The two species differ greatly in their size; however, the smaller invasive species, *C. fluminea*, appeared able to filter materials just as quickly as *E. crassidens*. When corrected for biomass, *C. fluminea* were much more efficient filter feeders. However, *E. crassidens* released a greater mass of materials through net excretion, which provides more food and nutrients to the benthic environment. In a natural stream environment, both species likely release larger materials (>10μm) that have relatively high organic content into the benthic environment, while retaining smaller materials. McLachlan *et al.* (1978) and Mattingly *et al.* (1981) showed positive relationships between the growth rate of chironomid larvae and organic matter availability, indicating that feeding and excretion by bivalves can lead to higher benthic productivity. Bivalve filtration is an important ecological service that results in greater stream benthic productivity (Vaughn *et al*., 2008, and references therein).

The two approaches combined allowed us to look closely at the mass of materials cycled and how these species feed and process specific components of the water column. Both species appeared to filter materials from mainly the smaller size classes as shown by the DM and AFDM values. In general, the concentration of the two smaller size classes stayed low over the latter portions of the experiment, while there was an increase in the largest size class. Both species utilized the smaller size classes, and then produced larger particles through their excretory processes. In the initial stages of the experiment both the native (*E. crassidens*) and the invasive

(*C. fluminea*) species showed preferences for living materials. Neither species significantly changed the concentration of inorganic materials in the containers. However, both species selected for organic materials as reflected by the decrease in the mass of organic materials in the first 3 hrs, and the mass of inorganic materials remaining fairly constant over the experiments. FC allowed us to demonstrate there were no differences in detritus abundance in the treatments and over time, while showing there were differences due to the bivalve filtration in many of the living components. This indicates these species select for living and organic components of suspended matter.

E. crassidens utilized the smaller 2 size classes, and especially selected for the medium size class as indicated by our gravimetric results. *E. crassidens* did not change the concentration of detritus and showed preference for many of the living materials in suspension, which have greater nutritional content than other particles (Ward & Cummins, 1979; Golladay *et al*., 1983). Brillant and MacDonald (2003) also found preferences for living materials by a filter-feeding sea scallop (*Placopecten magellanicus*). *E. crassidens* appeared to select heavily for nanoeukaryotes. In the *E. crassidens* treatments we noted an increase in the concentration of bacterial components with a concurrent decease in the concentration of nanoeukaryotes in the water column at the end of the treatment times. We hypothesize the feeding of nanoeukaryotes and production of larger materials by *E. crassidens* was stimulating microbial growth. Bacteria are the main diet of flagellates (Fenchel, 1982; Bratvold *et al*., 2000). *E. crassidens* may have been reducing flagellate numbers leading to a release of bacteria from their predators. *E. crassidens* is facilitating the production of bacteria by the reduction of predatory nanoflagellates and release of nutrient and labile organic matter (Christian *et al*., 2008), and the production of large particles due to excretion processes building structure for microbial colonization. There were less bacteria in the *E. crassidens* treatments at t =3 and t = 6 hrs than the control and *C. fluminea* treatments indicating *E. crassidens* may utilize some bacteria. The *E. crassidens* treatments also had less picoautotrophs at t = 1 hour than the control. These results indicate that *E. crassidens*, a dominant unionid species in the Ichawaynochaway Creek, is feeding on a mixture of nanoeukaryotes, some picoautotrophs, and bacteria. *E. crassidens* is likely getting a large proportion of energy from bacteria as hypothesized by many researchers (Nichols & Garling, 2000; Christian *et al*., 2004), but it appears as though most of the energy is through bacteriovores (i.e., nanoeukaryotes).

Corbicula fluminea utilized materials in the small size class as indicated by our gravimetric results. Our results also indicated that *C. fluminea* changed the abundance of living materials at the beginning of the experiment. However, there was not strong evidence that *C. fluminea* selects one portion of the living community over another. *C. fluminea* appeared to change the availability of bacteria at the beginning of the experiment, but these interactions were not strong. The *C. fluminea* treatments also had less picoautotrophs at t = 1 hr than the control. The concentration of all types of particles appeared to decrease (though not significantly) within the first hour of the experiment and then rebound. The filtering rate of *C. fluminea* has been shown to be greater than other unionid mussels (Leff *et al*., 1990; Silverman *et al*., 1997), and may lead to problems in comparing the particles retained by *E. crassidens*. They may be turning the water over quickly and stimulating more production through their excretory activities, making it difficult to observe lost particles at the rate we sampled. However, these results indicate that *C. fluminea* utilizes picoautotrophs, and is consistent with our gravimetric results and other results that this species utilizes very small materials (10 µm to ≤ 1 µm) (Way *et al*., 1990; Silverman *et al*., 1995).

Both native and invasive bivalve species co-occur within habitats in Ichawaynochaway Creek, and *C. fluminea* has become a dominant filter feeder. Both species appear to select for organic and living materials as indicated by our two methods. This has implications for the effect of this invasive species on aquatic food webs and native unionid species. Potentially, competition for food resources could be occurring due to their overlapping filtration activities. In addition, production of psuedofeces and feces occur and these species, and are adding to the pool of larger particulate matter in the benthic environment (especially *E. crassidens*) as they are taking up very small materials out of suspension from the water column. Native mussels excrete a great amount of larger materials that seem to be stimulating production of microbes in the latter portions of the experiment. For example, the stimulation of bacteria can increase the flow of bacterial carbon to higher trophic levels through the microbial loop (Meyer, 1994). However, *C. fluminea* also has the ability to pedal feed from the sediments (Reid *et al.*, 1992; Hakenkamp & Palmer, 1999), leading to the possibility that this species is also utilizing materials it has released into the benthic environment. We need to know more about the affects of invasive species in our environments to understand if they function similarly in our environment as the native species.

The use of both gravimetric and FC methods proved useful to determine changes in particle distribution and abundance to resolve how bivalves feed on natural particulate matter. We were able to determine preference for living materials as well as potential control of other trophic levels by bivalves and potential increases in production of bacteria due to bivalve excretory activities and bacterial release from predators. The gravimetric technique allowed us to ascertain how bivalve species use different size classes of particulate matter, and then in turn how they transform it through their excretory activities. The transfer of nutrients and energy resulting from bivalves' filtering activity, and then re-packaging of materials through their excretory activities has been shown to stimulate production across trophic levels (Howard & Cuffey, 2006; Spooner & Vaughn, 2006; Vaughn *et al*., 2007). In another laboratory study, FC was useful for elucidating feeding preferences of zebra mussels (Dionisio Pires *et al*., 2004). The gravimetric approach in combination with FC, we were able to resolve how *E. crassidens* alter the availability of materials in the water column and affect multiple trophic levels through top-down control. Many FC studies have been from lentic and marine systems. We have found that it was also a useful tool for studying how freshwater bivalves influence the concentration and distribution of particles from water in lotic systems. To our knowledge, this is the first study to look at selective filtration by bivalve species on natural stream particulate matter. FC has been a common approach to study grazing in marine systems (Cucci *et al*., 1985; Shumway *et al*., 1985; Reckermann & Veldhuis, 1997; Ward, 1997; Bratvold *et al*., 2000), and in the future, FC will continue to be a useful technique to study filtration by other freshwater bivalve species.

Acknowledgements:

We thank T.K. Muenz, M.S. Wiggers and J. McEntire for field assistance. We thank Josh Warren for help in the analytical laboratory. We thank Julie Nelson for her assistance in the flow cytometry laboratory. We thank Dr. Mike Conner for statistical advice. Dr. Amy Rosemond provided helpful comments during the editing process. Liz Cox helped locate key references. We would like to thank the R.W. Woodruff Foundation, J.W. Jones Ecological Research Center, and the University of Georgia Graduate School for funding and support.

Literature Cited:

Allen W.R. (1914) The food and feeding habits of freshwater mussels. *Biological Bulletin*, **27**, 127-147.

Bogan A.E. (2008) Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. *Hydrobiologia*, **595**, 139-147.

Bratvold D., Srienc F., & Raub S.R. (2000) Analysis of the distribution of ingested bacteria in nanoflagellates and estimation of grazing rates with flow cytometry. *Aquatic Microbial Ecology*, **21**, 1-12.

Brillant M.G.S. & MacDonald B.A. (2003) Postingestive sorting of living and heat-killed *Chlorella* within the sea scallop, *Placopecten magellanicus* (Gmelin). *Journal of Experimental Marine Biology and Ecology*, **290**, 81-91.

Brönmark C. & Malmqvist B. (1982) Resource partitioning between unionid mussels in a Swedish lake outlet. *Holarctic Ecology*, **5**, 389-395.

Caraco N.F., Cole J.J., Raymond P.A., Strayer D.L., Pace M.L., Findlay S.E.G. & Fischer D.T. (1997) Zebra mussel invasion in a large, turbid river: Phytoplankton reponse to increased grazing. *Ecology*, **78**, 588-602.

Christian A.D., Smith B.N., Berg D.J., Smoot J.C., & Findlay R.H.. (2004) Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca: Unionidae) in 2 small Ohio streams. *Journal of the North American Benthological Society*, **23**, 101-113.

Churchill E.P. & Lewis S.I. (1924) Food and feeding in fresh-water mussels. *Bulletin of the Bureau of Fisheries*, **39**, 439-471.

Conover W.J. & Iman R.L. (1981) Rank transformation as a bridge between parametric and nonparametric statistics. *The American Statistician*, **35**, 124-129.

Crosbie N.D., Teubner K., & Weisse T. (2003). Flow-cytometric mapping provides novel insights into the seasonal and vertical distributions of freshwater autotrophic picoplankton. *Aquatic Microbial Ecology*, **33**, 53-66.

Cucci T.L., Shumway S.E., Newell R.C., Selvin R., Guillard R.R.L., & Yentsch C.M. (1985) Flow cytometry: a new method for characterization of differential ingestion, digestion and egestion by suspension feeders. *Marine Ecology Progress Series*, **24**, 201-204.

del Giorgio P.A., Bird D.F., Prairie Y.T., & Planas D. (1996) Flow cytometric determination of bacterial abundance in lake plankton with the green nucleic acid stain SYTO 13. *Limnology and Oceanography*, **41**, 783-789.

Dionisio Pires L.M., Jonker R.R., Van Dork E., & Laanbroek H.J. (2004) Selective grazing by adults and larvae of the zebra mussel (*Dreissena polymorpha*): application of flow cytometry to natural seston. *Freshwater Biology*, **49**, 116-126.

Dudgeon D., Arthington A.H., Gessner M.O., Kawabata Z.-I., Knowler D.J., Lévêque C., Naiman R.J., Prieur-Richard A.-H., Soto D., Stiassny M.L.J., & Sullivan, C.A. (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Review*, **81**, 163-182.

Fenchel T. (1982) Ecology of heterotrophic microflagellates. 4. Quantitative occurrence and importance as bacterial consumers. *Marine Ecology Progress Series*, **8**, 211–223.

Golladay S.W., Webster J.R., & Benfield E.F. (1983) Factors affecting food utilization by a leaf shredding aquatic insect: leaf species and conditioning time. *Holartic Ecology*, **6**, 157-162.

Hakenkamp C.C. & Palmer M.A. (1999) Introduced bivalves in freshwater ecosystems: the impact of *Corbicula* on organic matter dynamics in a sandy stream. *Oecologia*, **119**, 445-451.

Horgan M.J. & Mills E.L. (1997) Clearance rates and filtering activity of zebra mussel *Dreissena polymorpha*: implications for freshwater lakes. *Canadian Journal of Fisheries and Aquatic Science*, **54**, 249-255.

Howard, J.K. & Cuffey K.M. (2006) The functional role of native freshwater mussels in the fluvial benthic environment. *Freshwater Biology*, **51**, 460-474.

Idrisi N., Mills E.L., Rudstam L.G., & Stewart D.J. (2001) Impact of zebra mussels (*Dreissena polymorpha*) on the pelagic trophic levels of Oneida Lake, New York. *Canadian Journal of Fisheries and Aquatic Science*, **58**, 1430-1441.

Leff L.G., Burch J.L., & McArthur J.V. (1990) Spatial distribution, seston removal, and potential competitive interactions of the bivalves *Corbicula fluminea* and *Ellipto complanata*, in a coastal plain stream. *Freshwater Biology*, **24**, 409-416.

Legendre L., Courties C., & Troussellier M. (2001) Flow cytometry in oceanography 1989-1999: environmental challenges and research trends. *Cytometry*, **44**, 164-172.

Li W.K.W. (1997) Cytometric diversity in marine ultraphytoplankton. *Limnology and Oceanography*, **42**, 874-880.

Littell R.C., Stroup W.W., & Freund R.J. (2002) *SAS for linear models, 4th ed.* SAS Institute, Cary, NC.

McLachlan A. J., Brennan A., & Wotton R. S. (1978) Particle size and chironomid (Diptera) food in an upland river. *Oikos*, **31**, 247–252.

McMahon, R.F. & Bogan A.E. (2001) Mollusca: Bivalvia. *Ecology and Classification of North American Freshwater Invertebrates, 2nd ed.* (Eds: J.H. Thorp and A.P. Covich), pp 331-416. Academic Press, San Diego.

Marie D., Partensky F., Jacquet S., & Vaulot D. (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Applied and Environmental Microbiology*, **63**, 186-193.

Mattingly R. L., Cummins K. W., & King R. H. (1981) The influence of substrate organic content on the growth of stream chironomids. *Hydrobiologia*, **77**, 161–165.

Moreira-Torcq P. & Martin J.M. (1998) Characterization of fine particles by flow cytometry in estuarine and coastal Arctic waters. *Journal of Sea Research*, **39**, 217-226.

Naddafi R., Pettersson K., & Eklöv P. (2007). The effect of seasonal variation in selective feeding by zebra mussels (*Dreissena polymorpha*) on phytoplankton community composition. *Freshwater Biology*, **52**, 823-842.

Nichols S.J., & Garling D. (2000) Food-web dynamics and trophic-level interactions in a multi-species community of freshwater unionids. *Canadian Journal of Zoology*, **78**, 871-882.

Otto F. & Tsou K.C. (1985) A comparative study of DAPI, DIPI, and Hoechst 33258 and 33342 as chromosomal DNA stains. *Stain Technology*, **60**, 7.

Parmalee P.W. & Bogan A.E. (1998) *The freshwater mussels of Tennessee.* University of Tennessee, Knoxville, TN.

Partensky F., Hess W.R., & Vaulot D. (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiology and Molecular Biology Reviews*, **63**, 106-127.

Paterson C.G. (1986) Particle-size selectivity in the freshwater bivalve *Elliptio complanata* (Lightfoot). *The Veliger*, **29**, 235-237.

Raikow D.F. & Hamilton S.K. (2001) Bivalve diets in a Midwestern U.S. stream: a stable isotope enrichment study. *Limnology and Oceanography*, **46**, 514-522.

Reckermann M. & Veldhuis M.J.W. (1997) Trophic interactions between picophytoplankton and micro- and nanozooplankton in the western Arabian Sea during the NE monsoon 1993. *Aquatic Microbial Ecology,* **12**, 263-273.

Reid R.G.B., McMahon R.F., Foighil D.O. & Finnigan R. (1992) Anterior inhalant currents and pedal-feeding in bivalves. *Veliger*, **35**, 93-104.

Sarmento H., Unrein F., Isumbisho M., Stenuite S., Gasol J.M, & Descy J. (2008) Abundance and distribution of picoplankton in tropical oligotrophic Lake Kivu, eastern Africa. *Freshwater Biology*, **53**, 756-771.

Shumway S.E., Cucci T.L., Newell R.C., & Yentsch C.M. (1985) Particle selection, ingestion, and absorption in filter-feeding bivalves. *Journal of Experimental Marine Biology and Ecology*, **91**, 77-92

Silverman H.S., Archberger E.E., Lynn J.W., & Dietz T.H. (1995) Filtration and utilization of laboratory-cultured bacteria by *Dreissena polymorpha, Corbicula fluminea* and *Carunculina texasensis*. *Biological Bulletin*, **189**, 308-319.

Silverman H.S., Nichols S.J., Cherry J.S., Achberger E., Lynn J.W., & Dietz T.H. (1997) Clearance of laboratory-cultured bacteria by freshwater bivalves: difference between lentic and lotic unionids. *Canadian Journal of Zoology*, **75**, 1857-1866.

Spooner D.E. & Vaughn C.C. (2006) Context-dependent effects of freshwater mussels on the benthic community. *Freshwater Biology*, **51**, 1016-1024.

Turgeon D.D., Quinn J.F., Bogan A.E., Coan E.V., Hochberg F.G., Lyons W.G., Mikkelsen P.M., Neves R.J., Roper C.F.E., Rosenberg G., Roth B., Scheltema A., Thompson F.G., Vecchione M., & Williams J.D. (1998) *Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks. 2nd edition*. Special publication 26. American Fisheries Society, Bethesda, Maryland.

Vanderploeg H.A., Liebig J.R., & Nalepa T.F. (1995) From picoplankton to microplankton: Temperature-driven filtration by the unionid bivalve *Lampsilis radiata siliquoidea* in Lake St. Clair. *Canadian Journal of Fisheries and Aquatic Sciences,* **52**, 63-74.

Vaughn C.C., Nichols S.J., & Spooner D.E. (2008) Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society*, **27**, 409-423.

Vaughn C.C., Spooner D.E., & Galbraith H.S. (2007) Context-dependent species identity effects within a functional group of filter-feeding bivalves. *Ecology*, **88**, 1664-1662.

Vaughn C.C. & Spooner D.E. (2006) Scale-dependent associations between native freshwater mussels and invasive *Corbicula*. *Hydrobiologia*, **568**, 331-339.

Veldhuis M.J.W. & Timmermans K.R. (2007) Phytoplankton dynamics during an in situ iron enrichment experiment (EisenEx) in the Southern Ocean: a comparative study of field and bottle incubation measurements. *Aquatic Microbial Ecology*, **47**, 191-208.

Ward G. M., & Cummins K. W. (1979) Effects of food quality on growth of a stream detritivore, *Paratendipes albimanus* (Meigen) (Diptera: Chironomidae). *Ecology*, **60**, 57–64.

Ward J.E., Levinton J.S., Shumway S.E., & Cucci T. (1997) Site of particle selection in a bivalve mollusc. *Nature*, **390**, 131-132.

Way C.M., Hornbach D.J., Miller-Way C.A., Payne B.S., & Miller A.C. (1990) Dynamics of filter feeding in *Corbicula fluminea* (Bivalvia: Corbiculidae). *Canadian Journal of Zoology*, **68**, 115-120.

Weitere M., Dahlmann J., Viergutz C., & Arndt H. (2008) Differential grazer-mediated effects of high summer temperatures on pico- and nanoplankton communities. *Limnology and Oceanography*, **53**, 477-486.

Table 3.1: The F-values, degrees of freedom, and P-values for all three size classes of the DM samples. The results from the ANCOVA and ANOVAs are shown. Bolded values denote significant differences at α = 0.05 (Tukey's HSD, Littell et al. 2002).

Comparison	**0.3-1.5μm**		**1.5-10μm**		**10-45μm**	
Statistic	***F***	***P***	***F***	***P***	***F***	***P***
Between Bivalves	$0.81_{2,37}$	0.452	$0.55_{2,37}$	0.579	$1.58_{2,38}$	0.22
t = 0	$0.06_{2,6}$	0.945	$0.51_{2,6}$	0.623	$1.57_{2,6}$	0.283
t = 1	$2.42_{2,6}$	0.169	$2.46_{2,6}$	0.166	$1.09_{2,6}$	0.396
t = 3	$1.26_{2,6}$	0.348	$0.02_{2,6}$	0.976	$0.80_{2,6}$	0.494
t = 6	$3.24_{2,6}$	0.111	$16.51_{2,5}$	**0.006**	$2.63_{2,6}$	0.152
t = 12	$3.38_{2,4}$	0.138	$1.05_{2,5}$	0.415	$7.55_{2,5}$	**0.031**
Between Times	$11.04_{1,37}$	**0.002**	$6.55_{1,37}$	**0.015**	$29.08_{1,38}$	**<0.0001**
Control	$9.10_{4,9}$	**0.003**	$4.67_{4,9}$	**0.026**	$0.12_{4,10}$	0.974
C. fluminea	$3.69_{4,10}$	**0.043**	$10.31_{4,10}$	**0.001**	$15.44_{4,10}$	**0.0003**
E. crassidens	$6.16_{4,9}$	**0.011**	$4.34_{4,9}$	**0.032**	$6.32_{4,9}$	**0.011**
Bivalve × Time	$0.21_{2,37}$	0.812	$1.37_{2,37}$	0.267	$5.92_{2,38}$	**0.006**

Table 3.2: The F-values, degrees of freedom, and P-values for all three size classes of the AFDM samples. The results from the ANCOVA and ANOVAs are shown. Bolded values denote significant differences at $\alpha = 0.05$ (Tukey's HSD, Littell et al. 2002). NA indicates data are not applicable.

Comparison	**0.3-1.5µm**		**1.5-10µm**		**10-45µm**	
Statistic	***F***	***P***	***F***	***P***	***F***	***P***
Between Bivalves	$1.01_{2,37}$	0.374	$1.10_{2,37}$	0.342	$1.73_{2,38}$	0.192
t = 0	$0.86_{2,6}$	0.469	na	na	$0.53_{2,6}$	0.616
t = 1	$0.42_{2,6}$	0.673	na	na	$0.39_{2,6}$	0.691
t = 3	$3.85_{2,6}$	0.083	na	na	$0.73_{2,6}$	0.519
t = 6	$0.72_{2,6}$	0.524	na	na	$0.46_{2,6}$	0.652
t = 12	$3.35_{2,4}$	0.14	na	na	$9.15_{2,5}$	**0.021**
Between Times	$9.43_{1,37}$	**0.004**	$1.55_{1,37}$	0.22	$8.64_{1,38}$	**0.006**
Control	$0.60_{4,9}$	0.674	na	na	$0.22_{4,10}$	0.924
C. fluminea	$1.04_{4,10}$	0.435	na	na	$3.42_{4,10}$	0.052
E. crassidens	$11.76_{4,9}$	**0.001**	na	na	$3.45_{4,9}$	0.057
Bivalve × Time	$1.51_{2,37}$	0.234	$0.28_{2,37}$	0.754	$3.03_{2,38}$	0.06

Table 3.3: The F-values, degrees of freedom, and P-values for all the parameters measured from the FC samples. The results from the ANCOVA and ANOVAs are shown. Bolded values denote significant differences at α = 0.05 (Tukey's HSD, Littell et al. 2002). NA indicates data that are not applicable.

Comparison	**Detritus**		**Heterotrophic Bacteria**		**Picoautotrophs**		**Nanoeukaryotes**		**Nanoautotrophs**	
Statistics	F	P	F	P	F	P	F	P	F	P
Between Bivalve	$0.35_{2,37}$	0.707	$2.54_{2,37}$	0.093	$0.22_{2,37}$	0.805	$0.92_{2,37}$	0.408	$0.79_{2,37}$	0.46
t = 0	na	na	$0.20_{2,6}$	0.828	$0.07_{2,6}$	0.935	$0.61_{2,6}$	0.575	na	na
t = 1	na	na	$1.02_{2,6}$	0.417	$9.06_{2,6}$	**0.015**	$1.63_{2,6}$	0.273	na	na
t = 3	na	na	$4.71_{2,5}$	0.071	$0.22_{2,5}$	0.221	$0.05_{2,5}$	0.956	na	na
t = 6	na	na	$29.16_{2,6}$	**0.0008**	$3.49_{2,6}$	0.099	$1.42_{2,6}$	0.313	na	na
t = 12	na	na	$0.74_{2,5}$	0.523	$1.51_{2,5}$	0.307	$1.22_{2,5}$	0.369	na	na
Between Time	$1.56_{1,37}$	0.219	$16.28_{1,37}$	**0.0003**	$5.37_{1,37}$	**0.026**	$4.12_{1,37}$	**< 0.05**	$1.53_{1,37}$	0.224
Control	na	na	$2.86_{4,10}$	0.081	$3.40_{4,10}$	0.053	$1.12_{4,10}$	0.402	na	na
C. fluminea	na	na	$6.85_{4,10}$	**0.006**	$4.41_{4,10}$	**0.026**	$0.69_{4,10}$	0.614	na	na
E. crassidens	na	na	$20.39_{4,8}$	**0.003**	$9.81_{4,8}$	**0.004**	$5.26_{4,8}$	**0.022**	na	na
Bivalve × Time	$0.21_{2,37}$	0.815	$0.54_{2,37}$	0.59	$1.06_{2,37}$	0.355	$3.12_{2,37}$	0.056	$1.16_{2,37}$	0.325

Figure 3.1: (A) FALS Signal of 1, 3 and 6 µm beads. (B) Histogram showing the entire planktonic population and gates. The objects in the FALS gate with the lowest signal were excluded from the analysis. (C) Gating based upon CHL and DAPI fluorescence of FALS Gates 1 – 2. Gates include detritus (low CHL and DAPI fluorescence), bacteria and picoautotrophs. (D) Gating based upon CHL and DAPI fluorescence of FALS Gates 3 – 4. Gates include detritus, heterotrophic nanoflagellates (HNF), pico- and nanoautotrophs.

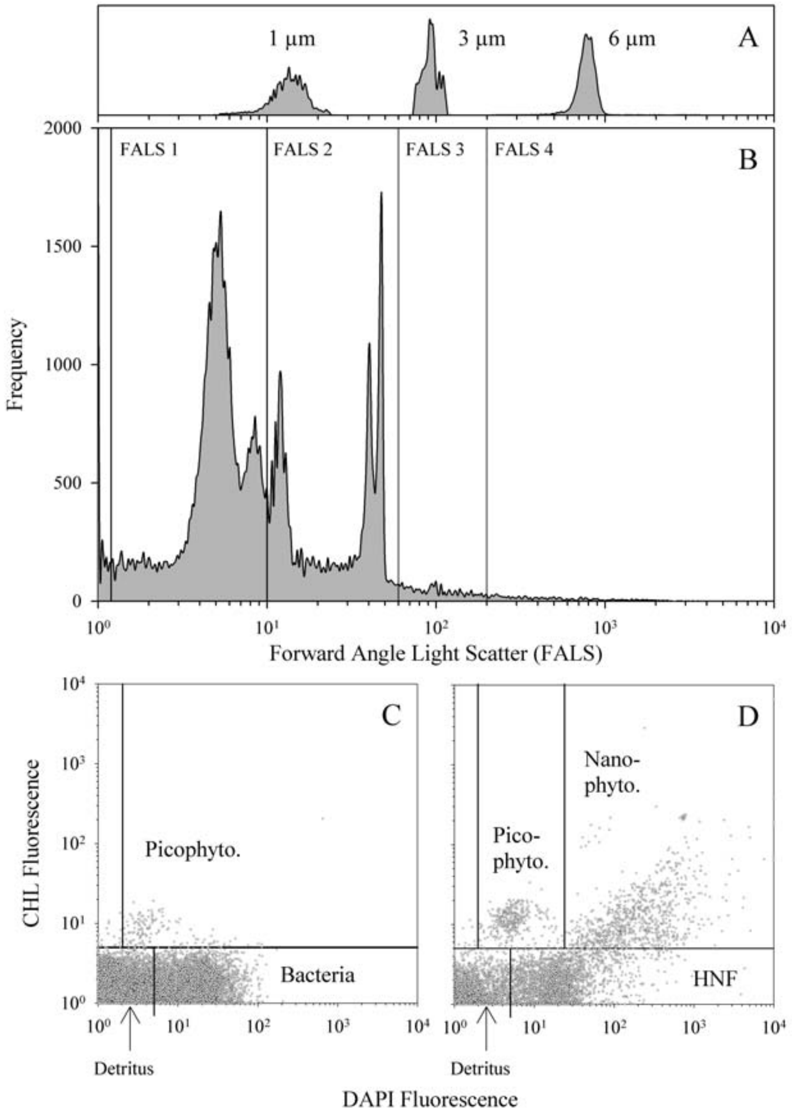
Figure 3.2: The mean DM remaining (± SE) over time in the (A) 10-45µm, (B) 1.5-10µm, and (C) 0.3-1.5µm treatments.

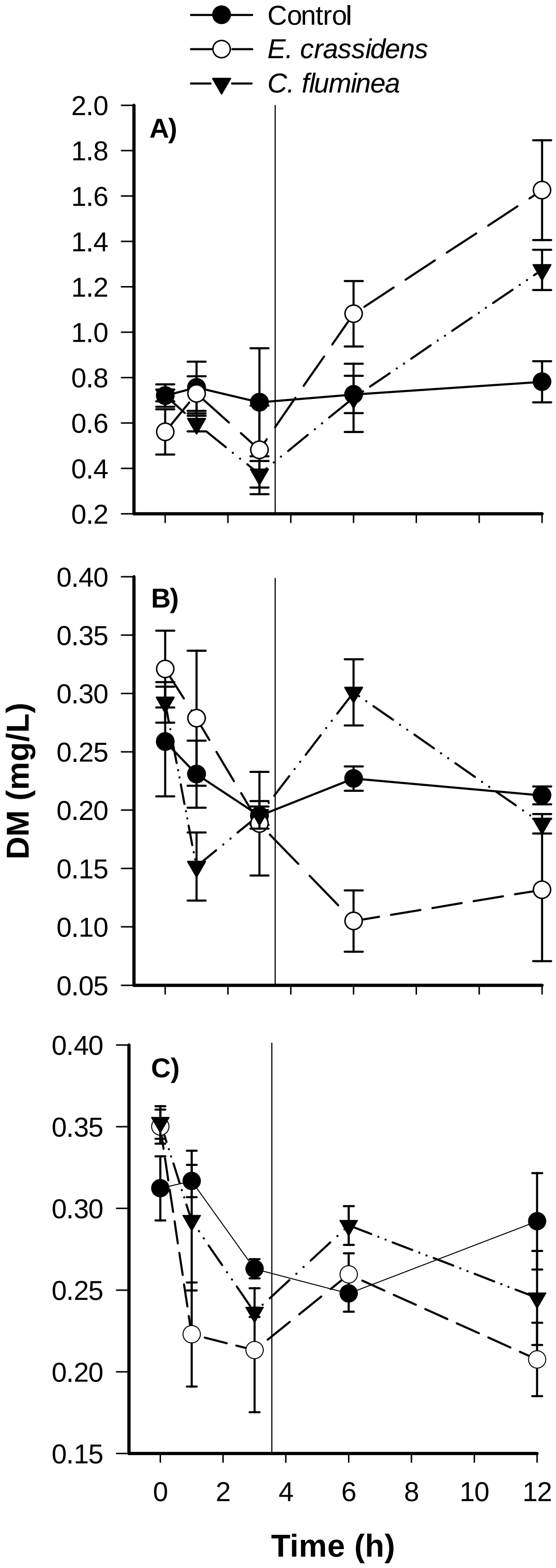
Figure 3.3: The mean AFDM remaining (± SE) over time in the (A) 10-45µm, (B) 1.5-10µm, and (C) 0.3-1.5µm treatments.

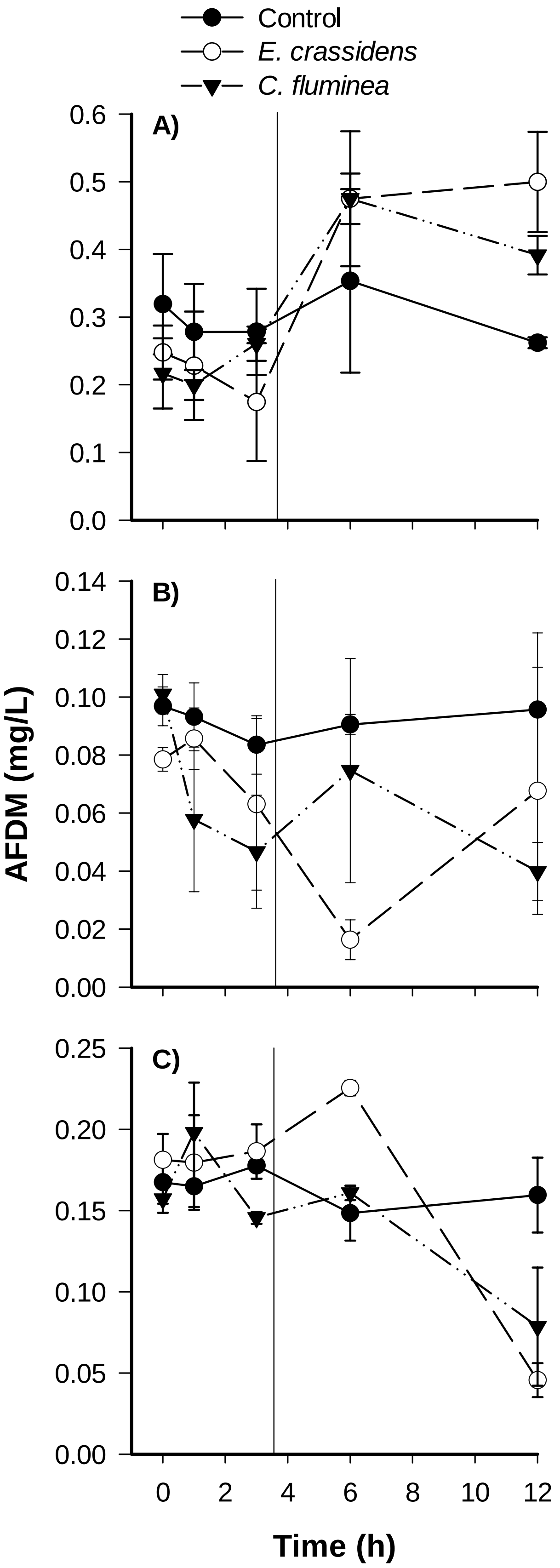
Figure 3.4: The mean abundances of organic and inorganic materials when all size classes are summed.

Figure 3.5: The mean abundances detritus, heterotrophic bacteria, picoautotrophs, nanoautotrophs, and nanoeukaryotes (± SE) found in the 3 treatments over time using FC.

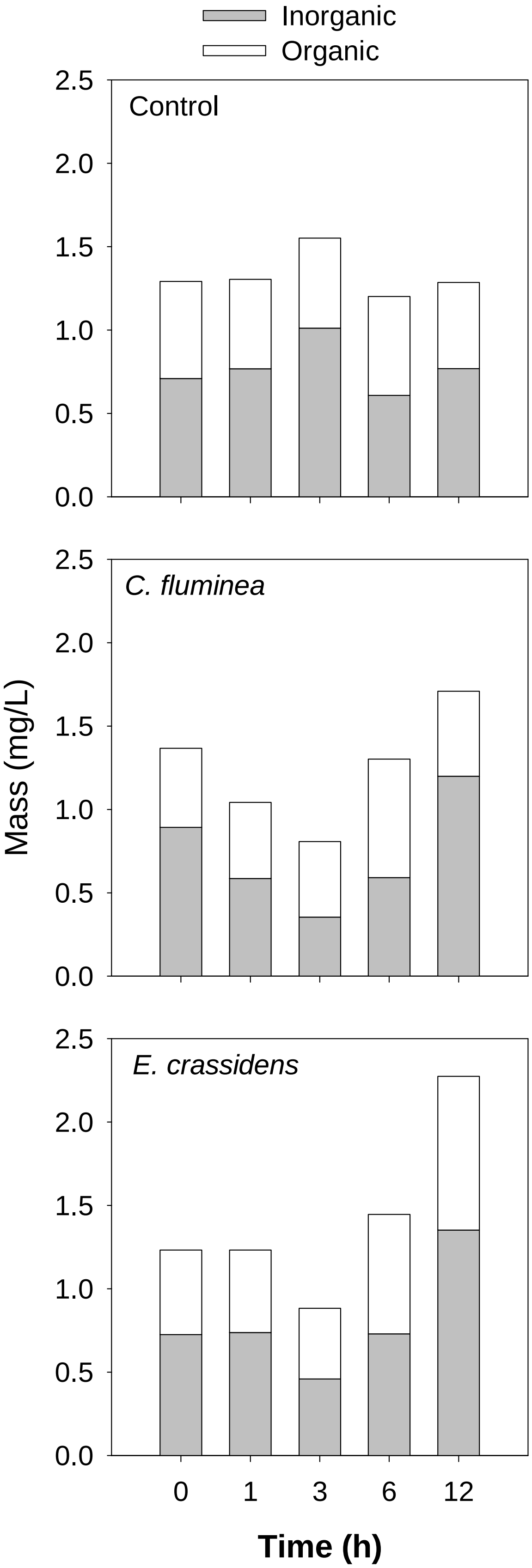
Figure 3.6: The concentration (particles/mL) of living and dead particles in the 3 treatments over time using FC.

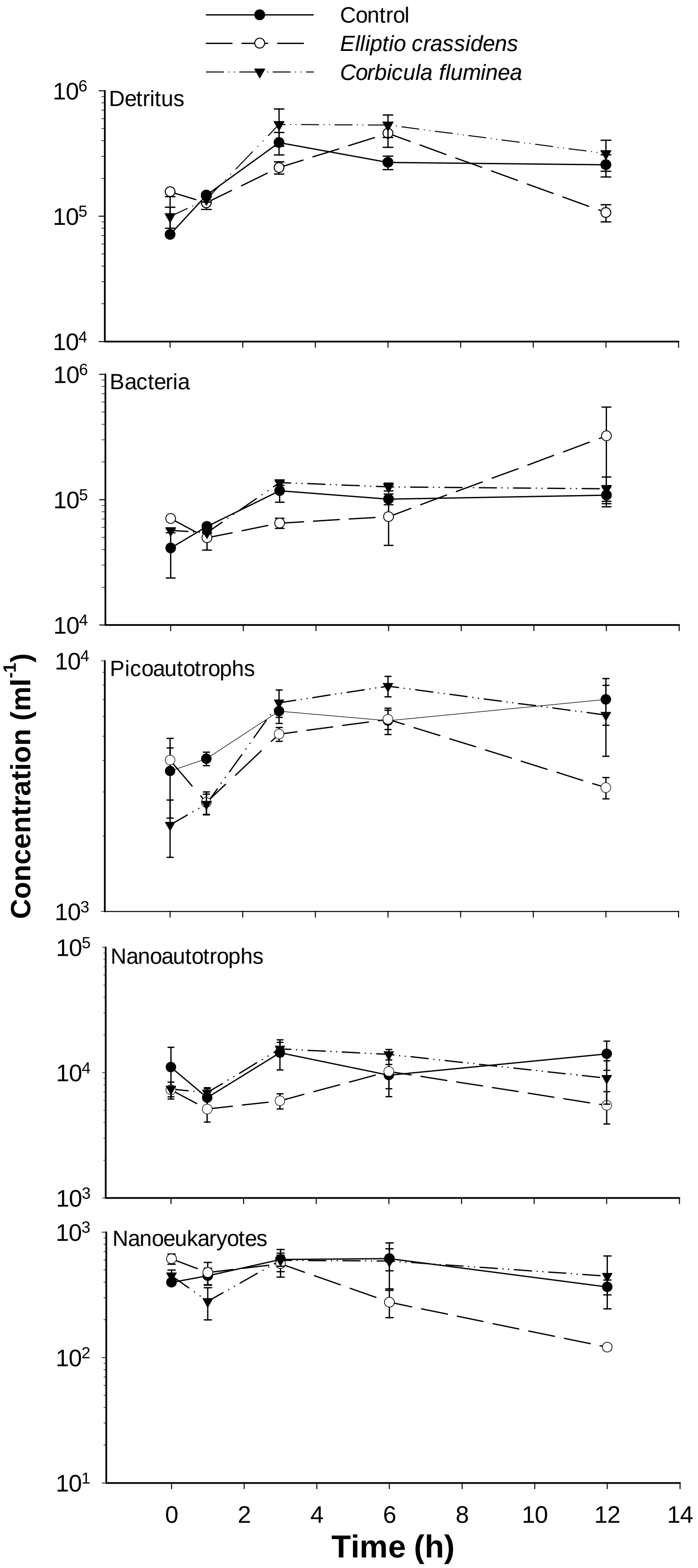

1 μm
3 μm
6 μm
A
2000
1500
1000
500
0
Frequency
FALS 1
FALS 2
FALS 3
FALS 4
B
10^0
10^1
10^2
10^3
10^4
Forward Angle Light Scatter (FALS)
CHL Fluorescence
10^4
10^3
10^2
10^1
10^0
C
Picophyto.
Bacteria
D
Nano-
phyto.
Pico-
phyto.
HNF
Detritus
Detritus
DAPI Fluorescence

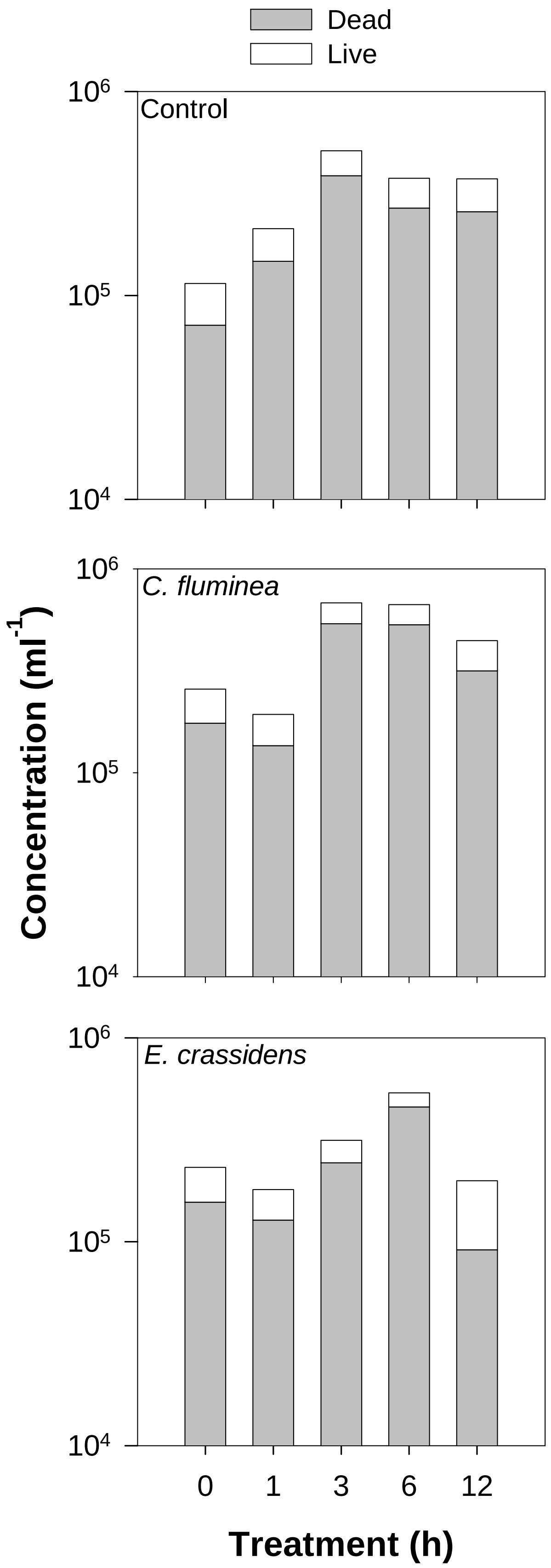

Control
E. crassidens
C. fluminea
A)
B)
C)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.40
0.35
0.30
0.25
0.20
0.15
DM (mg/L)
0
2
4
6
8
10
12
Time (h)



Control
E. crassidens
C. fluminea
A)
B)
C)
AFDM (mg/L)
Time (h)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.00
0.05
0.10
0.15
0.20
0.25
0
2
4
6
8
10
12


Inorganic
Organic
Control
C. fluminea
E. crassidens
Mass (mg/L)
0.0
0.5
1.0
1.5
2.0
2.5
0
1
3
6
12
Time (h)


Control
Elliptio crassidens
Corbicula fluminea
Detritus
Bacteria
Picoautotrophs
Nanoautotrophs
Nanoeukaryotes
10^6
10^5
10^4
10^3
10^2
10^1
Concentration (ml^{-1})
0
2
4
6
8
10
12
14
Time (h)


Dead
Live
Control
C. fluminea
E. crassidens
10^6
10^5
10^4
Concentration (ml^{-1})
0
1
3
6
12
Treatment (h)

CHAPTER 4

STABLE ISOTOPIC SIGNATURES, TISSUE STOICHIOMETRY, AND NUTRIENT CYCLING (C AND N) OF A NATIVE AND AN INVASIVE BIVALVE

Abstract:

Filter feeding mussels historically comprised the majority of benthic biomass in many streams and are important for stream ecosystem functioning by linking the water column and benthic zones in aquatic systems. The invasive bivalve, *Corbicula fluminea*, has the potential to profoundly alter organic matter dynamics and nutrient cycling in streams. However, little is known about differences in the feeding ecology and nutrient cycling of *C. fluminea* relative to native unionid mussels. In this study, we compared how a native freshwater mussel, *Elliptio crassidens,* and *C. fluminea* feed and cycle nutrients within a Coastal Plain stream. We hypothesized that *C. fluminea*, given their invasive properties, would assimilate a larger range of materials than the native species. We measured how these species differed in their use of suspended organic particulates when they were both feeding in the same location and when feeding in different locations of the drainage network. To determine dietary composition and potential overlap of *C. fluminea* and the native mussel *E. crassidens*, we measured the elemental and stable isotopic compositions (δ^{13}C and δ^{15}N) of their tissue, as well as 3 size classes of seston at different streamflows in Ichawaynochaway Creek, a tributary to the lower Flint River, Georgia, U.S.A. In the laboratory, we measured the C and N in biodeposits excreted by the two species. Our results indicate differences in the food resources assimilated and the nutrients deposited as feces and psuedofeces by these two bivalve species. *C. fluminea* has lower trophic fidelity, by being able to acquire and assimilate a multitude of resources as indicated by the large range of δ^{13}C values. Additionally, as indicated by *C. fluminea*'s more enriched δ^{15}N signatures,

they may be better able to acquire bacterial sources than *E. crassidens*. The presence of *C. fluminea* may also alter nutrient cycling in the benthic environment of coastal plain streams by retaining less nitrogen than *E. crassidens* as indicated by higher C:N of their tissue. Laboratory results indicated that *C. fluminea* releases more N back into the water through their excretion processes relative to *E. crassidens* by mass, implying that *C. fluminea* may modify nutrient cycling in streams. Furthermore, these results have implications for how invasive species, such as *C. fluminea*, may alter aquatic environments through differences in species traits within a functional group.

Introduction:

Freshwater mussels play an important role through filtration by transferring materials and nutrients from the water column to the surrounding benthic area, stimulating primary and secondary production (Howard and Cuffney 2006, Spooner and Vaughn 2006, Vaughn et al. 2007). Prior studies have primarily examined how abiotic factors affect biotic assemblages in Coastal Plain streams (Benke et al. 2000, Benke 2001, Martin and Paller 2008), but few have addressed how organisms are utilizing resources and, in turn, may be altering the availability of resources for other organisms. Historically, freshwater mussels were the most dominant invertebrate, in terms of biomass, in many eastern North American streams (Parmalee and Bogan 1998). Land-use changes, hydrologic alterations, invasive species, and declines in native fish species have caused declines in native mussel abundance and diversity in North America. The rates of ecological processes performed by mussels typically scale linearly with biomass (Strayer et al 1999, Vaughn et al. 2004). Understanding the role freshwater mussels play in terms of food web structure and nutrient cycling is essential for knowing how streams may be altered if mussel species continue to decline.

In general, particles in unionid guts resemble the suspended seston found in the habitats where they occur (Coker et al. 1921, Nichols and Garling 2000). Adult mussels can filter and assimilate a wide range of suspended fine particulate organic matter (FPOM) ranging in size from 1 μm up to at least 40 μm (Brönmark and Malmqvist 1982, Paterson 1986, Vanderploeg et al. 1995). These particulates can include algae, bacteria, zooplankton, and detrital matter (Nichols and Garling 2000). However, little is known about what is actually assimilated into bivalve tissue. Christian et al. (2004) hypothesized that microbes in FPOM are a large portion of diet in mussels after observing the $\delta^{13}C$ value of two unionid species was much lighter than the signature of the FPOM. The consumption of bacteria reduces the number of trophic transfers from microbes to higher trophic levels (such as algae and other plant or animal breakdown components), leading to a more efficient transfer of energy (Meyer 1994). Through the transfer of nutrients and particulates by filtration activities, bivalves have the potential to greatly affect nutrient cycling and food web dynamics.

Native and invasive species may utilize the same materials, but could process the materials differently. Small functional differences can lead to larger ecosystem-scale effects. If an introduced species can profoundly alter ecosystem-level processes such as primary or secondary productivity or nutrient cycling, then the properties of individual species can potentially control the functioning of whole ecosystems (Vitousek 1990, Taylor et al. 2006, Gonzalez et al. 2008). *Corbicula fluminea*, the invasive Asian clam, is widespread and has become “naturalized” in many river basins across the United States. *C. fluminea* are similar to native unionid mussels in that they are primarily filter-feeding consumers, which depend heavily upon microbes, algae, and detrital material available in the water column and benthic environment. *C. fluminea* can feed on particles in the water column of a particular size class (10 μm to ≤ 1 μm), that are typically smaller than what most native mussel species can utilize (McMahon and Bogan 2001). It has also been shown that *C. fluminea* can filter bacteria at a much higher rate than unionids (Silverman et al. 1995). Vaughn and Spooner (2006) found that *C. fluminea* abundance was negatively correlated to native mussel abundance at small spatial scales. As unionid numbers have decreased, *C. fluminea* has become a dominant filtering bivalve in many ecosystems. This change could lead to changes in the structure and function within the stream ecosystem despite similar functional roles of native unionid mussels and *C. fluminea*.

Very few studies have addressed the potential trophic role or "trophic niche space" that bivalve species occupy. Unionid mussels and *C. fluminea* are considered to be in the same functional group (filter-feeders). However, no study to date has examined the trophic and nutrient cycling roles these species play. In this study, we use a two-step statistical method to compare the trophic roles of species. We determined how similar two species were in their use of suspended organic particulates when feeding in the same location and when feeding in different locations of the drainage network. The measure of trophic fidelity has been proposed as a way to describe and compare species' trophic roles. Trophic fidelity describes the ability of an organism or group of organisms to adhere to its feeding habits through space and time. Our goal was to understand the trophic niche space filled by two species within the same functional group utilize and to quantify and compare trophic fidelity. To accomplish this goal, we determined how these two species change in isotopic signature (both the central tendency and dispersion of points) in relation to species identity and the position in the watershed or study site.

In this study, we examined the materials that a native unionid mussel, *Elliptio crassidens*, assimilated in comparison to an invasive bivalve species, *C. fluminea*, using stable isotopic analysis. We also determined the tissue stoichiometry (C and N) of these species and compared it to the nutrients excreted as biodeposited materials in their feces and pseudofeces. Many studies of bivalve diets have used laboratory studies to determine what materials these and related organisms utilize. However, these studies were not able to actually discern what materials these species were assimilating, or how these species alter these materials and transform them into usable forms for other consumers. Also, few studies have determined the tissue stoichiometry of these species and compared it to what these species are actually releasing back into the environment (but see Christian et al. 2008). We hypothesized that *C. fluminea*, given their invasive properties, would have lower trophic fidelity, by being able to utilize and assimilate a larger range of food materials than the native species, which is more specialized. Additionally, we hypothesized *C. fluminea* would cycle nutrients differently. This study allowed us to understand trophic and nutrient cycling roles these 2 species perform.

Methods:

Study sites:

Ichawaynochaway Creek (IC) is a 5th order, low-gradient tributary to the lower Flint River on the Gulf Coastal Plain of southwest Georgia (Golladay et al. 2000). Six sample sites were established in the IC drainage basin including two sites on a tributary, Chickasawhatchee Creek (Fig. 4.1, CC). Much of the IC basin is situated in the Dougherty Plain physiogeographic district where the mantled karst physiography controls the hydrology (Hyatt and Jacobs, 1996). The bedrock is primarily Ocala limestone, an extensively fractured and porous rock layer that has high hydraulic transmissivity, and is the principal water-bearing stratum for the Upper Floridian Aquifer (Hicks et al. 1981). Riparian areas are composed of maturing second growth hardwood forest (Golladay et al. 2000). About 10 km downstream from the confluence of CC and IC, the stream discharges into the Flint River (Fig. 4.1). Sample sites were chosen by the availability of access to the stream. *C. fluminea* occurs throughout the entire basin except for the headwaters. *E. crassidens* (native) appears to be associated with the Dougherty Plain region of this watershed, and is found primarily south of the boundary that divides the Fall Line Hills and the Dougherty Plain (Fig. 1, Brim-Box and Williams 2000).

Field Collection and Sample Analysis:

Seston samples were collected at the sample locations throughout the IC drainage between June 2007 and February 2008. Samples were collected seasonally during a variety of flow regimes. Material was fractionated into a series of 3 size categories using different sized filters to retain materials: small (0.3-1.5µm), medium (1.5-10µm), and large (10-45µm). Five to ten individuals of each species were collected at all sample locations between June 2007 and August 2007. Length (mm) and wet mass (g) including shell was recorded. Tissue samples were collected from these individuals and dried (45° C) and ground for each individual.

Total carbon and total nitrogen composition as well as the carbon and nitrogen stable isotopic signatures were determined for seston and mussel tissue samples using a Finnigan Delta Plus mass spectrometer in the University of Georgia's Ecology Analytical Laboratory. Isotope ratios were expressed in the delta (δ) format: $\delta^{13}C$ or $\delta^{15}N$ (units of ‰) = (R_{sample} - $R_{standard}$ /$R_{standard}$) x 1000, where R is the $^{13}C{:}^{12}C$ ratio or $^{15}N{:}^{14}N$ ratio. A bovine protein (peptone) lab standard referenced against an international standard (Fry 2006), and precision averaged 0.1‰ or less.

Laboratory:

Excretion experiments were used to determine the amount and composition of materials excreted by bivalves. Bivalves, *C. fluminea* (*n* = 12) and *E. crassidens* (*n* = 12), were collected in July 2008 at site 5. Immediately after collection, bivalves were taken to the laboratory in a bucket containing stream water. The bivalves were scrubbed and rinsed to remove any attached particles and then placed in individual containers containing deionized water and held at 25° C (typical summer stream temperature) in an incubator. The experiment was set up as a completely randomized block design. The main effect was bivalve treatment and with time as a covariate. There were 4 time treatments (0, 1, 2, and 3 hours) and 2 bivalve treatments (*E. crassidens* and *C. fluminea*), with each treatment being replicated 4 times (*n* = 32). The water at the end of the treatments was filtered through an ashed, pre-weighed GFF (nominal pore size 0.3μm, Sterlitech Corp., Kent, WA). The sample was dried and weighed to the nearest 0.0001 mg. The total amount of carbon and nitrogen on the filter was then analyzed on a Finnigan Delta Plus mass spectrometer. Mussels were then returned to the stream.

Data analyses:

Multiple response permutation procedures (MRPP) were used to compare the distribution of the two bivalves' stable isotopic signatures (Biondini et al. 1985, Zimmerman et al. 1985). MRPP allows one to compare bivariate data sets. To determine if *E. crassidens* and *C. fluminea* were significantly different from each other, MRPP was used to determine if the isotopic signatures (δ^{13}C and δ^{15}N) of the two bivalve species bivariate plots were distributed similarly. We tested whether the 2 species differed in their central tendency and dispersion of isotopic signatures and also determined whether the difference between the species was the same at all site locations. The MRPP was analyzed in the FORTRAN program FIDELITY (L.M. Conner, Joseph W. Jones Ecological Research Center, Newton, GA). We also calculated dispersion (i.e. mean distance between bivariate medians; Van Valen 1978) using FORTRAN in the program FIDELITY (L.M. Conner). This analysis allowed us to determine the dispersion of isotopic signatures of each species and a quantitative way to determine how large of a trophic niche space each species occupied. A small dispersion around this central tendency of all isotopic points of a species indicated a species that had high trophic fidelity across all the sites. A small trophic niche across all sites is also used as an indication of high trophic fidelity. We also determined the distribution and dispersion of the bivariate isotopic points for each individual site, which was another measure of trophic fidelity. The smaller the distance was for an individual species between the given sites, the greater the trophic fidelity. This approach allowed us to determine the distribution of both species at each site and the dispersion of these points at each site. Larger differences in dispersion indicated greater within site dispersion suggesting that the differences between the two species at that location were relatively large. Bootstrapping (sampling with replacement, 1000 bootstraps) was used to sample for the median values of each individual species' isotopic signatures at each sample sites to determine the distance between the bivariate medians (in ‰). Bootstrapped lower and upper confidence intervals of this distance were also determined. The confidence intervals of the two species were compared for each site combination. Non-overlapping confidence intervals indicated significance of the differences between species across sampling sites. In addition, larger values indicated greater distances between the medians of the given sample sites. A smaller value was indicative of greater central tendency which was characteristic of greater trophic fidelity. To illustrate the trophic niche space that these two species occupy, we plotted the isotopic signatures in ArcMap v9.1 (ESRI Inc. Redland, CA). We then used Hawth's Analysis Tools (Beyer 2004) to create minimum convex polygons around points representing one of the two species, and then around points representing individual sample sites as Layman et al. 2007.

To determine if C:N of the tissue of the two species varied, two-way analyses of variance were performed with species and site as the two main effects. Significant ANOVAs were followed by Tukey's HSD multiple comparisons to determine if significant differences existed between bivalve species and site ($\alpha = 0.05$; Littell et al. 2002). These analyses were followed by multiple comparison tests for one factor at each level using one-way ANOVAs to determine if there were differences within a species due to the sample site. Paired t-tests also followed these analyses to determine if there were significant differences between the species at each of the sites. For the laboratory studies, analyses of variance were performed with bivalve treatment as a main effect and time as a covariate to determine if there were significant differences in the mass of material and the %C, and %N excreted. Significant ANCOVAs were followed by Tukey's HSD multiple comparisons to determine if significant differences existed between bivalve treatment ($\alpha = 0.05$; Littell et al. 2002). These analyses were followed by multiple comparison tests for one factor at each level using one-way ANOVAs to determine if there were differences within time due to species or differences within a species due to the time. The composition of materials biodeposited was converted into a biodeposition rate (μmol C or N g^{-1} h^{-1}) for *E. crassidens* and *C. fluminea*. The biodeposition rates of C and N by *E. crassidens* and *C. fluminea* were examined using t-tests. All parametric statistical analyses were performed using SAS v9.1 (SAS Institute, Cary, NC).

Results:

Stable isotopes:

The stable isotopic data indicated that smaller seston size classes had a higher δ^{15}N, and in general had a more depleted δ^{13}C (Fig. 4.2). The small (mean values: $\delta^{13}C = -25.90 \pm 0.79$, $\delta^{15}N = 8.04 \pm 0.74$) and medium (mean values: $\delta^{13}C = -26.15 \pm 0.70$, $\delta^{15}N = 7.40 \pm 0.26$) size classes were more similar than the large (mean values: $\delta^{13}C = -22.66 \pm 0.96$, $\delta^{15}N = 6.75 \pm 0.25$) size class. The mean values of the *C. fluminea* stable isotope data (mean values: $\delta^{13}C = -28.98 \pm 0.28$, $\delta^{15}N = 9.69 \pm 0.08$) indicated that this species has a more enriched δ^{15}N and more depleted δ^{13}C signature than the native mussel species, *E. crassidens* (mean values: $\delta^{13}C = -28.23 \pm 0.11$, $\delta^{15}N = 8.38 \pm 0.03$).

Our trophic niche space analyses indicated that the stable isotopic signatures were significantly different between the two species (MRPP, $p < 0.00001$; See Fig. 4.3 for illustration). The Van Valen test also indicated that the two species differed significantly in the dispersion of their isotopic data ($t_{70} = 4.99$; $p < 0.00001$). The distance between group medians was 1.06 (95% bootstrap CL = -0.39 – 1.12). Dispersion was 1.72 (95% CI = 1.38 – 2.06) for all *C. fluminea* points and 0.79 (95% CI = 0.63 – 0.95) for all *E. crassidens* points. The difference in dispersion was 0.93 (95% CI = 0.56 – 1.31), with *C. fluminea* having greater dispersion between points. Because Van Valen's test was significant and distance between group bivariate medians was large, the significant MRPP statistic is reflective of both a change in dispersion of isotopic points and differences in central tendency between the two species, implying there were differences in feeding and trophic fidelity.

Differences in stable isotopic signatures of bivalves were also noted due to site location when species were grouped together. Using bootstrapping techniques we found significant differences between the distances of the medians between sampling sites when comparing the two species (Table 4.1; Fig. 4.4 for illustration). The distance between the bivariate medians of the sites within a species was significantly different for many site comparisons. *C. fluminea* had a significantly greater dispersion of isotopic points between sites than *E. crassidens.* In addition, the confidence interval values for both species were greater for sites located farther apart in geographic distance. This indicates isotopic signatures varied due to location. However, the values for *E. crassidens* were more clustered than the values of *C. fluminea* (Fig. 4.3) as indicated by the Van Valen test, indicating greater trophic fidelity across sites.

Nutrients:

The two species retained carbon and nitrogen significantly differently from the food sources they were assimilating as reflected by the differences in the two species in tissue C:N ($p < 0.0001$; Fig. 4.5). C:N values were significantly different due to the location of the site ($p < 0.04$) and the interaction between species and among sites ($p < 0.009$). In all cases, the C:N values of *C. fluminea* were always higher than the C:N values of *E. crassidens.* Differences in tissue C:N ratios between *E. crassidens* and *C. fluminea* were significant at sites 1 ($p < 0.0001$), 4 ($p < 0.001$), and 5 ($p = 0.005$). Significant differences were not noted between the two species at sites 2 ($p = 0.077$), 3 ($p = 0.734$), and 6 ($p = 0.235$). The C:N ratio of *C. fluminea* differed among the sites ($p < 0.05$), however Tukey's HSD was not able to elucidate those differences. The C:N ratio of *E. crassidens* differed significantly amongst the sites ($p < 0.0001$). The individuals at site 1 (upstream of Chickasawhatchee) were significantly lower in C:N than individuals at sites 2 and 3 (which are both on Chickasawhatchee Creek). *E. crassidens* individuals were significantly lower in C:N at site 4 (upstream of Chickasawhatchee) than individuals at site 2. *E. crassidens* individuals were significantly lower in C:N at site 5 than all sites except site 1.

These differences in C:N ratios of the tissue were consistent with the C:N ratios of the excretory products. Over time, *E. crassidens* excreted a greater mass of material than *C. fluminea* on an individual basis (Fig. 4.6). However, when this value was corrected for bivalve tissue wet mass, *C. fluminea* excrete more over time, although this difference was not found to be significantly different at $\alpha = 0.05$ ($p = 0.07$; Fig. 4.6). The %C and %N released in the biodeposits were significantly affected by bivalve species. *C. fluminea* released biodeposits significantly higher %C ($p = 0.004$; Fig. 4.7). Significant differences where found in the %C due to species ($p < 0.006$), but not due to time ($p < 0.09$) and the interaction between species and time ($p = 0.07$). Further multiple comparison revealed that the 2 species were different in the %C in biodeposits at $t = 2$ h ($p < 0.04$) and different at $\alpha = 0.1$ level at $t = 1$ h ($p = 0.056$), with *C. fluminea* excreting materials with higher %C. The %N the two species released was significantly different in their biodeposits ($p = 0.004$; Fig. 4.7). There were significant differences in %N in the biodeposits due to species ($p < 0.008$), time ($p < 0.05$), but the interaction between species and site ($p = 0.07$) was not significant at $\alpha = 0.05$. Furthermore, multiple comparisons revealed that the *C. fluminea* released more %N in their biodeposits at $t = 2$ h ($p < 0.04$) and at the $\alpha = 0.1$ level at $t = 1$ h ($p = 0.067$). In the materials that were excreted, there was significantly more C per mass released in biodeposits by *C. fluminea* than by *E. crassidens* ($p = 0.028$; Fig. 4.8). On average, *C. fluminea* released 1116 ± 473 μmol C g^{-1} DM h^{-1} more than *E. crassidens*. *C. fluminea* also released materials that were significantly greater in N than *E. crassidens* ($p = 0.033$). On average, *C. fluminea* released 171 ± 75 μmol N g^{-1} DM h^{-1} more in the form of biodeposits than *E. crassidens*.

Discussion:

Different food resources, as indicated by the differences in central tendency of the isotope data, were being assimilated by the two species. In the comparison of data from all locations, the dispersion of isotopic points was different between the two species as shown by the Van Valen's test. *C. fluminea* was utilizing a more diverse pool of resources shown by the larger dispersion of isotopic points, indicating that this species has lower trophic fidelity than *E. crassidens*. *E. crassidens* individuals, across all sites, exhibited lower dispersion of isotopic points, indicating that this species had higher trophic fidelity than *C. fluminea.* Differences in the materials utilized by a species varied among sites because sources vary across the watershed spatially. Isotopic signatures of basal resources can vary due to location, stream flow, and season (Finlay et al. 1999, McNeely et al. 2006). However, the isotopic signatures for consumers were less variable for the native species than *C. fluminea.* When sites were compared, the bivariate distance between sites for a species was always smaller for the native species, *E. crassidens*. These results demonstrate they have greater trophic fidelity because at all sites *E. crassidens* had less variability in isotopic signatures than the invasive bivalve. *C. fluminea's* lower trophic fidelity favors them as successful invaders because they can utilize a wider spectrum of food resources.

Due to the heterogeneity of the composition of materials in suspension at any given time it is difficult to discern exact sources of food to bivalve species. The signature of the small seston yielded δ^{13}C signatures that were more indicative of the signatures of bivalves in this system than did larger materials. However, the pool of materials in seston is large and contains an assortment of phytoplankton, bacteria, and nanoflagellates. Laboratory studies indicate that bivalves preferentially ingest living materials such as algae and bacteria (Brillant and MacDonald 2003). Both of these species seemed to assimilate smaller materials as indicated by their depleted δ^{13}C signature and enriched δ^{15}N signature, especially *C. fluminea*. A few studies have indicated that higher δ^{15}N signatures may be indicative of microbially enriched materials (Macko and Estep 1984; Angradi 1994; Goedkoop et al. 2006). Additionally, other researchers have hypothesized that bivalves selectively assimilate bacteria over the FPOM resource that they are attached (Nichols and Garling 2000; Christian et al. 2004). Bacteria are a more efficient energy source and their use results in direct transfers of energy within the stream food web (Meyer 1994). Our results also suggested that *C. fluminea* was more efficient at assimilating microbial sources as indicated by their more enriched δ^{15}N signature. We suspect both these species feed on multiple trophic levels, with *C. fluminea* feeding more heavily on higher trophic levels and microbial sources based on their more enriched δ^{15}N signature.

C. fluminea excreted materials that had higher concentrations of N than the native species. This is consistent with their tissue stoichiometry; *C. fluminea* had a higher C:N ratios, which indicates they retained less nitrogen in relation to the amount of carbon they retained. The native species, *E. crassidens,* released relatively less N into the water through their excretory activities, which is predicted by their tissue stoichiometry. The native species had a lower C:N ratio than *C. fluminea*. The higher C:N ratio in *C. fluminea* and the higher amounts of nitrogen in their excretory products has implications for stream nutrient cycling. Freshwater mussels were historically the dominant invertebrates in the terms of biomass in many southeastern streams. Our results suggest, the invasion of *C. fluminea* will likely change the concentration of available nutrients. Differences in bivalve tissue C:N also implications for stream food webs. A lower C:N value has been implicated in having higher nutritional quality due to the reduction of elemental imbalances (Frost et al. 2002, Gutseit et al. 2007). Impacts on food webs are also likely. For example, Barbour map turtles (*Graptemys barbouri*) consume bivalves as a primary diet component during part of their lives (Lindeman 2000, Lindeman 2006). A diet switch from native mussels to *C. fluminea* may have implications for growth of these species.

Invasive species can have other effects on the ecosystems, especially when they perform similar roles as native species. In this ecosystem, *C. fluminea* filter feed and assimilated food materials differently than the native species studied. In turn, *C. fluminea* also retained and deposited nutrients in a different way than the native species. The native species tissue composition had a lower C:N value than *C. fluminea*, indicating that *E. crassidens* may be better able to retain nitrogen than *C. fluminea*. Unionid mussels are typically long lived, while other freshwater bivalves (such as clams of the family Corbiculidae) are much shorter-lived (up to 3-4 years maximum age) (Vaughn and Hakenkamp 2001). The long lifespan of *E. crassidens* suggests that the native species may be storing these nutrients longer in biomass. As indicated by the %N in the biodeposits of these two species, *C. fluminea* excretes materials that contain more N than *E. crassidens*. *E. crassidens* was the most dominant native species throughout most of this watershed, however *C. fluminea* now outnumber this native species. *C. fluminea*'s invasion is relatively recent and may be having large affects on food web dynamics and nutrient cycling in the stream as this invasive has become the dominant filter feeder.

Few researchers have documented the functional affects of *C. fluminea* on North American waterways. We expect that while *C. fluminea* ingests similar materials as native species, it assimilates different materials. Additionally, the biodepostion of filtered materials into the sediments may potentially cause competition due to the reduction of materials in the water column. Also, *C. fluminea* has lower trophic fidelity implying they fill a larger trophic niche space and can change their feeding habits more readily given the availability of materials. In addition, *C. fluminea* may potentially alter nutrient cycling within these streams due to their feeding and excretion activities. An exotic species that alters ecosystem properties does not merely compete with native species; it also alters the fundamental rules of existence for all organisms in the area (Vitoseuk 1990). While having a similar trophic strategy (i.e., filter feeding) compared to native mussels, differential functional abilities suggest that *C fluminea* has the ability to alter nutrient cycling dynamics and the availability of materials in the water column. *C. fluminea* does not feed similarly, it is not as selective, or cycle nutrients the same as the dominant native species. More attention needs to be given to the functional roles invasive species play in aquatic ecosystems to fully understand the implications of their introduction.

Acknowledgements:

We thank T.K. Muenz, S. Allums, B. Cloninger, S. Sterrett, L. Sargent, M.S. Wiggers and J. McEntire for field assistance. We thank Dr. Mike Conner for his statistical genius. We thank Tom Maddox for his assistance in the stable isotope laboratory and Josh Warren for help in the analytical laboratory. Liz Cox helped locate key references. We appreciate the support of the R.W. Woodruff Foundation, J.W. Jones Ecological Research Center, and the University of Georgia Graduate School.

References:

Angradi, T.R. 1994. Trophic linkages in the lower Colorado River: multiple stable isotope evidence. Journal of the North American Benthological Society 13:479-495.

Benke, A.C. 2001. Importance of flood regime to invertebrate habitat in an unregulated river-floodplain ecosystem. Journal of the North American Benthological Society 20:225-240.

Benke, A.C., I. Chaubey, G.M. Ward, and E.L. Dunn. 2000. Flood pulse dynamics of an unregulated river floodplain in the southeastern U.S. coastal plain. Ecology 81:2730-2741.

Beyer, H. L. 2004. Hawth's Analysis Tools for ArcGIS. Available at http://www. spatialecology.com/htools.

Biondini, M.E., C.D. Bonham, and E.F. Redente. 1985. Secondary successional patterns in a sagebrush (*Artemisia tridentata*) community as they relate to soil disturbance and soil biological activity. Vegetatio 60:25-36.

Brillant M.G.S. & MacDonald B.A. 2003. Postingestive sorting of living and heat-killed *Chlorella* within the sea scallop, *Placopecten magellanicus* (Gmelin). Journal of Experimental Marine Biology and Ecology 290:81-91.

Brim-Box, J. and J.D. Williams. 2000. Unionid mollusks of the Apalachicola basin in Alabama, Florida, and Georgia. Alabama Museum of Natural History Bulletin 21. Tuscaloosa, Alabama.

Brönmark, C. and B. Malmqvist. 1982. Resource partitioning between unionid mussels in a Swedish lake outlet. Holarctic Ecology 5:389-395.

Christian, A.D., B.G. Crump, and D.J. Berg. 2008. Nutrient release and ecological stoichiometry of freshwater mussels (Mollusca: Unionidae) in 2 small, regionally distinct streams. Journal of the North American Benthological Society 27:440-450.

Christian, A.D., B.N. Smith, D.J. Berg, J.C. Smoot, and R.H. Findlay. 2004. Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca: Unionidae) in 2 small Ohio streams. Journal of the North American Benthological Society 23:101-113.

Coker, R.E., A.F. Shira, H.W. Clark, and A.D. Howard. 1921. Natural history and propagation of freshwater-mussels. Bulletin of the Bureau of Fisheries 37:77-181.

Colón-Gaud, C., S. Peterson, M.R. Whiles, S.S. Kilham, K.R. Lips, and C.M. Pringle. 2008. Allochthonous litter inputs, organic matter standing stocks, and organic seston dynamics in upland Panamanian streams: potential effects of larval amphibians on organic matter dynamics. Hydrobiologia 603:301-312.

Finlay, J.C., M.E. Power, and G. Cabana. 1999. Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. Limnology and Oceanography 44: 1198-1203.

Frost, P.C., R.S. Stelzer, G.A. Lamberti, and J.J. Elser. 2002. Ecological stoichiometry of trophic interactions in the benthos: Understanding the role of C:N:P ratios in lentic and lotic habitats. Journal of the North American Benthological Society 21:515-528.

Fry, B. 2006. Stable isotope ecology. Springer, New York, NY.

Goedkoop, W, N. Akerblom, and M.H. Demandt. 2006. Trophic fractionation of carbon and nitrogen stable isotopes in *Chironomus riparius* reared on food of aquatic and terrestrial origin. Freshwater Biology 51:878-886.

Golladay, S.W., K. Watt, S. Entrekin, and J. Battle. 2000. Hydrologic and geomorphic controls on suspended particulate organic matter concentration and transport in Ichawaynochaway Creek, Georgia, USA. Archiv für Hydrobiologia 149:655-678.

Gonzalez, A., A. Lambert, and A. Ricciardi. 2008. When does ecosystem engineering cause invasion and species replacement? Oikos 117:1247-1257.

Gutseit, K., O. Berglund, and W. Graneli. 2007. Food quality for *Daphnia* in humic and clear water lakes. Freshwater Biology 52:344-356.

Hicks, D.W., R.E. Krause, and J.S. Clarke. 1981. Geohydrology of the Albany Area, Georgia. U.S. Geological Survey, Atlanta.

Howard, J.K. and K.M. Cuffey. 2006. The functional role of native freshwater mussels in the fluvial benthic environment. Freshwater Biology 51:460-474.

Hyatt, J.A. and P.M. Jacobs. 1996. Distribution and morphology of sinkholes triggered by flooding following Tropical Storm Alberto at Albany, Georgia, U.S.A. Geomorphology 17:305-316.

Johnson, P.M. 2001. Habitat Associations and drought responses of mussels in the lower Flint River basin, southwest Georgia. MS Thesis, University of Georgia, Athens, GA.

Layman, C.A., D.A. Arrington, C.G. Montańa, and D.M. Post. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88:42-48.

Leff, L.G., J.L. Burch, and J.V. McArthur. 1990. Spatial distribution, seston removal, and potential competitive interactions of the bivalves *Corbicula fluminea* and *Ellipto complanata*, in a coastal plain stream. Freshwater Biology 24:409-416.

Lindeman, P. V. 2006. Diet of the Texas map turtle (Graptemys versa): Relationship to sexually dimorphic trophic morphology and changes over five decades as influenced by an invasive mollusk. Chelonian Conservation and Biology 5:25-31.

Lindeman, P. V. 2000. Resource use of five sympatric turtle species: effects of competition, phylogeny, and morphology. Canadian Journal of Zoology-Revue Canadienne De Zoologie 78:992-1008.

Littell, R.C. W.W. Stroup, and R.J. Freund. 2002. SAS for linear models, 4th ed. SAS Institute, Cary, NC.

Macko, S.A. and M.L.F. Estep. 1984. Microbial alteration of stable nitrogen and carbon isotopic compositions. Organic Geochemistry 6:787-790.

Martin, F. D., and M. H. Paller. 2008. Ichthyoplankton transport in relation to floodplain width and inundation and tributary creek discharge in the lower Savannah River of Georgia and South Carolina. Hydrobiologia **598**:139-148.

McMahon, R.F. and A.E. Bogan. 2001. Mollusca: Bivalvia: 331-416. In: J.H. Thorp and A.P. Covich. Ecology and Classification of North American Freshwater Invertebrates, 2nd Edition. Academic Press, San Diego.

McNeely, C., S.M. Clinton, J.M. Erbe. 2006. Landscape variation in C sources of scraping consumers in streams. Journal of the North American Benthological Society 25:787-799.

Meyer, J.L. 1994. The microbial loop in flowing waters. Microbial Ecology 28:195-199.

Nichols, S.J., and D. Garling. 2000. Food-web dynamics and trophic-level interactions in a multi-species community of freshwater unionids. Canadian Journal of Zoology 78:871-882.

Parmalee, P.W. and A.E. Bogan. 1998. The freshwater mussels of Tennessee. University of Tennessee, Knoxville, TN.

Paterson, C.G. 1986. Particle-size selectivity in the freshwater bivalve *Elliptio complanata* (Lightfoot). The Veliger 29:235-237.

Silverman, H.S., E.E. Archberger, J.W. Lynn, and T.H. Dietz. 1995. Filtration and utilization of laboratory-cultured bacteria by *Dreissena polymorpha, Corbicula fluminea* and *Carunculina texasensis*. Biological Bulletin 189:308-319.

Spooner, D.E. and C.C. Vaughn. 2006. Context-dependent effects of freshwater mussels on the benthic community. Freshwater Biology 51:1016-1024.

Taylor, B.W., A.S. Flecker, and R.O. Hall Jr. 2006. Loss of a harvested fish species disrupts carbon flow in a diverse tropical river. Science 313:833-836.

Vanderploeg, H.A., J.R. Liebig, and T.F. Nalepa. 1995. From picoplankton to microplankton: Temperature-driven filtration by the unionid bivalve *Lampsilis radiata siliquoidea* in Lake St. Clair. Canadian Journal of Fisheries and Aquatic Sciences 52:63-74.

Van Valen, L. 1978. The statistics of variation. Evolutionary Theory 4:33–43.

Vaughn, C.C. and C.C. Hakenkamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46:1431-1446.

Vaughn, C.C. and D.E. Spooner. 2006. Scale-dependent associations between native freshwater mussels and invasive *Corbicula*. Hydrobiologia 568:331-339.

Vaughn, C.C., D.E. Spooner, and H.S. Galbraith. 2007. Context-dependent species identity effects within a functional group of filter-feeding bivalves. Ecology 88:1664-1662.

Vitousek, P.M. 1990. Biological invasions and ecosystem processes – towards an integration of population biology and ecosystem studies. Oikos 57:7-13.

Zimmerman, G.M., H. Goetz, and P.W. Mielke. 1985. Use of an improved statistical method for group comparisons to study the effects of prairie fire. Ecology 66:606-611.

Table 4.1: The lower and upper confidence intervals found using 1000 bootstraps comparing the median distances of the isotopic signatures between sites for the two species. Bolded values indicate non-overlapping confidence intervals, which indicates significant differences in the difference of the bivariate medians of the site comparison between the two species. Larger values for the confidence intervals indicate the sites were more different in the stable isotopic signature for the individual species.

Site Comparisons	***C. fluminea***		***E. crassidens***	
	Lower CI	Upper CI	Lower CI	Upper CI
1 & 2	1.195	1.524	0.931	1.89
1 & 3	**1.725**	**2.742**	**0.623**	**0.848**
1 & 4	**4.035**	**4.596**	**1.116**	**1.448**
1 & 5	**3.508**	**3.750**	**1.377**	**1.66**
1 & 6	**3.399**	**3.667**	**1.096**	**1.413**
2 & 3	0.846	1.272	0.567	1.567
2 & 4	3.661	4.455	1.989	3.827
2 & 5	3.227	3.981	2.178	3.334
2 & 6	2.995	3.619	2.225	3.058
3 & 4	**2.251**	**3.118**	**1.307**	**1.793**
3 & 5	1.631	2.522	1.471	2.288
3 & 6	1.558	2.323	1.019	1.695
4 & 5	**0.278**	**0.885**	**-0.102**	**0.256**
4 & 6	**0.656**	**1.434**	**-2.079**	**0.138**
5 & 6	-0.243	9.166	-0.037	0.273

Figure 4.1: Map depicting the Ichawaynochaway Creek watershed in the lower Flint River Basin locations of sampling sites, and the physiogeographic provinces found within the basin. Sites 1, 4, 5, and 6 were on Ichawaynochaway Creek and sites 2 and 3 were on Chickasawhatchee Creek.

Figure 4.2: Stable isotope results depicting the mean values of three size classes of seston that were collected during all seasons and flow conditions and mean bivalve tissue signatures with S.E. bars around the means.

Figure 4.3: Isotopic signatures at all locations of *C. fluminea* (*n* = 42) and *E. crassidens* (*n* = 42) with a minimum convex hull drawn around each species.

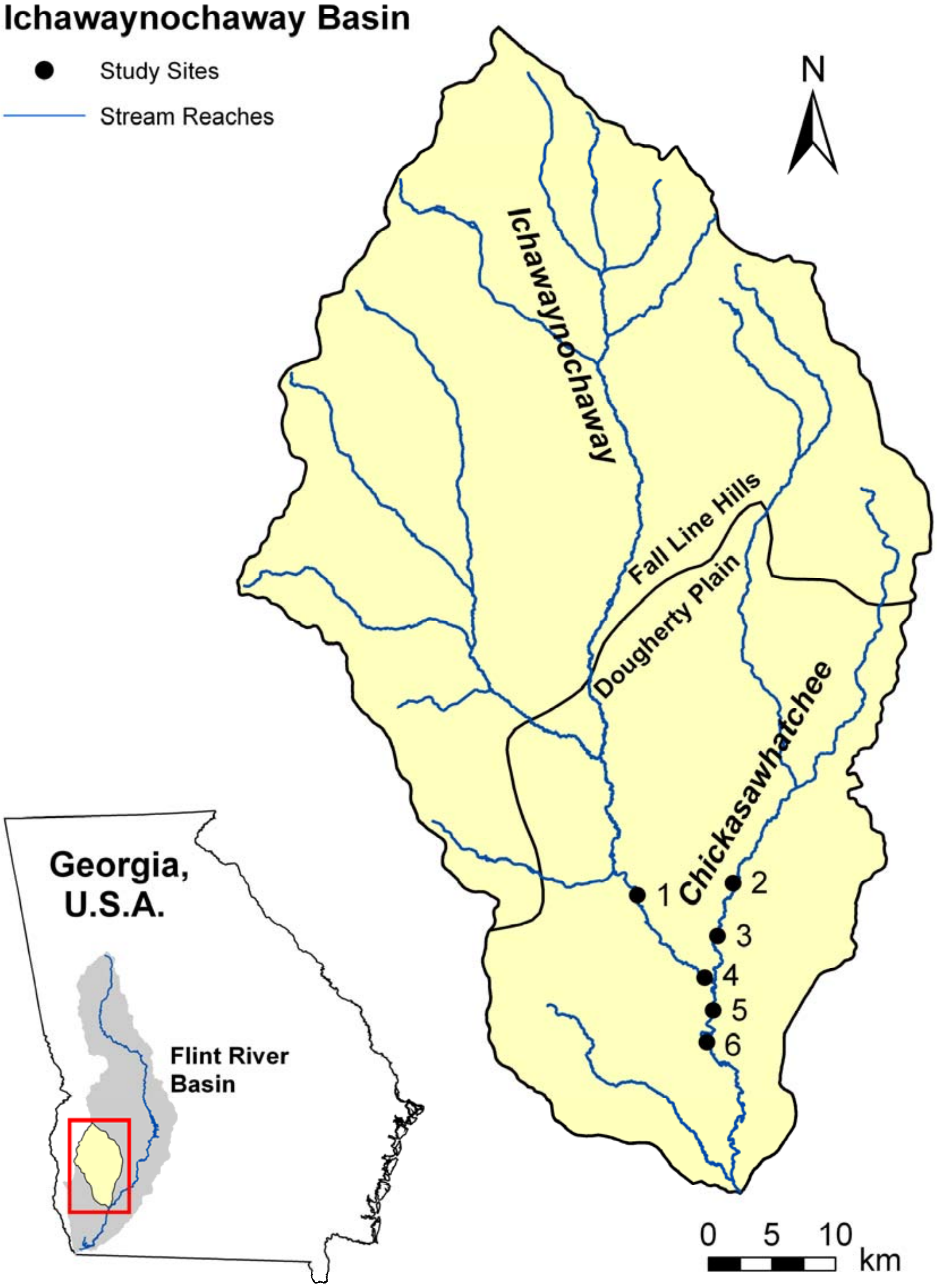
Figure 4.4: Isotopic signatures at all locations of *C. fluminea* (*n* = 42) and *E. crassidens* (*n* = 42) with a minimum convex hull drawn around both species for each sample site.

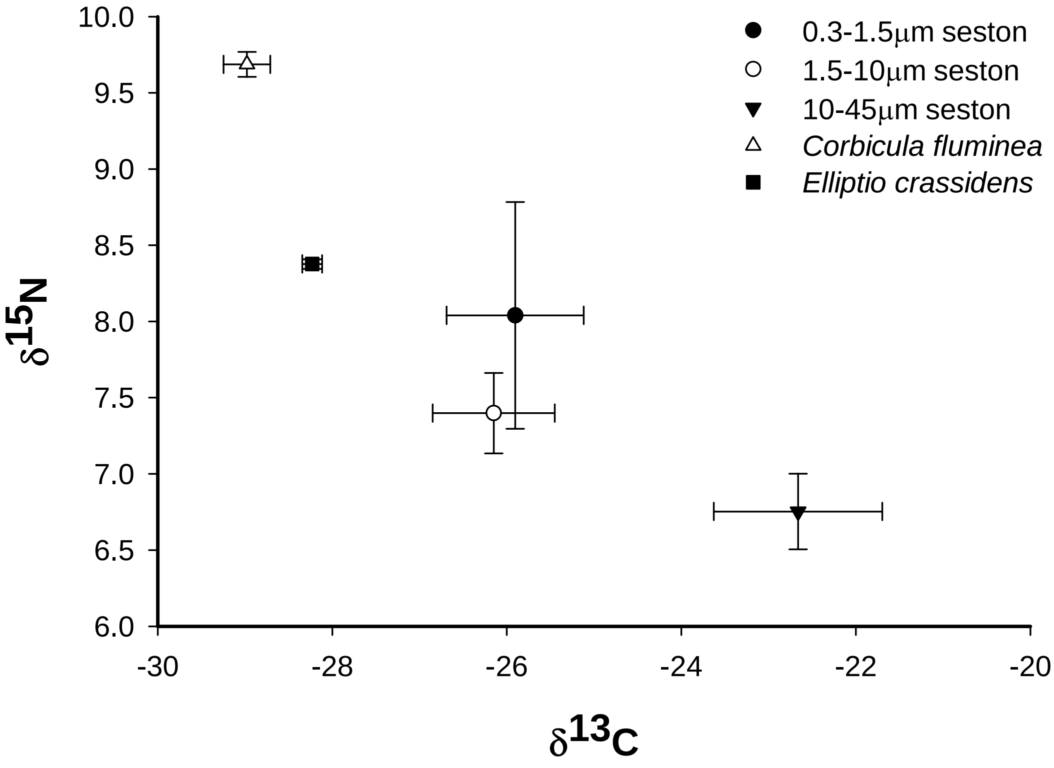
Figure 4.5: The C:N values of bivalve tissue at the 6 sampling locations. *C. fluminea* (*n* = 42) and *E. crassidens* (*n* = 42) were significantly different in C:N at sites 1, 4 and 5 at $\alpha = 0.05$ (Littell, 2000).

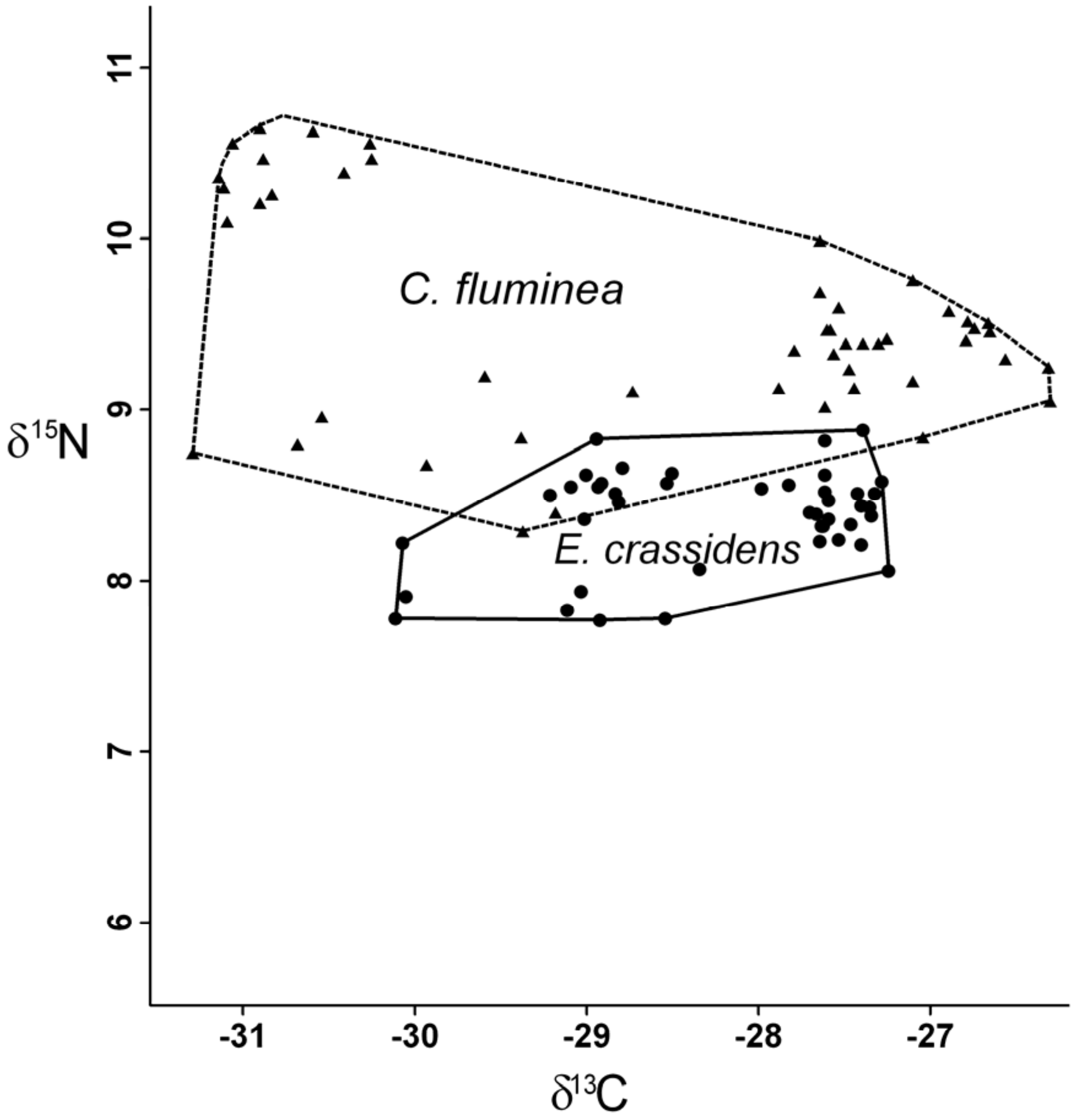
Figure 4.6: The total DM (bar plot) and the wet tissue bivalve biomass corrected DM (line plot) of materials bivalves were releasing as biodeposits. *E. crassidens* (*n* = 12) released significantly more DM than *C. fluminea* (*n* = 12) at $\alpha = 0.05$ (Littell, 2000). No significant differences were noted in the biomass corrected DM.

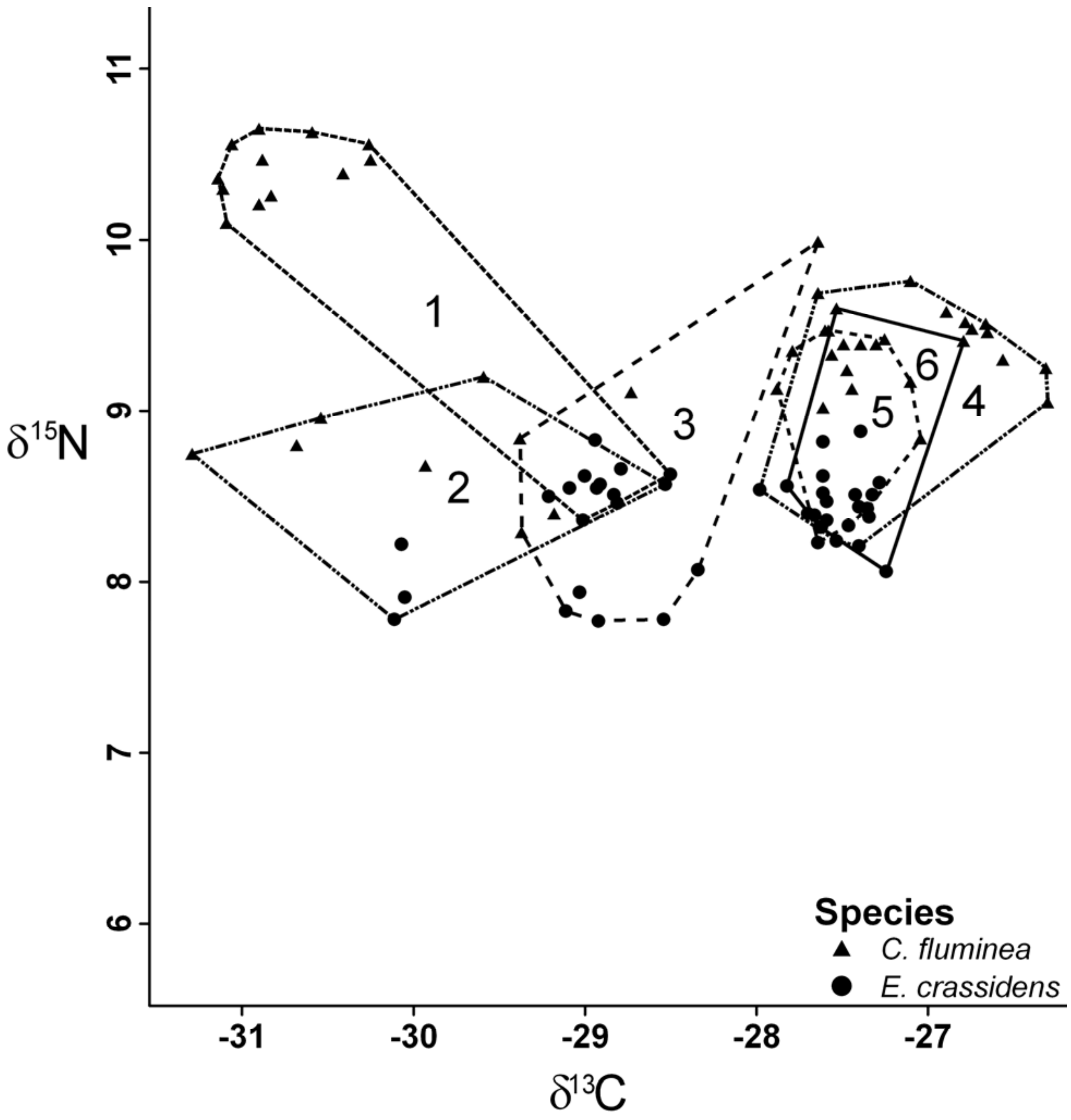
Figure 4.7: (A) The %C and (B) %N of biodeposits left by *C. fluminea* and *E. crassidens* over time. There is a linear relationship between %C ($y = -4.0657x + 21.31$; $r^2 = 0.94$) and %N ($y = -0.6807x + 3.0653$; $r^2 = 0.99$) and time for the *C. fluminea* treatments. No significant linear trend was seen in the *E. crassidens* treatments.

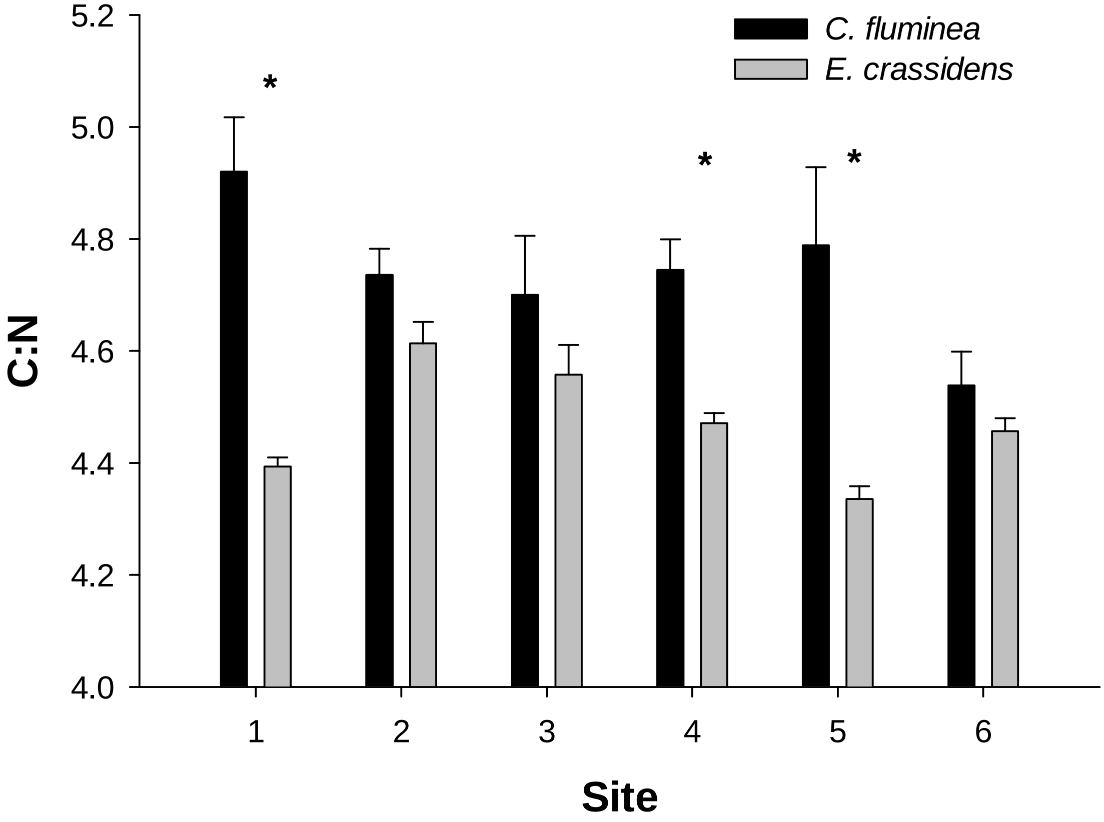
Figure 4.8: Biodeposition rates of *C. fluminea* and *E. crassidens*. *C. fluminea* leaves biodeposits with significantly more C and N than *E. crassidens* at $\alpha = 0.05$ (Littell, 2000).

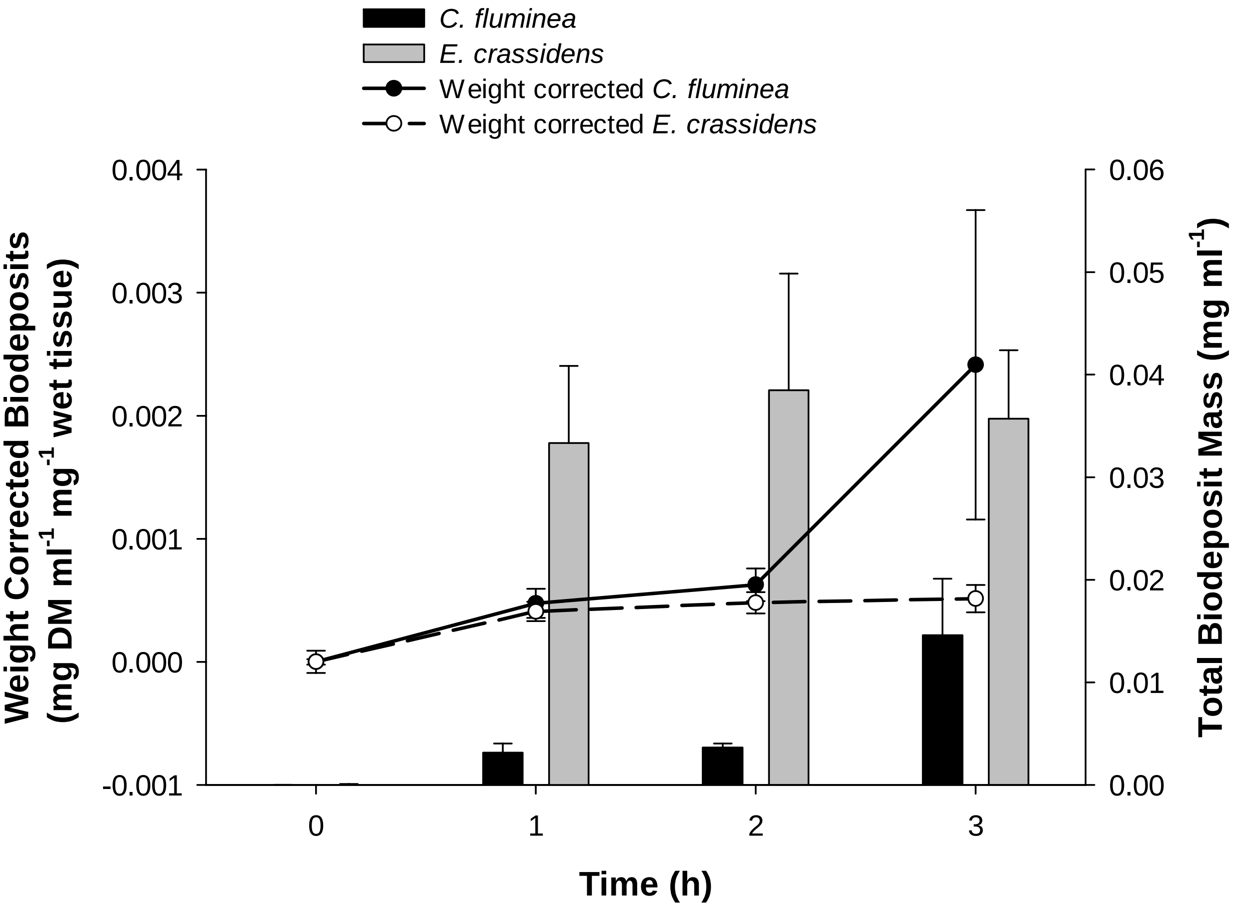

Ichawaynochaway Basin
● Study Sites
Stream Reaches
N
Ichawaynochaway
Fall Line Hills
Dougherty Plain
Chickasawhatchee
1
2
3
4
5
6
0 5 10 km
Georgia, U.S.A.
Flint River Basin

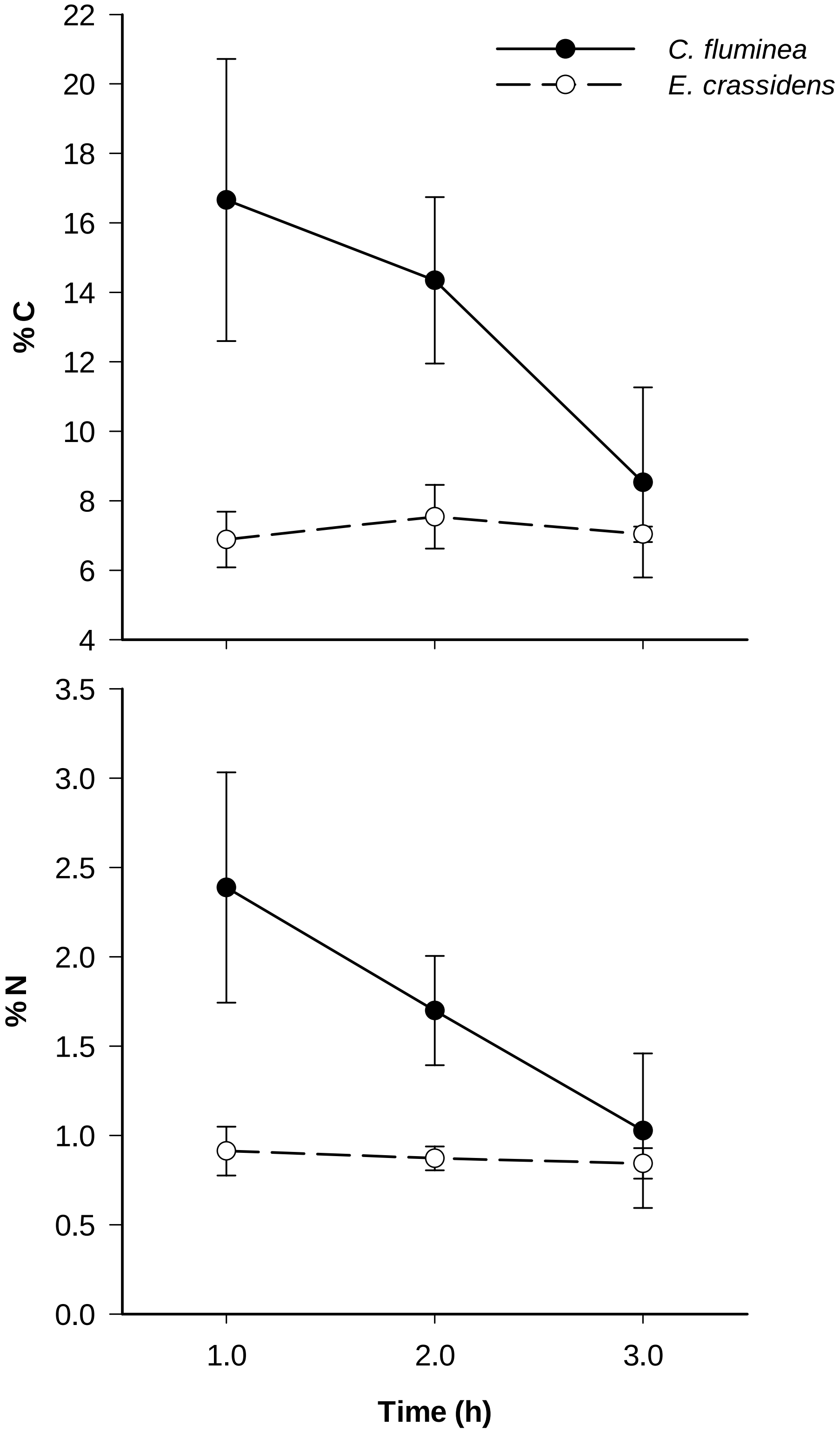

10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
$\delta^{15}N$
-30
-28
-26
-24
-22
-20
$\delta^{13}C$
● 0.3-1.5μm seston
○ 1.5-10μm seston
▼ 10-45μm seston
△ *Corbicula fluminea*
■ *Elliptio crassidens*

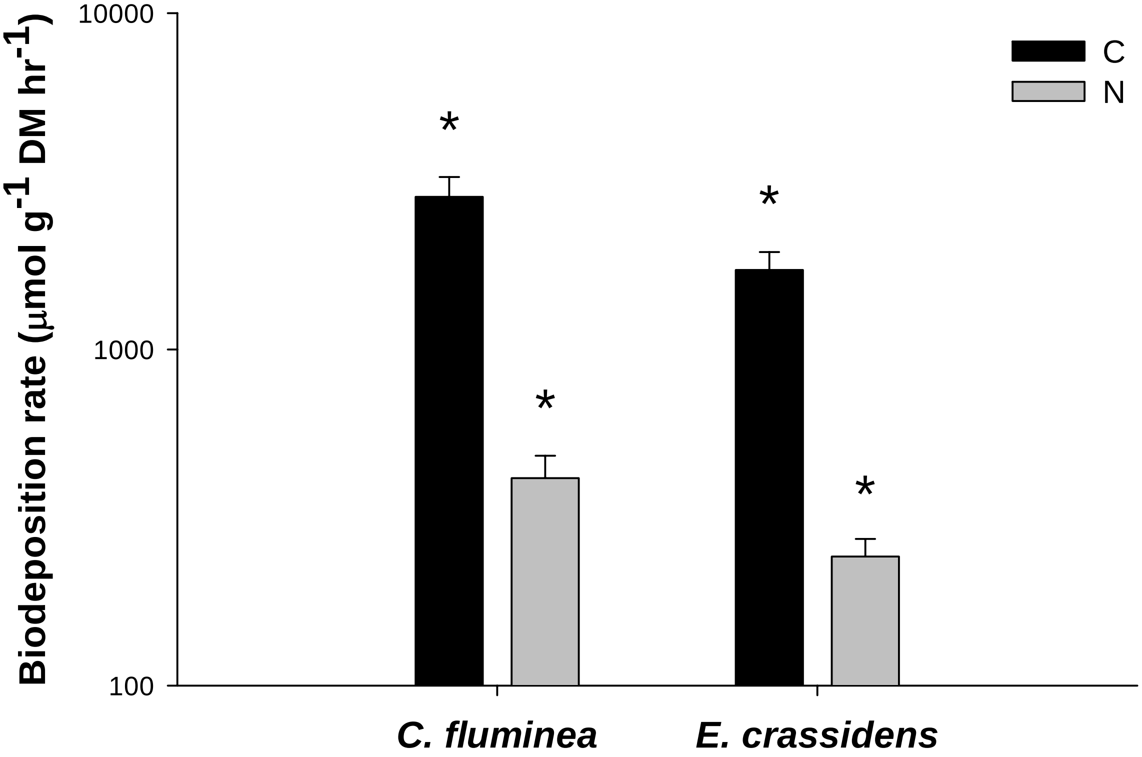

C. fluminea
E. crassidens
$\delta^{15}N$
$\delta^{13}C$
11
10
9
8
7
6
-31
-30
-29
-28
-27


δ15N
δ13C
11
10
9
8
7
6
-31
-30
-29
-28
-27
1
2
3
4
5
6
Species
▲ C. fluminea
● E. crassidens


5.2
5.0
4.8
4.6
4.4
4.2
4.0
C:N
1
2
3
4
5
6
Site
C. fluminea
E. crassidens
*
*
*


C. fluminea
E. crassidens
Weight corrected C. fluminea
Weight corrected E. crassidens
Weight Corrected Biodeposits (mg DM ml-1 mg-1 wet tissue)
0.004
0.003
0.002
0.001
0.000
-0.001
Total Biodeposit Mass (mg ml-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
1
2
3
Time (h)


22
20
18
16
14
12
10
8
6
4
% C
C. fluminea
E. crassidens
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
% N
1.0
2.0
3.0
Time (h)


Biodeposition rate (μmol g^{-1} DM hr^{-1})
10000
1000
100
C
N
*
*
*
*
C. fluminea
E. crassidens

CHAPTER 5

CONCLUSIONS AND SUMMARY

The combination of studies examining the abiotic and biotic factors structuring Ichawaynochaway Creek yielded new information on Coastal Plain streams and the roles of filter-feeding bivalves. Characterizing seston and relating it to hydrologic and geomorphic variables gave us a structural framework to build upon to learn about how the composition and quality of materials vary in a Coastal Plain stream. Combining this with experimental and field studies on how some of the most dominant consumers, freshwater bivalves, utilize and assimilate these materials informed us on how they may be structuring the availability of food and nutrient resources. Additionally, this information may help make predictions on how climate change may affect freshwater systems. *Elliptio crassidens* is the native species in this basin that is most prone to mortality during drought conditions. Consequently, as droughts become more frequent due to climate change, we may see a further reduction in the biomass of the dominant native species. If droughts and floods become more severe as predicted, we may be able to predict changes in basal resource quality and impacts on filter-feeding communities. Following are summaries of the findings of this research.

Part I:

The determination of the total carbon and total nitrogen composition as well as the nitrogen and carbon stable isotopic signatures of archived seston samples was informative to understand how the quality of basal resources in the Ichawaynochaway Basin varied. Suspended organic matter (seston) was collected throughout the watershed during varying flow regimes to determine quality and source of materials available over different temporal and spatial scales.

Material was fractionated into a series of 3 size categories using different sized plankton nets and sieves: large (>250 μm), medium (250-45 μm), and small (45 -10 μm). Quality, as indicated by a low C:N value, was greatest in the small size class and decreased significantly with increasing particle size. The $\delta^{13}C$ and $\delta^{15}N$ of the seston was the most enriched in the small and medium size classes and were significantly different than the large size class. Lower C:N and the $\delta^{15}N$ enrichment of smaller particles may indicate that those materials had higher microbial biomass associated. High flows were also responsible for higher quality seston with a less depleted $\delta^{13}C$ signature being in suspension; however during low flows, areas with high connectivity to the floodplain had lower quality materials in suspension. This was explained by the position in the watershed the samples were taken. The areas with high connectivity to the floodplain are in the upper portions of the basin where flow is highly reduced during low flows, while the confined portions are in the lower portions of the watershed that remain flowing during drought conditions. This is evident because the C:N of seston decreased, $\delta^{13}C$ was less depleted, and $\delta^{15}N$ increased in a downstream direction during low flow periods, suggesting that higher quality materials in downstream reaches was likely a result of greater primary production. While during high flows, regardless of position in the watershed, quality was always higher than during low flow periods, with $\delta^{13}C$ and and $\delta^{15}N$ being less depleted.

Results of these analyses indicate the importance of environmental flows and stream-floodplain connections. In general, these findings acknowledge the importance of multiple spatial and temporal scales contributing to variability in food quality and composition in stream ecosystems. While the spatial dimensions of the watershed lead to higher quality materials in the lower portion of the watershed during low flows when there is greater water clarity, the inundation of the floodplain is particularly important for supplying the stream with high quality food resources.

Part II:

The ecology of filter feeding mussels and the non-native Asian bivalve, *Corbicula fluminea* was investigated in this study. Results indicated that both the native (*E. crassidens*) and the invasive (*C. fluminea*) species showed preferences for living materials, which was indicated by both gravimetric and flow cytometry (FC) methods. The two approaches allowed us to examine how these species feed and process materials, and in turn how this affected suspended organisms. As these species were taking in and feeding on organic materials, they began excreting, which added organic materials to the substrate. Both species utilized small particles and transformed these resources into larger aggregates through excretory processes. Results indicated that *E. crassidens*, a dominant unionid species in the Ichawaynochaway Creek, was feeding on a mixture of nanoeukaryotes, picophytoplankton, and some bacteria. The FC results demonstrated that *E. crassidens* were filtering primarily nanoeukaryotes. The decrease of nanoeukaryotes occurred simultaneously with an increase in bacteria. *E. crassidens* was likely getting a large proportion of energy from bacteria as hypothesized by many researchers, but most of the energy is through bacteriovores (i.e., nanoeukaryotes).

Results for *C. fluminea* were not as clear; it appeared that they preferentially filtered picophytoplankton. Through selective filtration these organisms were potentially having a major effect on structuring these streams. This research showed further evidence for preference for living materials as well as potential control of other trophic levels by bivalves and increases in the production of bacteria due to bivalve excretory activities and bacterial release from predators.

To our knowledge, this was the first study to examine grazing by bivalve species on natural stream particulate matter. In the future, studies incorporating FC will likely yield information on additional resource-specific dynamics related to differences in filtration by these and other co-occurring species. Our results suggest that native and non-native mussels, both considered filter feeders, play differing functional roles in streams and rivers.

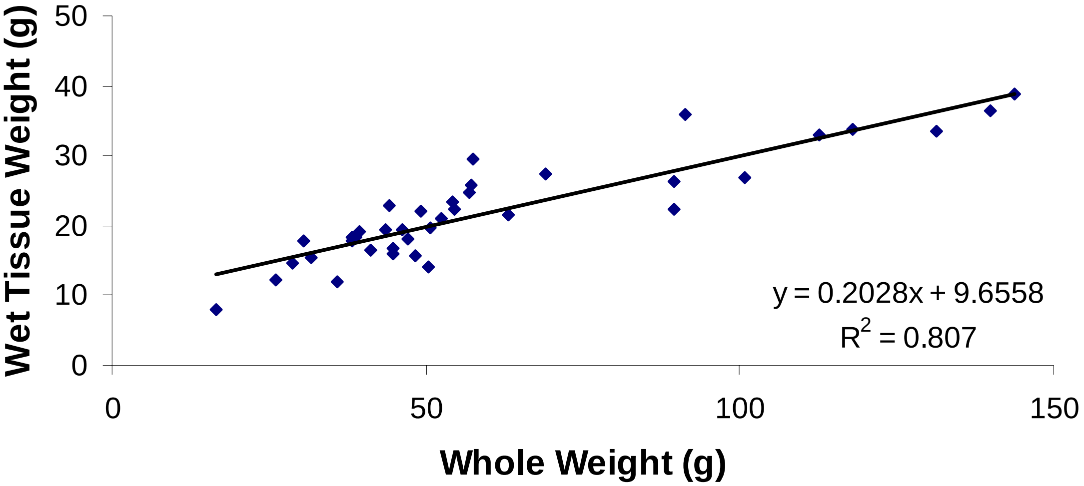
Part III:

To determine dietary composition and potential overlap of *C. fluminea* and the native mussel *Elliptio crassidens*, we measured the elemental and isotopic compositions of their tissue, as well as 3 size classes of seston across Ichawaynochaway Creek, a tributary to the lower Flint River, Georgia, U.S.A. In the laboratory, we measured the C and N in biodeposits excreted by the two species. Our results indicated differences in the food resources assimilated and the nutrients biodeposited by the two bivalve species. *C. fluminea* had lower trophic fidelity by being able to acquire and assimilate a multitude of resources as indicated by the large range of $\delta^{13}C$ values. Additionally, as indicated by *C. fluminea*'s more variable and enriched $\delta^{15}N$ signatures, they were better able to assimilate small materials, and may be better able to acquire bacterial sources than *E. crassidens*. *C. fluminea* were also retaining and biodepositing nutrients in an alternate way in comparison to *E. crassidens*. The native species tissue composition had a lower C:N value than *C. fluminea*. This indicates that *E. crassidens* may be better able to retain nitrogen than *C. fluminea*. Laboratory experiements indicated that *C. fluminea* deposited more nitrogen than *E. crassidens* through excretory processes.

As of yet, few researchers have documented the functional affects of *C. fluminea* on North American waterways. *C. fluminea* had lower trophic fidelity than *E. crassidens* implying they fill a larger trophic niche space and can change their feeding habits more readily given the availability of materials. In addition, *C. fluminea* may be altering nutrient cycling within these streams due to their feeding and excretion activities. Given the similar functional role *C. fluminea* is believed to possess in comparison to native mussels, it was not performing the same processes. More attention needs to be given to the functional roles invasive species play in comparison to native species in aquatic ecosystems to fully understand the implications of their introduction.

APPENDICES

APPENDIX A: Relationship between total mass (with shell) and wet tissue mass of *Elliptio crassidens* (*n* = 38).



APPENDIX B: Relationship between total mass (with shell) and wet tissue mass of *Corbicula fluminea* (*n* = 12).