ABSTRACT

BUCCI, JOHN P. Assessment of the Feeding Ecology of Native and Non-Native Freshwater Bivalves in a North Carolina River Basin. (Under the direction of William J. Showers).

There are several factors that contribute to the decline of freshwater bivalves, including the prevalence of storm water runoff, excess nitrogen inputs, low food quality and the destruction of aquatic habitats. The primary goals of this research were to: 1) characterize the water chemistry and hydrology of three watersheds with different land use characteristics in the Neuse River Basin (NRB), North Carolina, 2) to determine the dominant food sources and potential feeding strategy differences of native and non-native freshwater bivalves that reside concurrently in these habitats and 3) to better understand the efficacy of bivalves as biomonitors of nutrient and sediment contaminants. To address these goals, a combination of methods was utilized, including stable isotope ($\delta^{15}N$ and $\delta^{13}C$) analyses, high-resolution (hourly) water quality monitoring, and a laboratory dose-response experiment with a valve gape sensor.

Chapter 1 results of the long-term (36 month) analyses suggest that dominant watershed type was a predictor of mean $\delta^{15}N$ values of nitrate (NO_3^-). Also, mean $\delta^{15}N$ tissue values of *Elliptio complanata* from agricultural sites were significantly heavier than urban and forested watersheds, suggesting an assimilation of agricultural nutrient sources. Mean $\delta^{13}C$ bivalve tissue values were significantly depleted in forested compared to agricultural and urban watersheds. Short-term (3 to 5 day) storm event data indicated that the urban watershed showed greater changes in the quality of bivalve food sources ($\delta^{15}N$, $\delta^{13}C$ and C/N of SPOM) with discharge compared to the forested and agriculture watersheds. The combined isotopic and hydrologic data indicated that turbidity and low food quality (C/N >

10) were associated with increased discharge. The implications suggest that native mussels (*E. complanata*) may assimilate a majority of their food sources during low flow conditions.

Results of the second study showed a significant association between the isotopic composition of bivalve tissue (*Corbicula fluminea* and *E. complanata*) and watershed type. The implications are that *C. fluminea* bivalves may be more sensitive biomonitors than native mussel species of nitrate and suspended solids associated with turbidity found in disturbed watersheds. This study supports previous research and offers new information, which suggests that the decline of native mussel populations may be associated with poor water quality and hydrological conditions unique to urban and agricultural watersheds.

The third phase of this dissertation consisted of a laboratory study that utilized a state of the art valve gape sensor to evaluate feeding activity across distinct turbidity periods modeled after an urban storm event hydrograph. Results suggested that feeding strategies of the non-native bivalve, *C. fluminea* might be better adapted to tolerate moderate to high turbidity conditions associated with a compressed hydrograph. Conversely, the native mussel, *L. radiata* may have a disadvantage in frequent turbid environments, and this species may be better adapted in less disturbed watersheds (i.e., forested) where average food quality is higher and suspended solids change significantly less with discharge.

In summary, the results of this dissertation have provided valuable insights into native and non-native bivalve feeding ecology across different watershed types, and this work has generated new directions for future biomonitoring research.

ASSESSMENT OF THE FEEDING ECOLOGY OF NATIVE AND NON-NATIVE FRESHWATER BIVALVES IN A NORTH CAROLINA RIVER BASIN

by

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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the requirements for the Degree of
Doctor of Philosophy

MARINE, EARTH AND ATMOSPHERIC SCIENCES

Raleigh, NC

2006

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BIOGRAPHY

As a native Rhode Islander, John Bucci grew up with a deep appreciation for the natural mechanisms of marine and freshwater organisms and their relationship to oceanic processes. Summer sailing trips to Block Island and quahogging the mudflats of the intertidal zone in the Greenwich Bay have offered him valuable experiences that last a lifetime. John has had a diverse educational and professional background. In 1992, he earned a master's degree in behavioral science from the University of Hartford, Connecticut. After graduation, John worked with developmentally disabled children as a bio-behavioral intern at the University of Pennsylvania Children's Hospital. After spending several rewarding years conducting clinical health care research, he decided to embark on a career in marine science. He confirmed this decision by working at the Long Marine Laboratory and taking ecology courses at the University of California, Santa Cruz. In 2003, John received a master's degree (M.S.) in biological oceanography in the Marine, Earth and Atmospheric Sciences Department at North Carolina State University. During that time, he focused his research on estuarine food web processes and stable isotope ecology. John's educational and professional background has been unique. His experience has offered him a valuable perspective in which to pursue ecological research. The completion of his Ph.D. in biological oceanography has made this journey worth every step.

ACKNOWLEDGMENTS

I would like to thank my parents, my family and especially my wife Semra for their encouragement and lasting love throughout this effort. Many thanks go to my committee chair, Dr. Bill Showers for his support and inspiration as well as to my committee members (Drs. Dave DeMaster, Steve Rebach and Jay Levine) for their expert advice. I would like to express my appreciation for the funding and support of the RiverNet Research program at North Carolina State University (NCSU). This study was also made possible by the support of the Stable Isotope Laboratory (SIL) at NCSU, and the USDA Integrated Food Safety Research program (2003-51110-02084). Many thanks go to the laboratory and field staff (Bernie Genna, Harold Henion, and Jeff Siceloff) at NCSU-SIL for their critical input of study design and assistance in sampling. The College of Veterinary Medicine at NCSU, and the mussel barn team, Chris Eads and Erin Schubert, made additional support for this dissertation work possible. The author also thanks Nekton Research, and Ryan Moody, for the design support they have provided and access to the patented technology they have developed for monitoring the gape response of bivalves. Finally, thanks to my colleagues, friends and co-workers, Brian Usry, Geoff Bell, Darren Parsons, Donna Surge, Raj Butalia, Mathew Fountain, Brandon Puckett, Ted Wohn, and Bob Valentini for providing critical input to this project.

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Introduction

The United States has the greatest diversity of freshwater mussels in the world (Bogan, 1993; Williams et al., 1993). Of the five families and roughly 1,000 species occurring globally, nearly 300 species and subspecies in the families Unionidae and Margaritiferidae reside in the U.S. (Turgeon et al., 1988). The diversity of freshwater mussels in the Southeastern U.S. is unmatched by any other area in the world. A major issue for ecosystem ecologists is that freshwater mussels (Phylum Mollusca, Class Bivalvia) have suffered a greater decline than any other wide-ranging faunal group in North America. Master (1990) recognized 55% of North America's mussels as extinct or imperiled, compared to only 7% of the continent's bird and mammal species. There are several factors that contribute to this decline in freshwater systems, including the prevalence of storm water runoff, excess nitrogen inputs, low bivalve food quality and the destruction of aquatic habitats (Williams et al., 1993; Bogan, 1993). This dissertation study addressed factors associated with the link between feeding ecology and watershed water quality. The main purpose of this research was to examine important questions of bivalve feeding ecology: 1) what are the dominant food sources for stream bivalves from watersheds with different land uses; 2) to what extent is surface water hydrology and water chemistry associated with the quality of bivalve food sources and 3) how effective are native and non-native bivalves as biomonitors across a range of watershed types?

Aquatic habitat loss can occur from direct effects such as dams, dredging, and channelization, or from more indirect effects such as siltation and excess nitrogen associated with urbanization and intensive agriculture operations (Gao et al., 2004). Healthy populations of native unionids depend on a host fish to complete their complex reproductive cycle. Also, competition from non-native species such as the Asian clam (*Corbicula fluminea*) (introduced to the U.S. west coast in the 1930's) has become widespread in nearly every watershed since the 1980’s and they utilize resources (e.g., habitat space, food sources) that may contribute to the loss of native mussel populations (Bogan, 1993; Williams et al., 1993; McMahon, 2000). The high densities (up to thousands per square meter) of *Corbicula sp.* can decrease the oxygen available for native mussels (Belanger et al., 1990; Leff et al., 1990) and increase the amount of nutrients to surface waters (Cooper et al., 2005).

Freshwater bivalves are economically important members of freshwater communities and play a key role in the ecology of rivers and streams (Allan, 1995). The cost of habitat degradation can be high as the decrease in bivalve populations limits their ability to maintain healthy water quality. Bivalves are a vital connection in the food web between organic matter sources and fish (Gosling, 2003). Bivalves filter large volumes of water each day, thereby filtering contaminants from the water and serving as an early warning of water quality problems. These invertebrates are an important source of food for local fish and other carnivorous animals. Both freshwater native and non-native bivalves are sessile organisms and may be particularly sensitive to changes in water quality. Thus they are considered indicators of the health of aquatic ecosystems (Goldberg, 1975; Doherty, 1990; Williams et al., 1993).

There may be measurable differences in the food sources available and feeding strategies of native and non-native species across water chemistry and flow conditions, which can affect their success as a species and their bio-monitoring effectiveness. Stream invertebrates interact with the complex mixture of organic and inorganic nutrients (i.e., nitrate) as well as the transport of contaminant solutes to surface waters and benthic substrate

(Hauer & Resh, 1996). Physical factors include channelization from frequent higher than normal discharge in urban areas, which can destabilize the stream bottom and adversely impact bivalve populations (Wahl et al., 1997). The combined affect of biotic and abiotic variables necessitate a need for further research regarding the feeding ecology of bivalve species.

The definition of watershed used in this study is a pure hydrologically defined surface water drainage area (Chow et al., 1988; Cushing & Allan, 2001). The term "disturbed" watershed refers to the measure of habitat health, as defined by low water quality (high average suspended solids, low dissolved oxygen, excess nitrate concentration and low biotic diversity) and a non-supporting watershed classification (NCDWQ, 2002).

From a management perspective, bivalves' effectiveness as biomonitors may be misunderstood despite their ability to record contaminants as a time integrated measure. The concentration of contaminants (e.g., nitrogen, sediment, and bacterial) can change the composition of bivalve food quality as a function of flow (i.e., discharge) with episodic storm events. Given the advanced technological tools available to accurately measure changes in stream biota and the physical and chemical conditions in freshwater ecosystems, it is important to evaluate the quality of food sources and tissue bioaccumulation of nutrients related to storm event turbidity and the effects of sedimentation. Knowledge of these factors may improve our understanding of the major decline of freshwater mussel populations in North Carolina.

Research goals and objectives

Although the precise measurement of the physical, chemical and biological factors associated with stream habitats can be complicated, the aim of this dissertation research was to utilize a combination of sound methods to investigate associations between bivalve tissue assimilation of food sources and valve gape of freshwater bivalves with watershed hydrology. The goal was to assess these relationships and to contribute to our knowledge of the underlying mechanisms of bivalve feeding ecology in freshwater stream systems. The techniques used included stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses, high resolution (hourly) water quality monitoring, and a laboratory dose-response experiment. The primary goals were to: 1) characterize the water chemistry and hydrology of three different watersheds with dominant land use characteristics in the Neuse River Basin (NRB), North Carolina; and 2) to determine the foremost food sources and feeding strategies of native and non-native freshwater bivalves as a function of changes in water chemistry and hydrology (Appendix A, Map 1); and 3) to use this information to predict the efficacy of bivalve species as biomonitors of contaminants associated with high nitrate concentration and sedimentation across a range of watershed types.

The dissertation is organized as follows: Chapter 1 measures and characterizes the water chemistry and hydrological parameters of watersheds with different land use in the NRB. Additionally, storm events were examined across watershed type. The focus of chapter 1 was to evaluate the watersheds, based on long and short-term water chemistry data, and to examine the isotopic composition of native mussel food sources across different watersheds. This information was related to the food sources of the native freshwater mussel (*Elliptio complanata*). Of the several species of native mussels that are in decline in the southeastern, U.S., *E. complanata* is considered of special concern, but it is available for scientific collection. The NRB was unique as it offered a range of dominant percent land-use types (urban, agriculture, and forested) and provided an ideal field study environment in which to examine the feeding ecology of freshwater bivalves.

Chapter 2 extended this field research by describing a comparison between the isotopic ($\delta^{15}N$ and $\delta^{13}C$) compositions and feeding ecology (i.e., food sources) of native (*E. complanata*) and non-native (*Corbicula fluminea*) freshwater bivalves. Outcomes were evaluated based on long-term (36 month) and short-term (3 to 5 day) water chemistry data. Nutrient inputs of anthropogenic origin as well as microbial processes can affect food quality of suspended particulate matter in stream ecosystems (Martin & Merckx, 1992; Triska et al., 1984). The ecological importance of the biotic and abiotic retention of these sources relates to the transfer of organic carbon from the surface water, which can be partially or fully metabolized by heterotrophic microbial communities (Ward & Johnson, 1996). These transformations may depend on watershed type and affect the food quality of benthic invertebrates, such as bivalve consumers.

Complementing the field research, chapter 3 was a laboratory study that examined the difference in valve gape between native and non-native bivalves exposed to storm generated turbidity. Fine sediment associated with storm water runoff impairs the health of freshwater bivalve populations by interfering with feeding, growth, and reproduction (Aldridge et al., 1987; Ward & Shumway, 2004). This study evaluated the potential difference in feeding strategies used by both native (*Lampsilis radiata*) and non-native (*C. fluminea*) species. Designed to control several factors that cannot be controlled in the field survey; this study contributes toward our understanding of how each species selects particles of different food quality across distinct periods of a hydrograph during a storm event. There is reason to believe that native mussels may have a disadvantage in terms of food quality selection and tolerance of fine sediment associated with turbidity (Baker & Levinton, 2003). This laboratory study provided a controlled dose-response exposure to fine sediment to investigate this issue. Knowledge of a species-specific feeding strategy will improve our understanding of bivalve feeding mechanisms in response to pollutants (i.e., nitrate, sediment and heavy metals) and contribute to management practices that employ them as biomonitors.

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CHAPTER 1

THE USE OF STABLE ISOTOPES ($\delta^{13}C$ AND $\delta^{15}N$) AND WATERSHED HYDROLOGY TO ASSESS FRESHWATER MUSSEL (*ELLIPTIO COMPLANATA*) FOOD SOURCES

Abstract

Freshwater Unionidae mussels have suffered precipitous population declines over the last century in North America. Basic water chemistry questions regarding the relationship between isotopic tissue composition and diet of native freshwater mussels remain unanswered. This study compared the $\delta^{15}N$ and $\delta^{13}C$ composition of mussel (*Elliptio complanata*) tissue, suspended particulate organic matter (SPOM) and sediment to water chemistry in lower order creeks with different land use (forest, urban, and agricultural watersheds) in a North Carolina River basin. A significant relationship was observed between water chemistry parameters and $\delta^{15}N$ values of mussel tissue by watershed. A generalized linear regression model (GLM) for the long-term (36 month) data showed that watershed land use was a predictor of mean $\delta^{15}N$ values of nitrate (NO_3^-; DIN) surface water concentration. Using a Bonferroni multiple comparison test, mean $\delta^{15}N$ values of DIN were significantly more enriched from the agricultural (11.2‰ ± 2.5; N=43) compared to the forested (6.5‰ ± 2.3; N=35) and urban (8.0‰ ± 3.0; N=109) sites ($p < 0.05$). Mean $\delta^{15}N$ tissue values from the agricultural sites (8.5‰ ± 0.6; N=34) were also significantly more enriched than urban (6.6‰ ± 0.67; N=24) and forested samples (6.5‰ ± 0.5; N=30), suggesting an assimilation of agricultural sources ($p < 0.05$). Mean $\delta^{13}C$ mussel tissue values

(-33.2‰ ± 0.47; N=14) were significantly depleted in forested compared to agricultural (-29.9‰ ± 0.63; N=15) and urban (-30.5‰ ± 0.60; N=24) sites, suggesting a potential availability of depleted $\delta^{13}C$ sources, such as bacterial sources ($p < 0.05$). This material may contain a larger portion of dissolved organic carbon (DOC) available to bivalves, which is associated with a more negative $\delta^{13}C$ value of labile carbon. The short-term (3 to 5 day) storm event data indicated that the urban watershed showed greater changes with discharge in food source quality ($\delta^{15}N$, $\delta^{13}C$ and C/N of SPOM) than the forested and agriculture watersheds. The long-term data indicated that low food quality was associated with increased discharge across all watersheds. The combined isotopic and hydrologic data suggest that native mussels (*E. complanata*) may preferentially consume SPOM sources predominantly in low flow conditions. Implications of this study suggest that native mussels may be effective biomonitors of pollution from upstream sources in less disturbed, lower turbidity watersheds. However, more work is needed to better understand the feeding ecology of native compared to non-native bivalves by watershed to determine their effectiveness as biomonitors.

Introduction

The United States of North America is home to the richest freshwater mussel fauna in the world (Williams et. al., 1993; Neves et al., 1997). Precipitous declines in several species of freshwater mussels (Unionidae) native to the southeastern United States have been documented over the last century (Bogan, 1993, 1998; Lydeard et al., 2004). Nearly 72% of the United States' 297 native mussel species have become extinct, endangered, threatened, or of special concern (Turgeon et al., 1988; Williams et. al., 1993). This status places native freshwater mussels in greater peril than terrestrial vertebrates (including birds, mammals, reptiles and amphibians), of which < 20% of US taxa are classified as rare to extinct (Master, 1990). The reasons for this decline are related to a combination of factors including siltation associated with storm water runoff, point and non-point source pollution, and habitat destruction (Layzer et al., 1993; Cushing & Allan, 2004). Competition for food sources from non-native species has also contributed to the loss of native mussel populations (Leff et al., 1990; Silverman et al., 1995; Baker & Levinton, 2003).

Although native mussels have been recognized as suitable organisms for monitoring anthropogenic pollution (Doherty, 1990; Goldberg & Bertine, 2000; Smolders et al., 2004), their ability to monitor effectively, during variable sediment discharge conditions, has not been evaluated. As storm event flux of pollutants is associated with peak freshwater discharge in most fluvial systems, variations in nutrients and contaminants may affect bivalve feeding ecology. Nutrients such as NO_3^- and NH_4^+ are present in storm water runoff associated with wastewater, fine sediment and terrestrial organics and fertilizer from agricultural fields (Knowles & Blackburn, 1993; Kendall et al., 2001). The need to manage more effectively point and non-point source pollution associated with changes in the flux to freshwater ecosystems has been a priority of ecologists (Vitousek et al., 1997; Paerl et al., 2004). The $\delta^{15}N$ tissue composition of mussels has been used to assess the degree of anthropogenic nitrogen pollution in freshwater ecosystems (Vander Zanden & Rassmussen, 1999; Raikow & Hamilton, 2001; McKinney at al., 2002; Ulseth & Hershey, 2005).

Over the past 20 years, land use has changed in the Neuse River Basin (NRB). Cultivated croplands have decreased by 17%, forests have declined by 7%, and urbanized areas have increased by 89%. The predominant land use in the upper portion of the basin is urban (NCDENR, 2002). From 1980 to 2000, there has been a 28% increase of the human population in the basin, and this population is expected to double by 2020 to over 2.8 million people. Approximately 8% of the stream waters in 2002 were classified as impaired (NCDENR, 2004). The NRB was declared a nutrient sensitive watershed in 1988. Since that time, the North Carolina legislature mandated a 30% reduction in nitrogen loading to the NRE to improve water quality in the lower basin and estuary in partial response to frequent fish kills in the Neuse Estuary (NCDENR, 2002; Burkholder et al., 1999).

The purpose of this study was to measure the isotopic ($\delta^{15}N$ and $\delta^{13}C$) composition of freshwater native mussels (*E. complanata*) in watersheds with different dominant land use type. An additional goal was to evaluate the water chemistry and hydrology of three different watershed types in the Neuse River Basin (NRB). Native mussels primarily filter feed particulates; however, they may also deposit feed on sediment sources (Raikow & Hamilton, 2001; Ward & Shumway, 2004), it is important to gain knowledge across a range of flow conditions and watershed types.

Methods

The area of focus in this study is the Neuse River Basin (NRB), which contains approximately 24 species of Unionidae freshwater mussels (Bogan, 2002). The NRB is the third largest river basin in North Carolina and is entirely located in the state (Fig. 1).

Water chemistry and discharge parameters were sampled over a 36-month period (June 2000 to June 2003) on a monthly basis from Bear Creek (agricultural), Walnut and Crabtree Creek (urban) as well as Black and Marks Creek (forested) watersheds in the NRB (Fig. 1). Discharge (cfs) measurements were taken from USGS gauge stations and calculated directly from the multiplication of its velocity by its area. The creeks ranged in size from second to third order (NCDENR, 2002). The dominant land use in these watersheds varied from agriculture and forested to a significant amount of urbanization (Table 1). The $\delta^{15}N$ and $\delta^{13}C$ analyses were completed on the mussel foot tissue and suspended particulate organic matter (SPOM). In addition measurements of PON (suspended particulate organic nitrogen), POC (carbon) and C/N composition of SPOM and bottom sediment were made, along with the $\delta^{15}N$ of dissolved nitrate collected at the mussel sampling sites.

Unionid mussels

Ten specimens of *Elliptio complanata* were sampled from the Marks Creek watershed southeast of Raleigh, NC; the Bear Creek watershed east of Goldsboro NC; and the Crabtree Creek watershed located within Raleigh, NC (Fig. 1). Swimmers and waders collected the mussels by hand after verifying that they were alive at the time of collection. In the lab, shell length and width were measured, then the soft parts were removed, frozen and freeze dried.

Lyophilized tissue samples were finely ground in an agate mortar and pestle. The mussel tissue samples (100-400µg) were placed into tin boats and analyzed for $\delta^{13}C$ and $\delta^{15}N$

in a Finnigan MAT Delta + XL continuous flow isotope ratio mass spectrometer coupled with a Carlo Erba NC-2500 CNS Elemental Analyzer. Sample isotopic results for tissue $\delta^{13}C$ were calibrated against NBS 22, 21, and NIST 8545, 8542 and 5 internal lab standards. Isotopic results for tissue $\delta^{15}N$ were calibrated against NIST 8550, 8549, 8548, 8547, and 7 internal lab standards. The C/N ratio of tissue samples was determined from sample weights and integrated peak area calibrated against the 8 NIST/NBS standards and 7 internal standards. Organic matter contained in the carbonate shell material was obtained by placing 400 mg of carbonate shell powder in silver boats and exposing the samples to 12N HCl acid vapors in a glass desiccation jar for 12 hours. The decalcified shell samples were then frozen, and lyophilized prior to isotopic analysis. $\delta^{13}C$ and $\delta^{15}N$ IRMS analytical procedure for the shell organic material was the same as for the tissue samples.

Particulate organic matter and water chemistry

Sources of sewage in the Neuse River Basin include animal feeding operations in the agricultural watersheds (NCDENR, 2002, 2004), as well as municipal and industrial point sources in the urban watersheds (Table 2). Water samples were collected from surface waters in 1 and 4 liter acid washed (0.1M HCl) Nalgene® containers. Samples were collected on a monthly to biweekly basis in the center of the stream. Event water samples were collected hourly in the urban, agriculture and forested watersheds with an ISCO 3700c water sampler over a 3 to 5 day period. The surface water samples were filtered through a 0.45μm filter (Gelman AquaPrep 600) and kept at 4°C until isotopic analysis to prevent alteration by bacterial activity. An additional 250 ml of non-filtered water was collected in an acid washed Nalgene® container for particulate analysis and kept in a cooler or a 4°C cold room until processed.

Water samples were filtered (250 ml) through a pre-combusted Whatman GFF filter (0.7μm) to collect SPOM. Filters were placed in a tin boat and analyzed for C/N ratio, $\delta^{13}C$ and $\delta^{15}N$ in the elemental analyzer with the same procedure as the tissue samples.

Approximately 10 ml of filtered water were analyzed in an automated flow La Chat Quick-Chem 8000 nutrient flow injection analyzer for nitrate (NO_3^-, mgL^{-1}) and nitrite (EPA Method 353.2, USEPA, 1983) and ammonium (EPA Method 350.1, USEPA, 1983). During each La Chat analytical run, an external standard (EPA) and several internal quality control standards were run with 10 dilution standards and one spiked river water sample to quantify matrix effects.

The $\delta^{15}N$ of dissolved nitrate or ammonia was extracted from the water samples by a modification of the Chang et al. (1999) technique. Enough water to yield 15 μM of nitrogen was passed through a double ion exchange resin column (1^{st}- cation - 5 ml Biorad AG 50-WX8; 2^{nd}- anion - 2ml Biorad AG 2-X8). The cation column was pre-washed with RO (reverse osmosis) water; the anion column was pre-washed with 3N HCL, and then repeatedly washed with RO water to remove all acid residues. Pre-washing the anion column with the same strength acid as the elutant allows 15 μmoles dissolved samples to be analyzed without an isotopic correction (Showers et al., 2005, 2006). Nitrate was eluted from the anion column with 30 ml of 3N HCl. The HCL was neutralized with 15 gm of Ag_2O, the sample was filtered with a Whatman GFF filter to remove AgCl, and the filtrate was freeze dried. The sample was placed in a tin boat and combusted in a Carlo Erba NC2500 Elemental Analyzer and isotopically analyzed with a Finnigan Mat Delta+ XLS CF-IRMS to determine the $\delta^{15}N$ of nitrate (NO_3^-, mgL^{-1}).

Watershed designations were based on a combination of point source data, LULC calculated percentages and NCDENR 2002 reports (Table 1 and 2). The dominant percent land use land cover (LULC) in each watershed was determined from the Neuse River Basin, data set (USEPA, 2000). The time period covered by the satellite data was October 1998 to October 1999. The sum of the area of each land use category for the different watersheds was compiled using Arcinfo® GIS 9.1 software (ESRI, 2004). Hydrologic units were defined on a 1:250,000 scale. The aerial extent of each land use was summed for each watershed, and the percentage of land use of the total area of the watershed was calculated.

Statistical analyses

A generalized linear regression model (GLM), analogous to a one-way analysis of variance was used to estimate the conditional response variables given the values of explanatory variables. A Bonferroni pairwise, corrected t-test was used to measure the differences between mean values of the isotopic composition of water chemistry and the response variables (e.g. isotopic composition of mussel tissue and food sources) by watershed (Olejnick et al., 1997; Scheiner & Gurevitch, 2001). The main explanatory variables (i.e., NO_3^- concentrations, $\delta^{15}N$ of dissolved nitrate) and the isotopic composition of POC and PON were examined across different watershed types (e.g., agricultural, urban and forested). Suspended solids (i.e., associated with turbidity) were also examined between watershed types using a linear Pearson correlation coefficient (r) across discharge. The statistical comparisons were examined during the 36-month study.

Results

Water chemistry and discharge

A clear trend in the isotopic values of nitrate was observed across all three watersheds. A generalized linear model (GLM) showed that watershed land use type (e.g., forested) was a predictor of mean nitrate (NO_3^- mgL^{-1}) concentrations across watershed sites ($F = 139$; $p < 0.05$) (Fig. 2a). A post hoc Bonferroni pairwise comparison test showed that the mean nitrate (NO_3^- mgL^{-1}) concentrations were significantly higher in the agricultural (2.3 mgL^{-1} ± 0.6; N=46) as compared to the forested (0.09 mgL^{-1} ± 0.7; N=35) and urban (1.2 mgL^{-1} ± 0.7; N=111) watersheds sites (Fig. 2a, Table 3) ($p < 0.05$). Generalized linear models showed significantly more enriched mean $\delta^{15}N$ values of nitrate (DIN) observed in the agricultural (11.2‰ ± 2.5; N=43) sites ($F = 15.5$; $p < 0.05$) compared to the forested (6.5‰ ± 2.3; N=35) and urban (8.0‰ ± 3.0; N=109) sites (Fig. 2b, Table 3). There was a higher mean suspended solid value in the agricultural (11.3 mgL^{-1} ± 22.1; N = 45) as compared to urban (8.1 mgL^{-1} ± 14.5; N = 108) and forested (7.5 mgL^{-1} ± 7.0; N = 35) watershed sites (Table 3; $p < 0.01$). Related to this, both the agricultural and urban watersheds showed large variability in suspended solids (20-100 mgL^{-1}) with discharge (Fig. 3). To further evaluate these parameters, the Pearson correlation coefficient showed stronger relationships among the urban ($r = 0.67$) and agricultural ($r = 0.60$) compared to the ($r = 0.30$) forested watersheds. Both the urban and agricultural relationships were significant in the positive direction ($p < 0.05$).

Isotopic composition of food sources

The $\delta^{13}C$ and $\delta^{15}N$ isotopic composition of suspended particulate sources (POC, PON) sampled from watersheds sites were examined as a potential food source for resident

E. complanata mussels. The linear regression analyses showed that watershed was a predictor of mean $\delta^{13}C$ values (F = 14.9; $p < 0.05$). Post hoc tests (i.e. Bonferroni) showed that the mean $\delta^{13}C$ composition of POC was significantly more depleted in the forested (-29.7‰ ± 2.4; N=34) compared to the urban (-26.5‰ ± 2.6; N=101) and agricultural (-27.1‰ ± 1.4; N=39) sites (F = 14.9; $p < 0.05$) (Fig. 4b, Table 3). The mean $\delta^{15}N$ values of PON from the urban watershed (4.5‰ ± 2.2; N=101) were significantly different than the agricultural (6.1‰ ± 1.5; N=40) and forested (5.7‰ ± 2.0; N=35) samples (F = 12.3; $p < 0.05$) (Fig, 4a, Table 3). Further analyses showed that the mean $\delta^{15}N$ values of PON were more variable in urban watersheds, which were significantly different from the agricultural and forested sites (F = 8.1; $p < 0.05$) (Fig. 4b). The mean C/N for SPOM was significantly higher for the urban (11.4% ± 5.1; N = 101) compared to the forested (9.4% ± 2. 6; N = 34) and agricultural (9.3% ± 2. 6; N = 40) sites (F = 5.6; $p< 0.05$) (Table 3). The sediment $\delta^{15}N$ values were more enriched in the agricultural compared to the urban and forested sites, although not significantly different (Fig. 5a). The sediment $\delta^{13}C$ isotopic composition did not vary significantly (ranging from -27 to -27.7‰) among watershed types (Fig. 5b, Table 3).

$\delta^{15}N$ and $\delta^{13}C$ composition of mussels

The mean $\delta^{15}N$ values of mussel tissue from agricultural sites (8.5‰ ± 0.6; N=34) were significantly more enriched than the values from the urban (6.6‰ ± 0.67; N=24) and forested sites (6.5‰ ± 0.5; N=30) (F =114.7; $p < 0.05$) (Fig. 5a). Regression analyses of the mean $\delta^{15}N$ values for each trophic level sampled (e.g., primary producers and consumers) were graphically represented between watersheds (Fig. 6a). A GLM analyses found that watershed type was significantly associated with the dependent variable, mean $\delta^{13}C$ of mussel foot tissue (F =139.3; $p < 0.05$). The mean $\delta^{13}C$ mussel tissue values (-33.2‰ ± 0.47; N=14) sampled from the forested sites were significantly more depleted than those from the agricultural (-29.9‰ ± 0.63; N=15) and urban (-30.5‰ ± 0.60; N=24) sites ($p < 0.05$) (Fig. 5b, Table 3). Similarly, mean values of $\delta^{13}C$ shell organic carbonate material sampled from forested watersheds (-31.8‰ ± 2.4; N = 3) were more depleted than the agricultural (-28.8‰ ± 0.7; N = 3) and urban (-28.9‰ ± 0.1; N = 3) samples ($p < 0.05$) (Fig. 5b). Mean $\delta^{15}N$ values of shell organic material sampled from agricultural sites (7.5‰ ± 0.9; N =3) were significantly more enriched than samples from the forested (5.3‰ ± 0.7; N =3) and urban (5.6‰ ± 1.8; N =3) watersheds (F = 12.5; $p < 0.05$) (Fig. 6, Table 3).

Watershed hydrology

The watersheds varied in size from 29 to 106 square miles (75 to 275 sq. kilometers) in area (Table 1, Fig.1). According to GIS percent land use calculations, Bear Creek watershed was predominantly agricultural (67%) compared to the predominantly forested 40-63% land use in both Marks and Black Creek. The Crabtree and Walnut Creek watersheds contained 27-30% urban watershed land use. The number of point and non-point sources from the agricultural watershed sites was higher (14) compared to the forested watershed (Marks and Black Creek) (8) but not higher than the urban (15) sites (Table 2).

Hourly discharge measurements (cfs, cubic feet per second) from the urban watershed (Walnut Creek) over a short term (3-5 day) storm event showed an increase in suspended solids and SPOM C/N ratio during peak discharge (100cfs) and a sharp decrease across time as the hydrograph fell (Fig. 7a). The $\delta^{15}N$ and $\delta^{13}C$ values of SPOM changed quickly across the storm event. A short-term storm event conducted in a forested watershed site (Marks Creek) also showed an increase in suspended solids across rain discharge. There was much less variability in C/N ratios as well as $\delta^{15}N$ and $\delta^{13}C$ values of SPOM (Fig. 7b). The Bear Creek short-term event showed variability in these food quality variables at the start of the storm (Fig. 7c).

Discussion

The use of environmental stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) combined with high resolution hydrologic data has both confirmed prior research (Cabana & Rasmussen, 1996; Fry, 1999; Nichols & Garling, 2000; Raikow & Hamilton, 2001; McKinney et al., 2002) and yielded new insights into the feeding ecology of native mussels. The assessment of mussel food sources and their trophic relationships can provide valuable information about the ecosystem and its organisms (Peterson & Fry, 1987; Vander Zanden et al., 1999). Primary consumers tend to be enriched in $\delta^{15}N$ (3.4‰ ± 1.0) and $\delta^{13}C$ (1.0‰ ± 0.5) values relative to their food sources (DeNiro & Epstein, 1978, 1981; Minagawa & Wada, 1984; Nichols & Garling, 2000).

Streams are important sinks of nitrogen along hydrologic flow paths across the landscape, removing nitrogen through both assimilative uptake by consumers and bacterial denitrification of nitrate (Hamilton et al., 2001). However, in spite of programs aimed at reducing excess nutrient inputs, nitrate flux from inland waters continues to alter aquatic habitats (Vitousek et al., 1997; Mayer et al., 2002; Howarth, 2004; Paerl et al., 2004). The present study showed that surface water nitrate concentrations, $\delta^{15}N$ of DIN, $\delta^{15}N$ of SPOM, and sediment were significantly higher at sites sampled in the agricultural watershed compared to the forested watershed. Mean $\delta^{15}N$ *E. complanata* tissue values were more positive in the agricultural compared to the forested and urban watersheds ($p < 0.05$) (Fig. 5a). These results support the claim that freshwater mussels may reflect nutrient sources (i.e., animal waste) with relatively heavier $\delta^{15}N$ values originating from agricultural land use (Peierls et al., 1991; Ulseth & Hershey, 2005). In both freshwater and estuarine systems, trophic level $\delta^{15}N$ tissue enrichment from primary producer sources (e.g., SPOM, detritus and terrestrial organics) to primary consumers has been documented as a likely response to anthropogenic nutrient loading (Canuel et al., 1995; McClelland & Valiela, 1998; Micheli et al., 2001; Soucek et al., 2001; Carmichael et al., 2004; Savage et al., 2004).

The long-term (36-month) water chemistry results showed significant differences among food sources across different watershed types. Food quality was significantly higher in the forested watershed (C/N ratios of SPOM < 10) compared to the urban and agricultural watersheds (Table 3). The mean C/N values of sediment from the forested watershed were also of higher food quality. The results suggest that suspended particles in the forested watershed may contain higher food quality, which is consistent a mixture of isotopically depleted suspended sources such as bacteria (Nichols & Garling, 2000; Raikow & Hamilton, 2001). During the microbial decomposition process, there is a depletion of the heavier isotope (i.e., more negative $\delta^{13}C$) compared to higher trophic level processes (Coffin et al., 1989). Microbes generally have lower C/N ratios than the detritus to which they attach, so they can enhance detrital quality (Tenore et al., 1982) and improve the nutritional content for bivalves (Jardine et al., 2005). In the present study, the SPOM $\delta^{13}C$ was significantly depleted in the forested watershed ($p < 0.05$; Fig 5b, Table 3). Since *E. complanata* tissue was also significantly depleted in the forested watershed, there may be a connection between primary producer and bivalve consumer sources within this watershed type (Fry & Sherr, 1983). The combined results of the food source data confirmed previous results (Peterson & Fry, 1987; Michener & Schell, 1994; Fry, 1999; Kendall et al., 2001) that suggest refractory organic matter, contaminants attached to particulates, and heavier SPOM $\delta^{13}C$ sources (C_3 plant detritus) may dominate suspended solid load in urban and agricultural watersheds.

Elliptio complanata tissue was significantly depleted in $\delta^{13}C$ values (-33‰) sampled from the forested watershed, which imply that microbial sources may comprise a dominant percentage of their diet ($p < 0.05$) (Fig. 6, Table 3). The importance of bacteria as a food source to native mussels has been examined (Nichols & Garling, 2000). Bacterial sources have been considered an adequate source of carbon and nitrogen for aquatic invertebrates (Conway et al., 1989; Kendall et al., 2001). Studies have shown that native freshwater mussels (*Pyganodon cataracta* and *Amblema plicata*) ingest unicellular cyanobacteria preferentially to phytoplankton species (Baker & Levinton, 2003) and have difficulty assimilating bulk material (Aldridge et al., 1987; Summers et al., 1996). Stable isotopes have been used to detect sources of carbon used by bacterioplankton in estuarine systems (Coffin et al., 1989; Kelley et al., 1998; Krisgard et al., 2004). McMahon (1991) lists bacteria and fine detritus as principal dietary components of freshwater mussels. Growth of bacteria on decomposed particles of allochthonous origin may also account for their negative $\delta^{13}C$ composition (Hall et al., 2000; Nichols & Garling, 2000). The present study suggests that native mussels feed on microbial sources. Whether this is related to forested or urban watersheds is yet to be understood, as the food quality of SPOM and re-suspended sediment may change as a function of flow (Hornbach et al., 1984).

Dominant land use type, point sources, and anthropogenic alterations of stream flow affect food quality across watershed types (Cushing & Allan, 2001). The flux of fine sediment from storm water (agriculture and urban land use) runoff may decrease the success and dispersal of unionid larvae to natural fish hosts and favor the proliferation of non-native bivalve species (e.g. *Corbicula fluminea*). These conditions degrade habitat and contribute to the decline of freshwater mussel populations (Busch et al., 1992; Williams et al., 1993; Box & Mossa, 1999). A mixture of detritus and labile algal sources at higher flow may limit adult unionids' capacity for particle selection (Singh et al., 1991; Boltovskoy et al., 1995; Raikow & Hamilton, 2001). The significant difference found in urban and forested watersheds between $\delta^{13}C$ of SPOM and mussel tissue highlight the need for a more complete investigation of food sources (i.e., microbial heterotrophs) across flow. In forested watersheds, leaf litters broken down by microbial decomposition to fine particulate matter may contribute to the amount of dissolved organic carbon (DOC) (Meyer & Wallace, 1998; Wallace et al., 1999). The $\delta^{13}C$ of SPOM composition may include a mixture of microbial carbon and detritus, which is often utilized by a bivalve's digestive process (Hall & Meyer, 1998; Willows, 1992; Christian et al., 2004). Hetertrophic microbes (bacteria) are thought to use allochtonous carbon sources (Findlay et al., 2002). The result of which may contribute to a transfer of energy to higher trophic levels. A reasonable interpretation of the present study results is that a portion of microbial decomposed leaf litter (i.e., allochthonous source) may be available to mussel consumers in the NRB, forested watershed.

To better understand shorter time scale changes in water quality, a 3 to 5 day discharge event study was measured across watershed type and the isotopic composition of mussel food sources was examined (Fig. 7a, b and c). Storm generated event flows in stream habitats contain elevated concentrations of fine sediment, dissolved organic nutrients (i.e., carbon and nitrogen) and toxic contaminants (Williams & Melack, 1991; Wass & Leeks, 1999; McGlynn & McDonnell, 2003; Tran et al., 2002). Pre-event groundwater, not storm water, can also contribute to the amount to stream flow during precipitation events in forested and agricultural watersheds (Butte, 1994; Renshaw et al., 2003). The sensitivity of aquatic biota to these episodic pulses of contaminates is poorly understood (Dauer et al., 2000).

Descriptive observations of the storm event data showed little change in the isotopic composition of food sources at the forested compared to the urban watershed. The C/N, $\delta^{13}C$ and $\delta^{15}N$ values of SPOM varied with discharge at the urban watershed site (Fig. 7a). Specifically, the $\delta^{13}C$ values increased to an average of -26‰ once the peak discharge occurred, and then remained high during the next 48 hours. The $\delta^{15}N$ values were less clear; however, there was a decrease observed as the C/N values increased. The suspended solids decreased to nearly pre-event levels in 12 hours, but the C/N of SPOM took over 36 hours to return to pre-event levels. The $\delta^{13}C$ and $\delta^{15}N$ of SPOM trends observed in the urban site were in contrast to those during the forested event (Fig. 7b). An increase in food quality (C/N) with discharge was evident in both the urban and forested watershed event; however, the values ranged from 6 to 10 in the forested, which was considerably lower than the urban site (10 to 20). Complementing these results, the long-term data indicated that suspended solids were significantly lower with discharge in the forested compared to the urban and agricultural watersheds ($p < 0.05$) (Table 3). The agricultural watershed event was smaller in discharge and thus more difficult to draw definitive conclusions regarding the compositional changes in food quality.

During short-term discharge events, storm sewers and a high proportion of impervious surfaces in urban environments can increase the proportion of surface runoff and associated contaminates (Booth & Jackson, 1997). Conversely, prolonged low flow conditions in agricultural watersheds may adversely affect stream ecosystems as a result of a concentration of livestock contaminates (Caruso, 2002). These low flow conditions can reduce the contribution of non-point source contaminates (Tavener & Iqbal, 2003). Bivalve biomonitors can integrate pollutant sources over time. A biweekly water chemistry-sampling schedule may underestimate pollution levels from storm discharge events. The results of the present study support previous assertions that the short timescale of storm events prohibits accurate quantification of contaminant concentrations by conventional water quality monitoring (Brassard et al., 2000; Van der Hoven et al., 2002; Chanat & Hornberger, 2003).

Both long and short-term data showed consistently higher food quality and lower average suspended solids in the forested watershed. In the agricultural and urban watersheds, higher discharge is thought to be associated with prolonged exposure of suspended solids, which may overload a native mussels' gill capacity (Morton, 1971; Riisgaard & Randlov, 1981) and physically impede food particles across gill membranes (Aldridge et al., 1987; McMahon, 1991). Unionids are thought to feed continuously with a limited ability to sort particles in the mantle cavity prior to ingestion (Ward & Shumway, 2004). During high flow and low food quality, digestive efficiency and assimilation can be low for native mussels, and much of the ingested material (e.g., algae) passes through the stomach alive and unaltered (Miura & Yamashiro, 1990; McMahon, 1991; Iglesias et al., 1992). Routine monitoring of the isotopic composition of mussel tissue may be an effective way to detect long-term water chemistry impacts on a watershed level (Fry, 1999).

In summary, this field study has raised new questions that challenge our traditional view of freshwater mussel feeding ecology. From a conservation perspective, an impaired ability of native mussels to sort nutritious particles during higher flow may result in decreased assimilation of higher food quality in disturbed watersheds. A reduction in energy intake, if not compensated for, may eventually lead to a decline in growth and reproduction. Also, native mussels' population success might be dependant on competition for food resources with non-native bivalves (e.g., *Corbicula fluminea*). More laboratory studies of bivalve feeding strategies across flow conditions are needed to better understand how these threatened invertebrate's function as land use changes (Chapter 3, Bucci doctoral dissertation).

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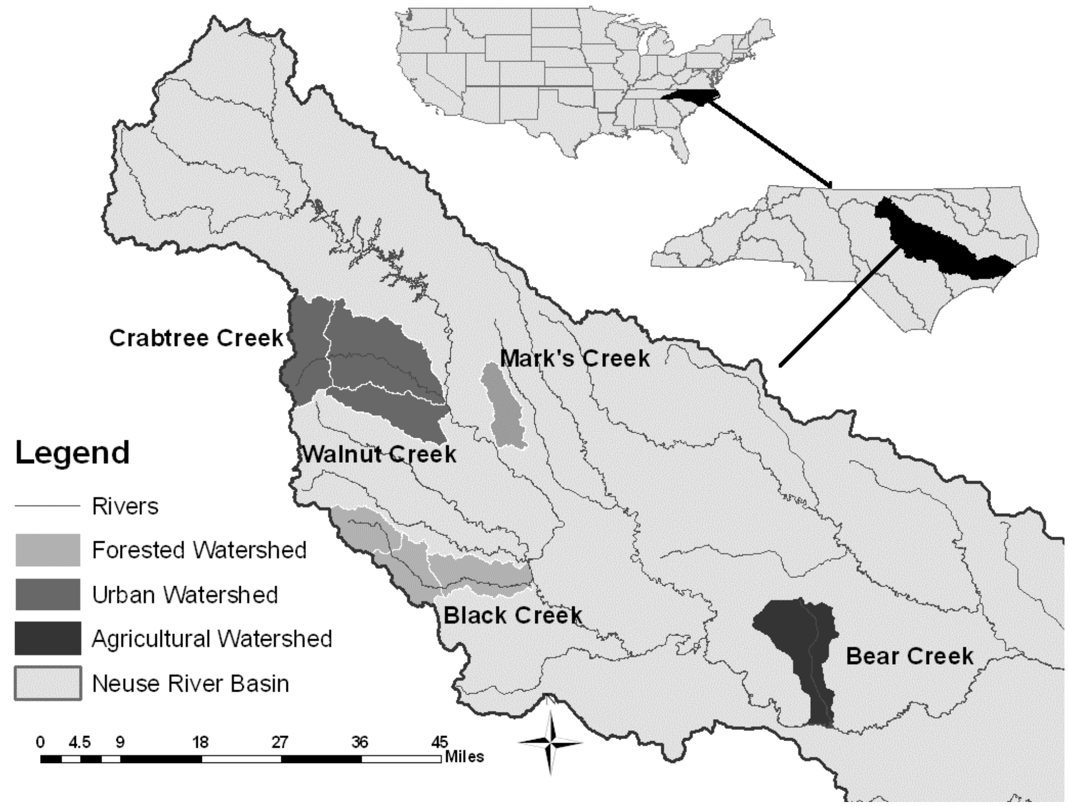
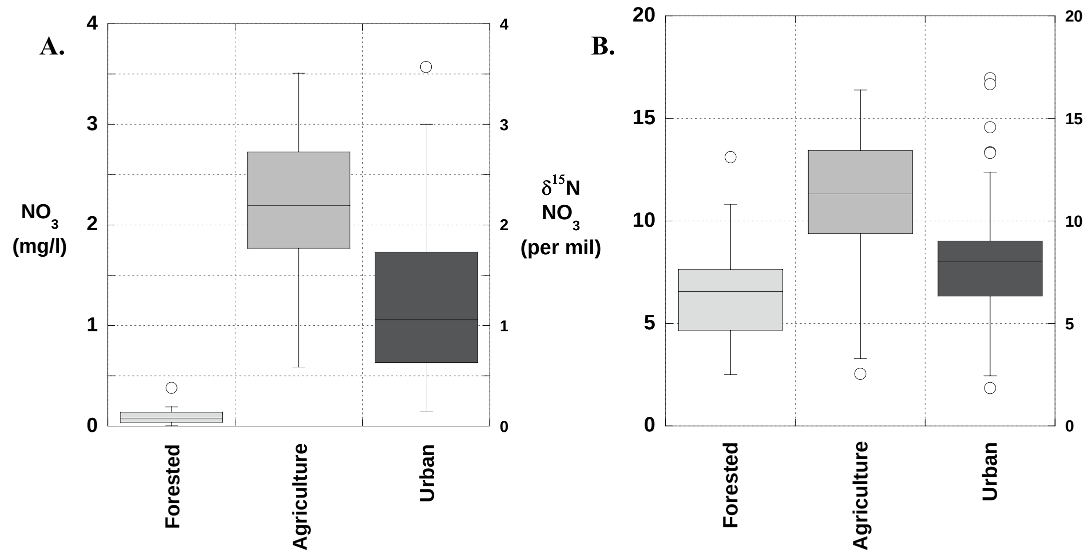


Figure 1: Sampling watersheds classified by different land use characteristics in the Neuse River Basin, North Carolina. Water chemistry and mussels were collected at sites from each watershed type.



Figures 2a: Water chemistry relationships showed that watershed was a predictor of NO_3^- concentration. Specifically, samples from the agricultural watershed was significantly higher in nitrate concentrations than urban and forested watersheds ($p < 0.05$). **2b**: Mean $\delta^{15}N$ values of NO_3^- from the agriculture watershed were significantly heavier than in the urban and forested watersheds ($p < 0.05$).

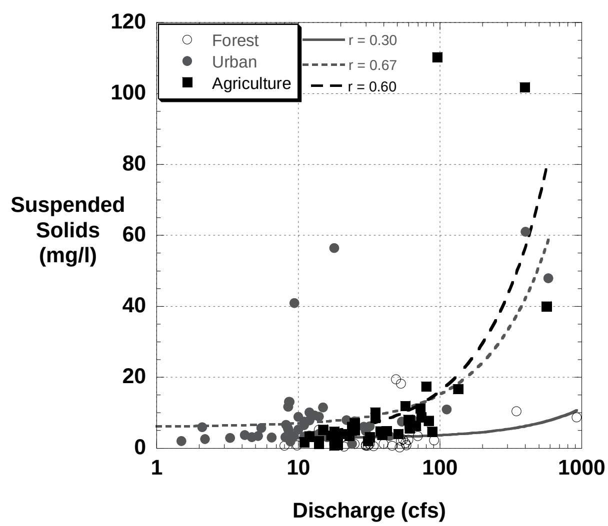


Figure 3: Suspended solids were measured across discharge by watershed across a 36-month period. The Pearson correlation coefficient (r) was stronger among the urban (gray dashed line, r = 0.67*) and agricultural (black dashed line, r = 0.60*) compared to the forested (r = 0.30) watershed. Significance *($p < 0.05$)

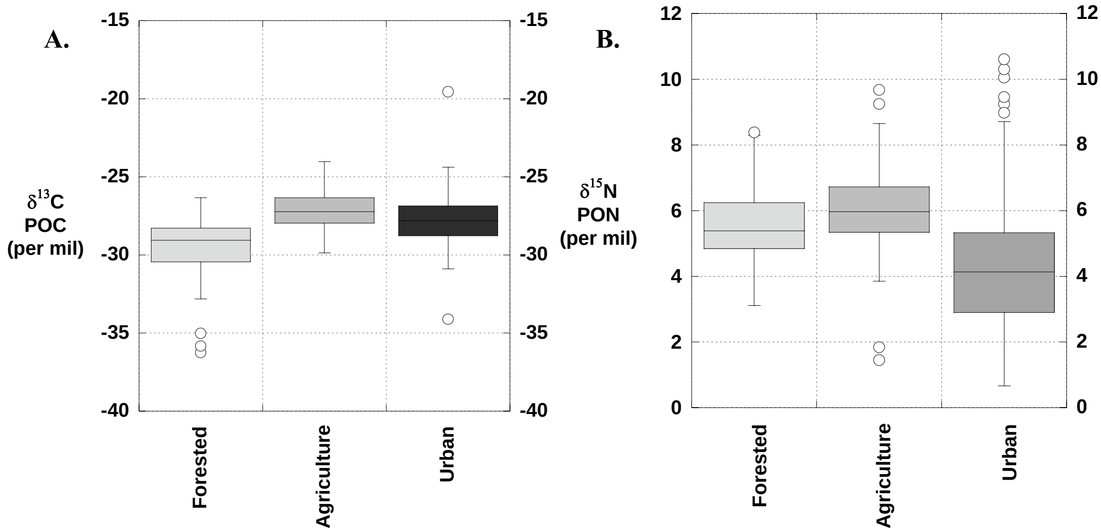
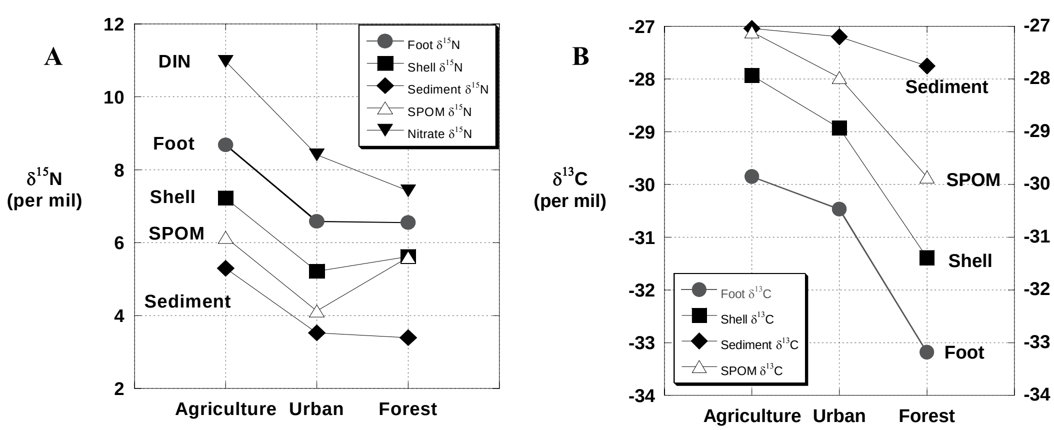


Figure 4a: Data was expressed using a box plot quartile analysis of isotopic composition of SPOM across watershed types. A general linear model (GLM) of $\delta^{13}C$ of POC (SPOM) showed that forested mean values were significantly different than the urban and agricultural watersheds ($p < 0.05$). **4b:** Mean $\delta^{15}N$ values of PON were significantly heavier in the agriculture watershed compared to forested and urban watersheds ($p < 0.05$). A box plot of $\delta^{15}N$ SPOM samples showed the highest variation across the 36-month period was in the urban watershed.



Figures 5a: Mean isotopic ($\delta^{15}N$) composition of *Elliptio complanata* tissue compared to organic matter in the shell, sediment, SPOM, and nitrate (mgL^{-1}) between watersheds. **5b:** A stronger $\delta^{13}C$ diet-consumer relationship was observed between *E. complanata* tissue and SPOM compared sediment as a potential food source. See Table 3 for standard deviations.

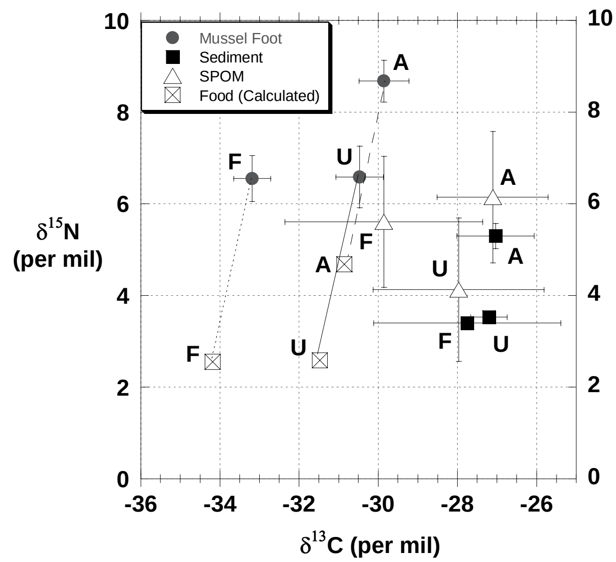


Figure 6: The $\delta^{15}N$ and $\delta^{13}C$ composition of foot tissue of *Elliptio complanata* specimens from agricultural (A), urban (U), and forested (F) watersheds. Error bars are one standard deviation (σ_1) of the variance of replicate analyses. Lines connect the isotopic composition of the foot tissue to the predicted food source composition (☒).

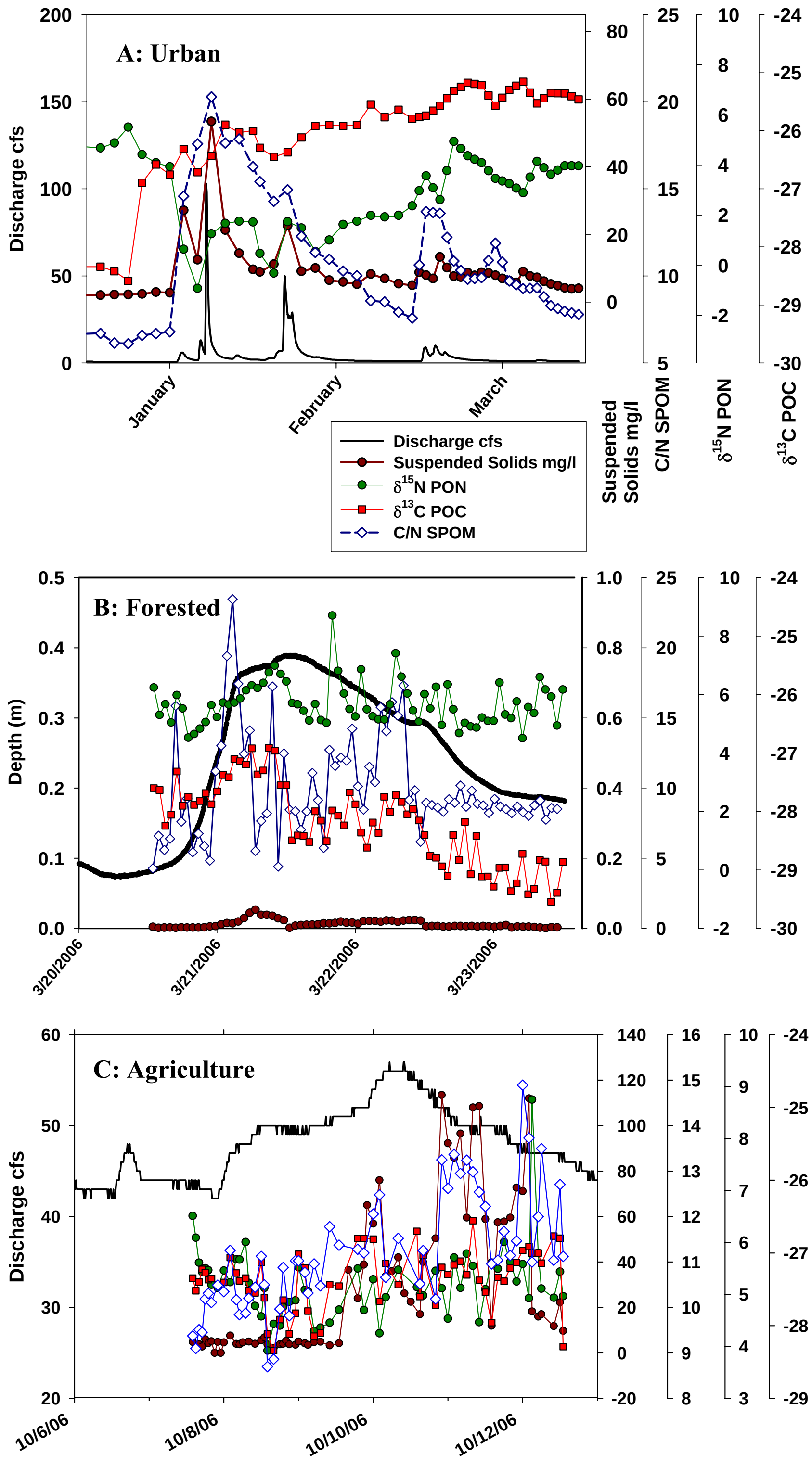


Figure 7: Hourly storm event samples of $\delta^{13}C$ and $\delta^{15}N$ of SPOM and suspended solids from the forested (Marks Creek) urban (Walnut Creek) and agriculture (Bear Creek) watersheds during a 3-7 day discharge event.

Table 1: Percent land use land cover (LULC 2000) across watersheds where *E. complanata* mussels were collected. Watershed designations were based on a combination of point source data, LULC calculated percentages and NCDENR 2002 reports.

	Forested	Forested	Agricultural	Urban	Urban
Watershed	**Marks Creek**	**Black Creek**	**Bear Creek**	**Crabtree Creek**	**Walnut Creek**
Size (sq. miles) (sq. kilometers)	**28.5 73.8**	**105.7 273.8**	**59.0 152.8**	**92.8 240.3**	**46.3 119.9**
% LULC					
Urban	**2.0**	**3.4**	**3.4**	**29.7**	**26.8**
Agricultural	**28.7**	**45.7**	**66.7**	**1.0**	**4.1**
Woody	**63.1**	**40.3**	**23.9**	**55.4**	**50.2**
Herbaceous	**0.8**	**1.2**	**0.7**	**10.7**	**13.7**
Water	**0.8**	**1.6**	**0.7**	**0.9**	**0.9**
Wetlands	**4.1**	**7.2**	**4.6**	**1.1**	**3.3**

Table 2: Point and non-point sources depicted in each of the study watersheds.
Note: NPDES = National Pollutant Discharge Elimination System.

Watershed	NPDES Large (point source)	NPDES Small (point source)	Animal Farms (non-point)	Summary of sources
Marks Creek	**0**	**1**	**0**	**1**
Black Creek	**0**	**2**	**5**	**7**
Bear Creek	**0**	**0**	**14**	**14**
Crabtree Creek	**1**	**10**	**3**	**14**
Walnut Creek	**0**	**0**	**1**	**1**

Table 3: Water chemistry, *E. complanata* tissue and food sources estimated across three watersheds in the NRB.

Parameter estimate	***Watershed***					
Water chemistry	**Agriculture**		**Urban**		**Forested**	
	Mean	Sample Size	Mean	Sample Size	Mean	Sample Size
NO_3^- (mgL^{-1})	2.3 ± 0.6	46	1.2 ± 0.7	111	0.09 ± 0.7	35
$\delta^{15}N$ of NO_3^- (mgL^{-1})	11.2 ± 2.5	43	8.0 ± 3.0	109	6.5 ± 2.3	35
Suspended solids (mgL^{-1})	11.3 ± 22.1	45	8.1 ± 14.5	108	7.5 ± 7.0	35
$\delta^{15}N$ *E. complanata* foot tissue (‰)	8.5 ± 0.61	34	6.6 ± 0.67	24	6.5 ± 0.51	30
$\delta^{15}N$ *E. complanata* shell organic (‰)	7.5 ± 0.9	3	5.3 ± 0.7	3	5.6 ± 1.8	3
$\delta^{15}N$ SPOM (‰)	6.1± 1.5	40	4.5 ± 2.2	101	5.7 ± 2.0	35
$\delta^{15}N$ Sediment (‰)	5.3 ± 0.12	3	3.5 ± 0.16	3	3.4 ± 0.39	3
$\delta^{13}C$ *E. complanata* foot tissue (‰)	-29.9 ± 0.63	15	-30.5 ± 0.60	24	-33.2 ± 0.47	14
$\delta^{13}C$ *E. complanata* shell organic (‰)	-28.8 ± 2.4	3	-28.9 ± 1.7	3	-31.8 ± 2.4	3
$\delta^{13}C$ SPOM (‰)	-27.1 ± 1.4	39	-26.5 ± 2.6	101	-29.7 ± 2.4	34
$\delta^{13}C$ Sediment (‰)	-27.0 ± 0.09	3	-27.2 ± 0.41	3	-27.7 ± 0.2	3
C /N SPOM	9.3 ± 2.6	40	11.4 ± 5.1	101	9.4 ± 2.6	34
C/ N Sediment	15.1 ± 5	2	19.9 ± 2.3	3	9.9 ± 2.8	2

CHAPTER 2

A COMPARISON OF FOOD SOURCES FOR NATIVE (*ELLIPTIO COMPLANATA*) AND NON-NATIVE (*CORBICULA FLUMINEA*) FRESHWATER BIVALVES IN NORTH CAROLINA

Abstract

The main objective of this study was to compare the isotopic composition of muscle tissues and food sources of native (*Elliptio complanata*) and non-native (*Corbicula fluminea*) freshwater bivalves across watersheds with different hydrology and water chemistry in a North Carolina River Basin. Through the use of stable isotope analyses and watershed level water chemistry assessment, the present study identified an association between dominant watershed type and bivalve isotopic ($\delta^{15}N$ and $\delta^{13}C$) tissue composition. Results suggest that the urban watershed may be non-point source dominated at higher flow and point source dominated at lower flow. This was evident as nitrate concentration decreased with discharge indicating a possible dilution effect ($p < 0.05$; $r = -0.31$). Food quality as measured by C/Nratio of particulate matter increased significantly with discharge in the agricultural watershed ($p < 0.01$), but showed little change in the forested watershed. A general linear model (GLM) showed a significant association between isotopic composition of species and watershed ($p < 0.05$; $F = 151$). Both *C. fluminea* (10.6‰ ± 0.13; N = 13) and *E. complanata* (8.5‰ ± 0.61; N = 3) $\delta^{15}N$ mean values were significantly more enriched in the agricultural than the urban and forested watersheds. Both *C. fluminea* (-33.8‰ ± 1.2; N = 5) and *E. complanata* (-33.2‰ ± 0.47; N = 14) $\delta^{13}C$ mean values were significantly more depleted in the forested compared to the urban and forested watersheds ($p < 0.05$; $F = 147$). The

implications are that non-native (*C. fluminea*) bivalves may better tolerate low food quality associated with disturbed watersheds (i.e., urban, agriculture) where discharge and non-point runoff increase suspended solids and turbidity.

Introduction

North American freshwater mussels (Family Unionidae) have been in decline, with nearly 70% of species extinct, endangered, threatened, or of special concern as a result of increased sedimentation, habitat destruction, loss of fish hosts, and exploitation (Riccardi & Rasmussen, 1999; Bogan, 1993; Williams et al., 1993). Little is known of the feeding ecology of these important mussels as changing land use contributes to increased water pollution and sediment loads coupled with a reduced availability of nutritious food sources (Penrose et al., 1980; Vaugh & Hakencamp, 2001; Baker & Levinton, 2003 Lydeard et al., 2004). Information on the feeding strategies across watershed type is needed to understand the causes of their decline and to better understand their effectiveness as biomonitors. In North Carolina, the effect of increased urbanization and construction activities have been associated with higher than average sediment erosion rates, increased storm runoff and increased peak discharge (Leopold, 1994; NCDWQ, 2002; White & Greer, 2006). Streams in the piedmont area of North Carolina are particularly susceptible to sediment problems because erodible soils contain a high percentage of clay-sized particles (Lenat & Crawford, 1994). Agriculture runoff is also a source of sediment and nutrient problems because of the large amount of animal and fertilizer waste contributing to adjacent stream systems (Cooper 1993; Henze et al., 2002).

Combined with this issue, the introduction and spread of the non-native bivalve species (*Corbicula fluminea*) [Müller, 1774] throughout the eastern United States has had an adverse impact on biotic and abiotic characteristics in freshwater ecosystems (Carlton & Geller, 1993; McMahon, 2000; Bogan, 2002). The ecosystem wide impacts on nutrient cycling and native species feeding ecology are not fully understood (Newell & Ott, 1999). Non-native bivalves reside in a wide range of habitats (especially tolerant in urban and agricultural watersheds) and may compete for suitable habitat and food resources with native unionids (Pennack, 1989; Belanger, 1991; Bogan, 1998). There is a need to investigate the feeding ecology of both native and non-native bivalve species where they both reside as land use continues to alter allochthonous and autochthonous food sources.

Freshwater ecosystems experience a range of contaminant levels in stream habitats flowing from large urban areas toward coastal riverine waterways (Aldridge et al., 1987; Hornberger et al., 2000; Cushing & Allan, 2001; Cole et al., 2004). The characterization of water chemistry (i.e., suspended solids, flow, nutrient concentrations) related to contaminants by watershed is essential in evaluating bivalve feeding ecology, (Doherty, 1990; Metcalfe & Charlton, 1990; Soucek et al., 2001).

Since flow, nutrients, and food quality are related (Kendall et al., 2001), an important question regarding the conservation of native bivalve species is how they feed and thus bioaccumulate contaminants compared to non-native bivalves in watersheds with variable flow. It has been suggested that bivalves may preferentially select nutritive particles more effectively at different points across discharge of a storm event (Belanger, 1991; Cloern et al., 2002; Rogers, 2003). Watersheds dominated by non-point sources tend to disperse contaminants with rainfall in intensive urban and agricultural land use, which are more difficult to control than point sources (USEPA, 2002; Old et al., 2006). These substances adsorb to suspended solids from stream erosion and surface runoff and enter waterways contaminating the sediments. In addition, elevated discharge may be associated with a reduction in contaminant concentrations in urban watersheds (Williams & Melack, 1991; Bales et al., 1993).

Stable isotope studies have been used to examine the role and relative importance of food sources in supporting bivalve diet (Incze et al., 1982; Thorp et al., 1998; Raikow and Hamilton, 2001). Since hydrological and water chemistry parameters are known to influence these factors (Fry, 1999), potential differences in dominant food sources for bivalves across watersheds with different land use may influence growth, reproduction and population success. In addition, species differences in isotopic signatures may reflect potential differences in resource availability and contributions to bivalve nutrition. The main objective of this study was to compare the isotopic tissue and food sources of native (*E. complanata*) and non-native (*C. fluminea*) freshwater bivalves across watersheds with varied hydrology and water chemistry in North Carolina. The specific goal was to test whether there was a difference in mean $\delta^{15}N$ and $\delta^{13}C$ tissue values between non-native (*C. fluminea*) and native (*E. complanata*) tissue collected from watersheds over different flow regimes.

Methods

Bivalve collection and processing

Non-native bivalves (*Corbicula fluminea*) were sampled from first to third order creeks in three watersheds with different land use; Crabtree Creek (urban), Bear Creek (agricultural), and Marks Creek (forested, NCDWQ, 2002). Waders collected resident bivalves with stainless steel clam rakes or by hand from the benthic substrate. Ten adult specimens from each site were sampled during a 12-month period (Spring 2005-06). Previously collected (2001-03) isotopic data of *Elliptio complanata* tissue were used in the comparison analyses in this study. The size of *C. fluminea* specimens was measured with digital calipers (± 0.1mm accuracy) and kept to an average range of 15 mm to 19 mm in height and (Gosling, 2003). Native mussels (*E. complanata*) ranged in size from 20 to 28 mm in height.

The samples were placed on ice in the field to minimize digestion processes. Whole bivalve samples were rinsed in de-ionized water and kept frozen prior to isotopic analyses. The foot tissue of each individual specimen was extracted and washed with de-ionized water, then treated with 10% HCl (to remove carbonate material), and rinsed again with de-ionized water. Lyophilized tissue samples were ground in an agate mortar and pestle. The ground tissue samples (100-400μg) were placed into tin boats and analyzed for $\delta^{13}C$ and $\delta^{15}N$ in a Finnigan MAT Delta + XL continuous flow isotope ratio mass spectrometer coupled with a Carlo Erba NC-2500 CNS Elemental Analyzer. Sample isotopic results for tissue $\delta^{13}C$ were calibrated against NBS 22, 21, and NIST 8545, 8542 and 5 internal lab standards. Isotopic results for tissue $\delta^{15}N$ were calibrated against NIST 8550, 8549, 8548, 8547, and 7 internal lab standards.

Independent measures

Water samples (250ml) were collected and analyzed from surface waters in 1 and 4-liter acid washed (0.1 M HCl) Nalgene® containers. Water chemistry samples were collected bi-monthly and analyzed for temperature, pH, nitrate concentrations (mgL^{-1}), $\delta^{15}N$ of dissolved nitrate (NO_3^-), $\delta^{15}N$, $\delta^{13}C$ and C/N of SPOM and suspended solids mgL^{-1} at NC State University. The discharge (cubic feet per second, cfs) data were taken from USGS gauging stations located at all sites. Water chemistry and discharge parameters were sampled over a 36-month period (June 2000 to June 2003) on a monthly basis from Bear Creek (agricultural), Walnut Creek (urban) as well as Black and Marks Creek (forested) watersheds in the NRB (Fig. 1, Table 1). Previously collected water chemistry measurements (May 2000 to May 2005) were also considered in the analyses (NCDWQ, 2002; USGS, 2005). Surficial sediment was sampled at the time of bivalve collection and measured isotopically as a potential food source.

The $\delta^{15}N$ of dissolved nitrate (DIN) was analyzed using a modification of the technique of Chang et al. (1999) (Karr et al., 2001; Savage et al., 2004) Water samples (200ml to 1L) were collected in acid cleaned Nalgene® bottles. Nitrate was extracted from a water sample on prepackaged 2 ml ion exchange columns at the collection site. The nitrate was diluted from the loaded ion exchange columns and converted to silver nitrate. The silver nitrate was analyzed for $\delta^{15}N$ and $\delta^{18}O$ with a Finnigan MAT Delta XL IRMS, coupled to a NC2500 elemental analyzer and a TCEA pyrolysis analyzer. The %C and %N of particulate samples were determined from sample weights and integrated peak areas calibrated against the 8 NIST/NBS standards and 7 internal standards. The C/N ratios are conventional but indirect measures of food quality of particulate matter (Iglesias et al., 1996). Generally, phytoplankton has lower C/N ratios and detritus have higher (less nutritious) values (Valiela, 1995).

Statistical analyses

Variations in isotopic tissue ($\delta^{15}N$ and $\delta^{13}C$) data were examined as a function of nitrate concentrations (mgL^{-1}), $\delta^{15}N$ of dissolved inorganic nitrogen, and suspended solids associated with discharge. Pearson's (r) correlation coefficient analyses were used to measure the variability between independent variables (i.e., water chemistry) across watershed types (Cohen, 1988). The strength and direction of these long-term (36-month) water chemistry trends were evaluated. General linear regression models (GLM) were used to measure differences between isotopic tissue values by species and potential food sources (i.e., $\delta^{15}N$ and $\delta^{13}C$ of SPOM and sediment). A GLM also was used to evaluate relationships between water chemistry and bivalve tissue ($\delta^{15}N$ and $\delta^{13}C$ mean values) from the various watershed types and species in the (NRB) Neuse River Basin (SAS, 2002). Interactions between species and watershed were also assessed with the model (Scheiner & Gurevich, 2001).

Results

Watershed type was a significant predictor of NO_3^- concentration ($p < 0.05$; Bucci doctoral dissertation, Chp. 1). Mean nitrate concentrations of surface water samples were significantly higher in the agricultural watershed (2.3 mgL^{-1} ± 0.6; N=46) compared to the urban (1.2 mgL^{-1} ± 0.7; N=111) and forested (0.09 mgL^{-1} ± 0.7; N=35) watersheds (Table 1). Discrete water samples taken in 2006 supported this trend as the forested watershed showed low mean NO_3^- values (0.03 mgL^{-1} ± 0.02; N=7). Dissolved inorganic nitrogen ($\delta^{15}N$ of NO_3^-) showed mean values from the forested watershed (6.5‰ ± 2.3; N=35) that were significantly more depleted than the agriculture (11.2‰± 2.5; N=43) and urban (8.0‰ ± 3.0; N=109) watersheds ($p < 0.05$). Also, there was a significantly more variation in mean suspended solids (mgL^{-1}) in the urban and agricultural compared to the forested watersheds over a 36-month period ($p < 0.05$) (Table 1). Further analyses showed that mean suspended solids were lowest in the forested watershed ($p < 0.05$; 7.5 mgL^{-1} ± 7.0; N=35; Fig. 2).

Nitrate and suspended solids versus discharge relationships showed a moderately strong correlation in the agricultural watershed ($r = 0.47$ and 0.61, respectively) (Fig. 2a; $p < 0.05$). In the urban watershed, there was an inverse relationship between nitrate and discharge ($r = -0.31$) and a positive correlation between suspended solids and discharge ($r = 0.67$) (Fig. 2b; $p < 0.05$). A positive correlation was observed in the forested watershed for both nitrate ($r = 0.16$) and suspended solids ($r = 0.46$) by discharge, however it was not significant (Fig. 2c).

The C/N of SPOM increased significantly with discharge in the agricultural watershed ($p < 0.01$), but showed little change in the forested watershed. SPOM C/N ratios reached maximum values (>25) at intermediate discharge levels in the urban watershed, and this watershed also had the highest particle C/N compositions of the three watersheds with different land use (Fig. 3). The isotopic food source data (36-month) was evaluated across discharge levels. The mean values for $\delta^{15}N$ of SPOM showed a moderately strong significant correlation in the forested watershed with respect to discharge ($r = -0.39$; $p < 0.05$) (Fig. 4a). Nitrogen isotopic values of particulate food sources from urban watershed showed no significant relationship ($r = 0.003$), which was similar to the agricultural watershed ($r = 0.037$) (Fig. 4a). In the forested ($r = 0.38$) and agriculture ($r = 0.40$) watersheds, there was a moderately positive significant relationship between $\delta^{13}C$ values of SPOM versus discharge (Fig. 4b; $p < 0.05$). This relationship was not significant in the urban watershed ($r = 0.047$).

A comparison of food sources from the water column (SPOM) and surficial sediment with bivalve tissue showed that mean $\delta^{15}N$ values of SPOM were significantly more enriched in the agricultural compared to the urban watersheds ($p < 0.05$; $F = 12.5$) (Fig. 5a). The mean $\delta^{15}N$ values of *C. fluminea* tissue from the agricultural watershed were reflective ($\delta^{15}N$ >10‰) of DIN sources derived from heavily enriched point sources such as animal waste (Fig. 6a; Kendall et al., 2001). *C. fluminea* tissue was 2‰ more enriched in $\delta^{15}N$ mean values than *E. complanata* in the agriculture watershed and 1.7‰ more depleted in the forested watershed ($p < 0.05$). The mean $\delta^{13}C$ values of *C. fluminea* tissue were more closely associated (within 1‰ ± 0.5) with SPOM as a food source in the urban compared to the agricultural and forested watersheds (Fig. 5b, Table 1). The mean $\delta^{13}C$ of SPOM values were significantly more depleted in the forested (-29.7‰ ± 2.4) compared to the agricultural (-27.1‰ ± 1.4) and urban (-26.5‰ ± 2.6) watersheds ($p < 0.05$) (Fig. 5b).

According to the GLM results, the effect of isotopic $\delta^{15}N$ ($p < 0.0001$; $F = 151$) and $\delta^{13}C$ ($p < 0.0001$; $F = 220$) tissue depended on the watershed (Fig. 6). In terms of $\delta^{13}C$ mean values, both *C. fluminea* (-33.8‰ ± 1.2; N = 5) and *E. complanata* (-33.2‰ ± 0.47; N = 14) were significantly more depleted in the forested compared to the urban and agricultural watersheds ($p < 0.0001$; $F = 13.5$; Fig. 6c and d). Further multivariate analyses showed a significant interaction between watershed and species with respect to $\delta^{15}N$ ($p < 0.0001$; $F = 21$) (Table 1). Specifically, *C. fluminea* $\delta^{15}N$ mean values (10.6‰ ± 0.13; N = 13) were significantly more enriched than *E. complanata* $\delta^{15}N$ mean values (8.5‰ ± 0.61; N = 3) in the agricultural watershed compared to the urban and forested watersheds (Fig. 6a and b; $p < 0.05$). In the urban watershed, $\delta^{13}C$ mean values for *C. fluminea* (-28.8‰ ± 0.08; N = 9) were significantly more positive than *E. complanata* (-30.5‰ ± 0.6; N = 24) (Fig. 6c and 6d).

Finally, the isotopic composition of SPOM was examined against C/N ratios by watershed (Fig. 7). A significant relationship was observed between $\delta^{13}C$ and C/N of SPOM in the forested watershed ($p < 0.01$; $F = 7.06$).

Discussion

Both *C. fluminea* and *E. complanata* showed isotopic ($\delta^{15}N$ and $\delta^{13}C$) tissue differences between watershed types. The results of this study showed significant differences in bivalve tissue related to hydrology across watershed types. Although food source quality can vary across watersheds seasonally, water chemistry and hydrology appear to interact and affect bivalve feeding ecology. Knowledge of potential differences in feeding across different turbidity levels and flow conditions observed in the NRB may have implications for native bivalve declines prevalent in the Southeastern U.S.

Watershed level differences in hydrology and food quality were apparent in the present study. The increased turbidity and high C/N ratios of SPOM and sediment at urban and agricultural sites suggest that food quality decreased with increased discharge. Similarly, nitrate concentrations and $\delta^{15}N$ of dissolved nitrogen were highest at the agricultural site, suggesting an animal waste source. Suspended sediment yield was greatest in the urban watershed and least in the forested watershed. These results were consistent with a land use study of three streams in the North Carolina piedmont (Lenat & Crawford, 1994). However, at low to moderate flow, suspended solids concentrations were greatest at the agricultural site, unlike the present study where this condition was associated with the urban watershed.

The characterization of potential trends in bivalve feeding ecology and their exposure to sediment is essential to better understand differences between native and non-native species. Urbanization-induced impacts (i.e., increased sediment load) to streams are related to changes in impervious land surface and channel incision (Wahl et al., 1997; Ryan & Packman, 2006). Intense urbanization leads to an increase in the fraction of fine sediment, wastewater effluent discharge (Paul & Meyer, 2001) and non-point source run-off with discharge (Rose & Peters, 2001). Thus, the composition of bivalves' diet may be impacted by storm event driven changes in urban watersheds. The long-term water chemistry data from the urban watershed showed that nitrate decreased with discharge indicating a dilution effect ($p < 0.05$; $r = - 0.31$; Fig. 2b). As previously suggested (Old et al., 2006), urban watersheds may be non-point source dominated at higher flow and point source dominated at lower flow. Conversely, the results from the forested watershed indicated that water quality did not vary substantially with discharge.

The present study highlights the need to better understand how event driven contaminant load can impact bivalve feeding and thus bioaccumulation of contaminants (Nixon et al., 1986; Howarth, 1996; McClelland & Valiela, 1998; Vander Zanden et al., 2004). Previous studies have not adequately examined the potential differences in feeding ecology between native and non-native bivalves, which include their ability to discriminate nutritive food sources and bio-accumulate pollutants (Osenberg et al., 1994; Jardine & Cunjak, 2003). Significantly heavier mean $\delta^{15}N$ values for both species were positively correlated with sites that showed significantly higher nitrate concentrations (i.e., agricultural) ($p < 0.05$; Fig. 5a). Furthermore, mean $\delta^{15}N$ values of NO_3^- (DIN) were significantly heavier the agriculture compared to the urban and forested watersheds ($p< 0.05$) (Chapter 1, Bucci doctoral dissertation). As previously reported, the isotopic composition of bivalve tissue can reflect anthropogenically (i.e., animal wastewater, nitrification of fertilizer nitrate) derived nitrate in aquatic systems (Lenat & Crawford, 1994; McClelland & Valiela, 1998; Soucek et al., 2001; Kendall et al., 2001; Savage et al., 2004). The association between heavy $\delta^{15}N$ values of nitrate and bivalve tissue support previous food web studies conducted in San Francisco and North Carolina estuaries (Fry, 1999; Ulseth & Hershey, 2005; Bucci et al., 2007). These relationships indicate that anthropogenic nitrogen loading has the potential to impact nutrient dynamics in these food web ecosystems (Minigawa & Wada, 1984).

Both species reflected significantly more negative $\delta^{13}C$ values in the forested compared to agriculture and urban watersheds, indicative of a bacterial food source, which indicates a potential influence of DOC in these watersheds (Fig. 5b, 6c and d). The microbial portion of organic matter has shown to be an important carbon source for bivalves (Roditi et al., 2000; Nichols & Garling, 2000; Christian et al., 2004). In freshwater, forested systems, microbial decomposition of organic matter (Cushing & Allan, 2001; Kelley et al., 1998) has been linked to depleted values (-30 to -33‰). Bacterial assemblages associated with fine organic fractions may utilize a portion of bulk particulate organic carbon, such as suspended colloidals (Blair et al., 1985, Jahnke et al., 1995). Organic matter particles dominated by microbial activity have lower C/N values than detritus to which they attach, and can enhance detrital food quality (Odum et al., 1979; Tenore et al., 1982). The present study used smaller sized (0.7 µm) filters, which confirm similar isotopic results of SPOM sources (Raikow & Hamilton, 2001).

The carbon and nitrogen isotopic compositions (C/N, $\delta^{15}N$ and $\delta^{13}C$) of SPOM sources have been examined on a larger spatial scale in major rivers in the United States (Kendall et al., 2001). The data suggest that bivalve food sources can vary (Ahlgren & Hyenstrand, 2003) as nutrient uptake by primary producers varies with flow (Elser et al., 2000; Tank & Dodds, 2003). Biological degradation of organic matter can also affect the nature of these sources, producing a higher percentage of detritus based particulates (Caraco et al., 1998). Researchers agree that the composition of these sources include live and dead phytoplankton algae, terrestrial organics (decomposed leaf litter), soil organic matter and macrophytes (Cushing & Allen, 2001). However, more work is needed to understand the complex factors that control bivalve food quality and water chemistry in a range of watershed types.

In forested streams, the $\delta^{13}C$ measurements of dissolved organic carbon (DOC) have been shown to support bacterial production (Fogel et al. 1992; Lenat & Crawford, 1994). This connection may appear counter-intuitive since light limits algal growth with increased tree cover in forested watersheds (Lowe et al., 1986; Cushing & Allan, 2001). However, large amounts of labile carbon are associated with plant leaf litter and microbial decomposition (Cushing & Allan, 2001), which contributes to a homogeneous mixture of bulk carbon, suggesting allochthonous energy sources in forested watersheds (Whitford, 1960; Kritzberg et al., 2004). A significant relationship between the compositions of $\delta^{13}C$ SPOM versus the C/N of SPOM in the forested watershed may support the claim that a more homogeneous food source is evident in the forested watershed ($p < 0.01$; Fig. 7a). Conversely, the relationships observed between $\delta^{15}N$ and $\delta^{13}C$ of SPOM and C/N (Peterjohn & Correll, 1984) may include a mixture of bulk SPOM in the agricultural and urban watersheds (Fig. 7b and c). The combined results of $\delta^{13}C$ SPOM values (-26‰ ± 0.8) in the agriculture and urban watershed suggest that bivalve food quality may be predominantly heterogeneous (e.g., soil organic matter, terrestrial plants, microbes, and detritus) (Boutton, 1996; Table 1). Thus, autochthonous productivity (i.e., plankton) combined with a large detritus component are likely important energy sources for both native and non-native bivalve species in these streams; especially in summer when light is abundant and high concentrations of nitrate are available (Schlosser & Karr, 1981; Lenat & Crawford, 2004).

The relatively lower C/N particulate values observed in the forested watershed suggest that both native and non-native bivalves can ingest higher quality particles across a range of flow regimes (Fig. 3). Native bivalves in particular may not ingest nutritive sources at higher flow levels where food quality is consistently low. The $\delta^{13}C$ SPOM results across all three watersheds showed enrichment with discharge; however the relationship was strongest in the agriculture watershed ($r = 0.40$; $p < 0.05$) (Fig. 4b). Since $\delta^{13}C$ SPOM was more positive at higher flow, it is plausible that *C. fluminea*, which showed significantly more positive $\delta^{13}C$ tissue values may show the unique ability to sort particles and feed in watersheds that experience higher flow (urban and agricultural) (Fig. 6c and d). However, it is clear that C. *fluminea* and *E. complanata* exhibited a range of $\delta^{15}N$ and $\delta^{13}C$ tissue values in the forested and agricultural watersheds, indicating assimilation of a variety of food sources (Fig. 5a and b). Specifically, the stronger relationship between *C. fluminea* and *E. complanata* $\delta^{13}C$ tissue values in the urban watershed suggests that non-native bivalves may assimilate a lower food quality and more positive $\delta^{13}C$ SPOM source associated with urban watershed types. Our study has provided a baseline characterization of the isotopic composition of bivalve food sources and confirmed previous results that suggest watershed type may influence the relationships between bivalve species' diet and isotopic tissue fractionation (Adams & Sterner, 2000, Vanderklift & Ponsard, 2003).

The multivariate (GLM) analyses showed significant interactions of $\delta^{15}N$ and $\delta^{13}C$ tissue values between species and watershed ($p < 0.05$) (Fig. 6; Table 2). More enriched $\delta^{15}N$ values were evident for *C. fluminea* compared to *E. complanata* tissue values (Fig. 6a, b).

The notion that watershed hydrology may influence food sources and thus contaminant levels (i.e., nutrients, heavy metals) for bivalve consumers is not new (Fry, 1999; Hornberger et al., 2000; Croteau et al., 2004). Variable exposure to these sources could complicate the bio-monitoring usefulness of bivalves (Allen et al., 2002). The $\delta^{13}C$ values for *C. fluminea* were closer to a predicted SPOM food source compared to *E. complanata* in the urban watershed (Fig. 5b). Although $\delta^{13}C$ of consumer tissue is closely related to that of its food source (DeNiro & Epstein, 1978; Fry & Sherr, 1984), there are negligible differences as a result of tissue isotopic fractionation, with the animal being 0.7-1.4‰ heavier than its food (Rau et al., 1983). However, in the present study, differences were within the limits of typical analytical error (Lancaster & Waldron, 2001; Christian et al., 2004).

The results suggest that *C. fluminea* may select smaller sized particles of higher nutritious value such as bacteria, which may be attached to detritus during higher turbidity and flow (Jorgensen, 1990; Sierszen & Frost, 1992; Nichols & Garling, 2000). Conversely, *E. complanata* may not possess this selectivity response but consume food sources that are readily available. Implications are that non-native (*C. fluminea*) bivalves may have better population success in disturbed watersheds (i.e., urban) and show a tolerance for increased suspended solids with greater flow (Kiorboe & Mohlenberg, 1981; Boltovskoy et al., 1995; Baker et al., 1998; Sylvester et al., 2005). Native mussels may be less efficient at retaining smaller sized particles (0.5 -1µm) at higher clearance rates throughout their digestive process than non-native species (Way et al., 1990; Tankersley, 1996; Gosling, 2003; Baker & Levinton, 2003). Thus, native mussels may be better suited as biomonitors in lower flow, which was found predominantly in the forested watershed.

A more detailed examination of water chemistry across seasonal periods is needed to determine a more complete description of the food sources available to bivalves. Also, the present study was limited to one size fraction (0.70µm) in the isotopic identification of SPOM. A range of finer fractions (< 0.70 to 0.30µm) of suspended particulates could strengthen the present study results. As an additional method of calculating food sources, isotopic measurements of DOC are needed to understand the relationship of groundwater inputs (Christian et al., 2004). Further evaluation of biochemical markers (e.g., lipids, fatty acids) present in digestive glands of bivalves may be used in combination with isotopic assays to further identify dominant bivalve food sources (Wolf-Rainer et al., 1998; Narbonne et al., 1999).

The importance of the present study is that it identified an interaction between watershed type and bivalve bioaccumulation of $\delta^{15}N$ and $\delta^{13}C$ sources between native and non-native species. This could lead to an improved understanding of the role of bivalves as biomonitors across different turbidity and flow conditions. Also, an understanding in feeding ecology differences by species may contribute our understanding of how non-native bivalves compete for food sources and contribute to the decline in native mussel populations in the Southeast U.S. (McMahon, 1991; Bogan, 1993). Evolutionarily, *C. fluminea* may respond better to higher sediment load compared to *E. complanata*, since they originate from larger river drainage basins proximal to mountainous regions (Asian continent) that are associated with higher average turbidity (Milliman & Meade, 1983).

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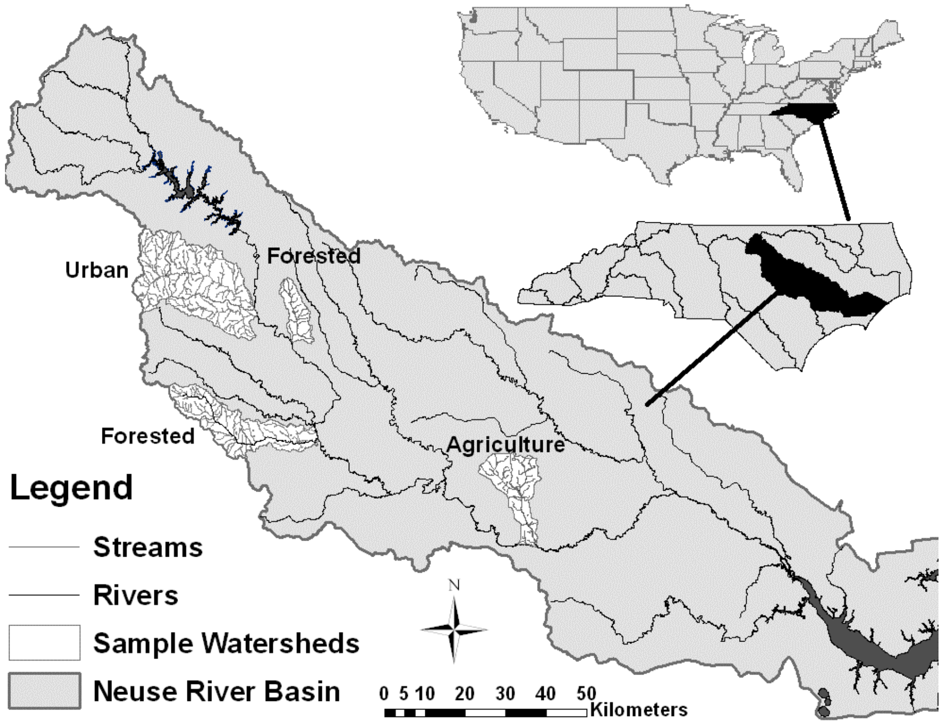

Figure 1: Map of sampling watersheds by different land use characteristics in the Neuse River Basin, North Carolina. See Map 1A in appendix for detailed color version of watershed land cover.

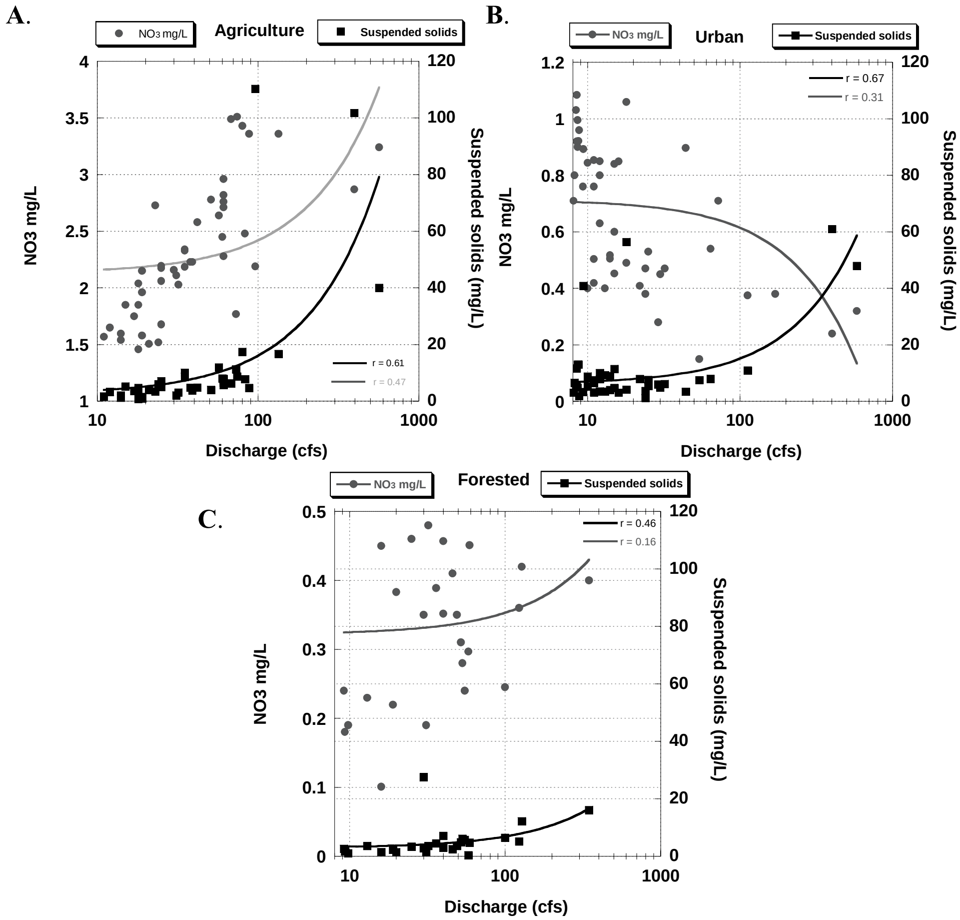

Figure 2: Nitrate concentration and suspended solids versus discharge were evaluated using the long term (36-month data) to characterize watershed hydrology. A Pearson correlation coefficient (r) showed a positive relationship (r = 0.61 and 0.47) in the agricultural (A) watershed ($p < 0.05$). In the urban watershed (B), a point source dilution is suggested as nitrate decreased with increasing discharge and suspended solids (r = - 0.31 versus r = 0.67) ($p < 0.05$). (C) No significant relationship was found.

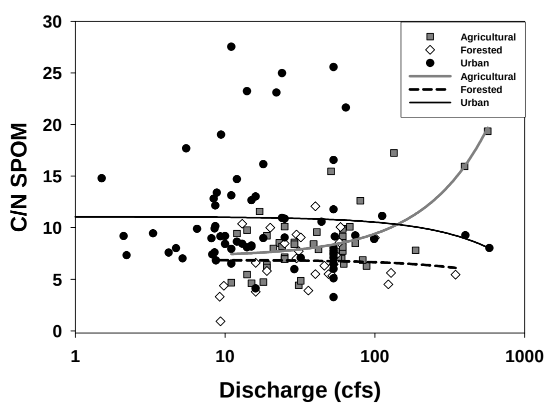

Figure 3: Variation of C/N ratio of SPOM by discharge in the three study watersheds with different land use type. Linear regressions showed that there was a significant relationship between C/N SPOM and discharge for the agricultural watershed ($p < 0.01$).

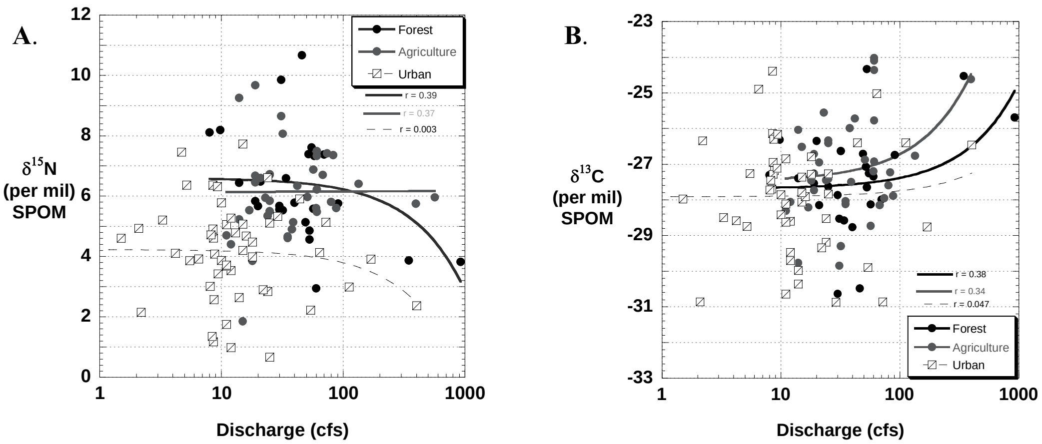

Figure 4a: Pearson correlations were used to measure the relationship between $\delta^{15}N$ values of SPOM versus discharge across watersheds. There was a moderately strong inverse relationship in the forested watershed ($r = -0.39$; $p < 0.05$). The agriculture watershed showed no significant relationship ($r = 0.037$) and the urban sites were more variable ($r = 0.003$). **4b:** Isotopic $\delta^{13}C$ values of SPOM versus discharge showed a moderately positive relationship in the forested ($r = 0.38$; $p < 0.05$) and agriculture ($r = 0.40$; $p < 0.05$) compared to the urban watershed ($r = 0.047$).

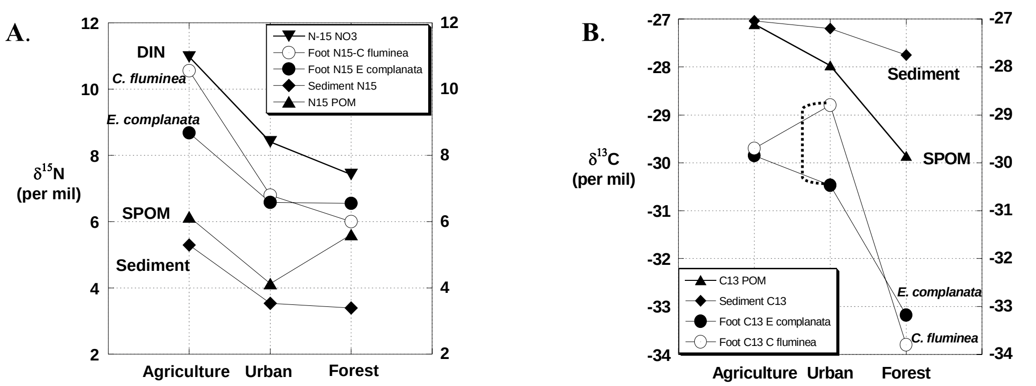

Figure 5a: Mean values of the isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition of *E. complanata* and *C. fluminea* compared to sediment organic matter, SPOM, and nitrate concentrations (mgL^{-1}) by watersheds. **Figure 5b:** The $\delta^{13}C$ values for *C. fluminea* compared to *E. complanata* were significantly different (bracket) and closer to the SPOM predicted food source (1‰ ± 0.5) in the urban watershed *$p < 0.01$. See Table 1 for standard deviations.

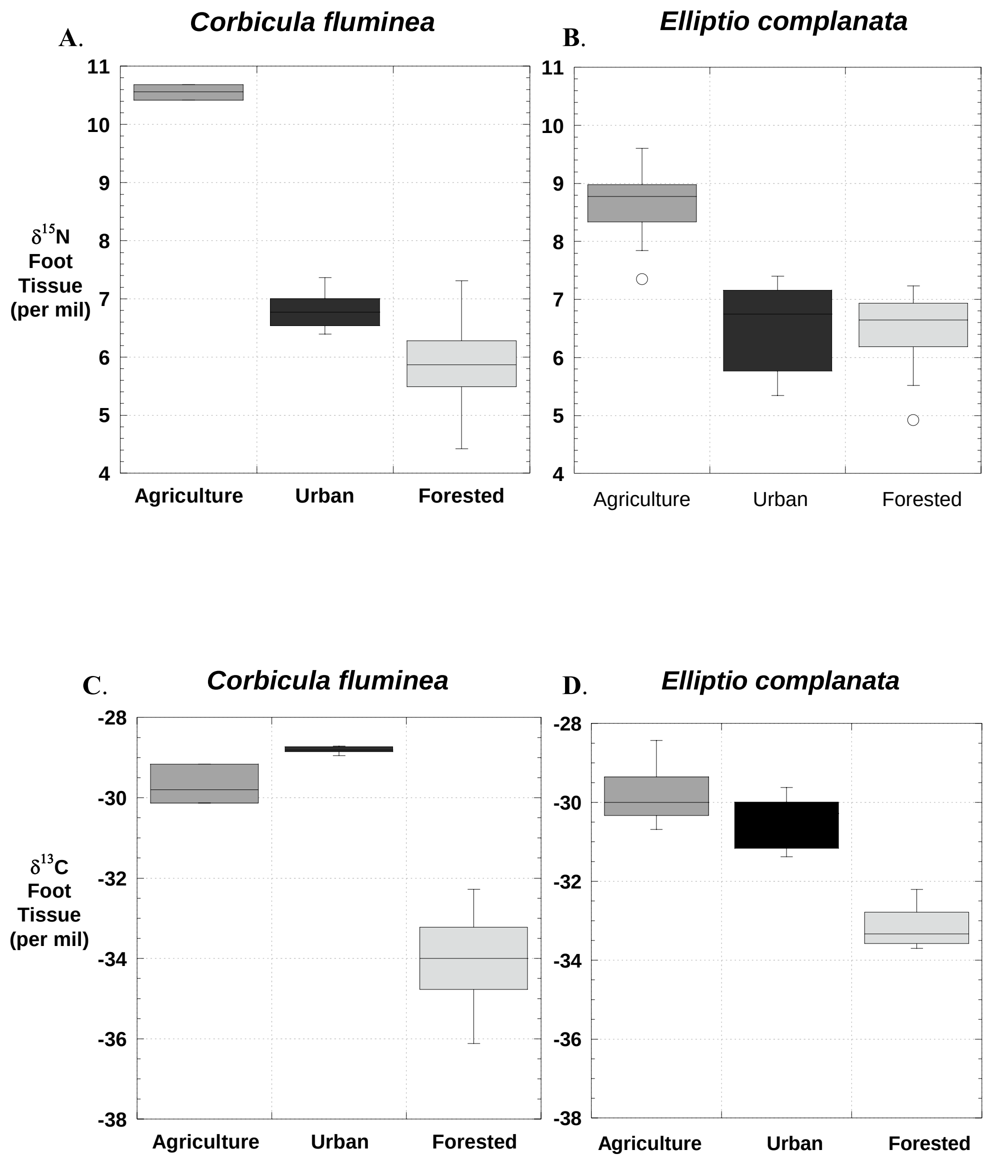

Figure 6a-d: Box plots of mean tissue values by watershed. The multivariate (GLM) analyses showed significant interactions of $\delta^{15}N$ and $\delta^{13}C$ tissue values between species and watershed ($p < 0.05$) The $\delta^{15}N$ mean values for both species were significantly heavier in the agriculture compared to the forested watershed ($p < 0.05$).

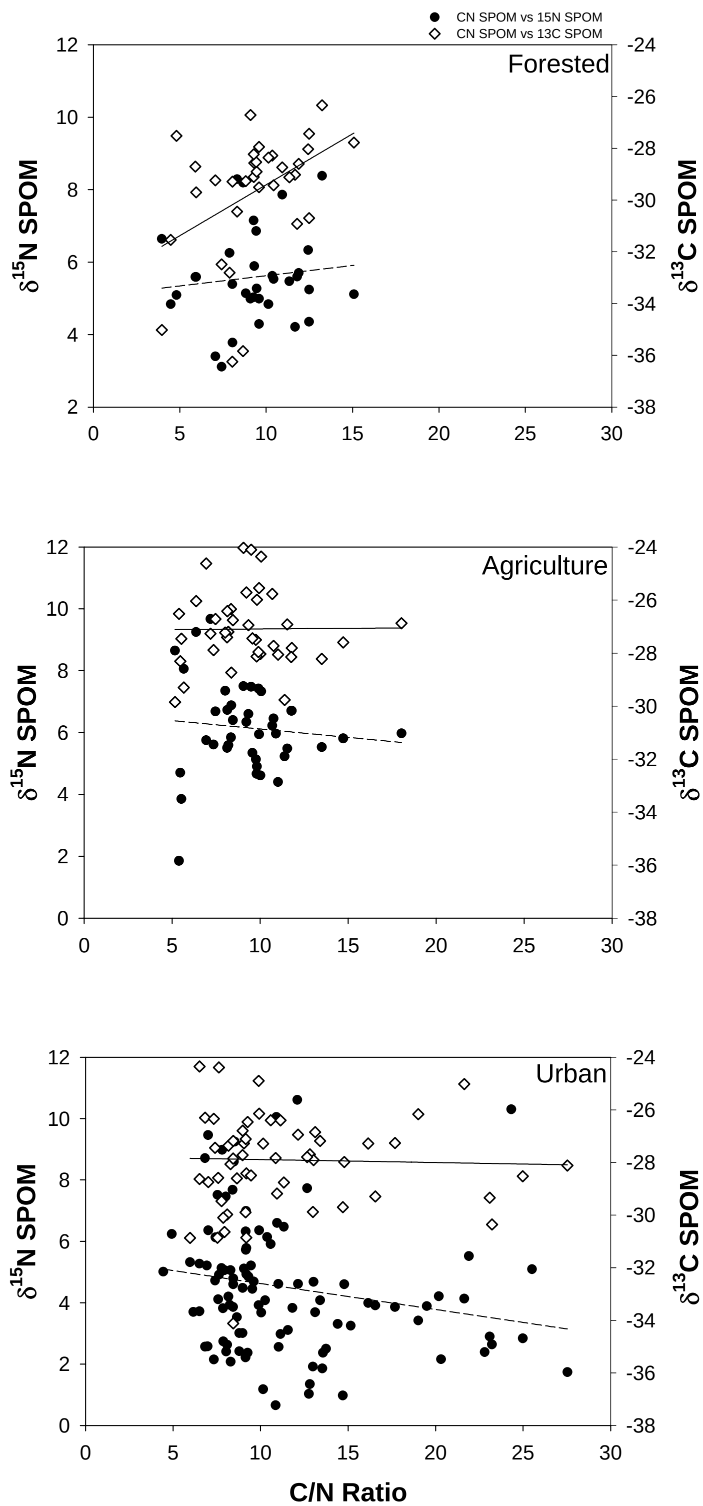

Figure 7: Isotopic ($\delta^{15}N$, $\delta^{13}C$) versus elemental compositions (C/N) of SPOM across watersheds. Box plots of mean tissue values by watershed. The solid lines are $\delta^{13}C$ SPOM versus C/N linear regressions. The forested watershed relationship of $\delta^{13}C$ and C/N of SPOM was significant ($p < 0.01$).

Table 1: Isotopic data for bivalve foot tissue of *C. fluminea* and *E. complanata* are shown with previously measured water chemistry parameters and food sources. (n = sample size)

Parameter Estimate	Collection Period	Watershed					
		Agriculture Mean	n	Urban Mean	n	Forested Mean	n
NO_3^- (mgL^{-1})	2000-03	2.3 ± 0.6	46	1.2 ± 0.7	111	0.09 ± 0.7	35
$\delta^{15}N$ of NO_3^- (mgL^{-1})	2000-03	11.2 ± 2.5	43	8.0 ± 3.0	109	6.5 ± 2.3	35
Suspended solids (mgL^{-1})	2000-03	11.3 ± 22.1	45	8.1 ± 14.5	108	7.5 ± 7.0	35
$\delta^{15}N$ *E. complanata* foot tissue (‰)	2000-03	8.5 ± 0.61	34	6.6 ± 0.67	24	6.5 ± 0.51	30
$\delta^{15}N$ *C. fluminea* foot tissue (‰)	2006	10.6 ± 0.13	3	6.8 ± 0.34	9	6.0 ± 0.8	5
$\delta^{15}N$ SPOM (‰)	2000-03	6.1± 1.5	40	4.5 ± 2.2	101	5.7 ± 2.0	35
$\delta^{15}N$ Sediment (‰)	2000-03	5.3 ± 0.12	3	3.5 ± 0.16	3	3.4 ± 0.39	3
$\delta^{13}C$ *E. complanata* foot tissue (‰)	2000-03	-29.9 ± 0.63	15	-30.5 ± 0.60	24	-33.2 ± 0.47	14
$\delta^{13}C$ *C. fluminea* foot tissue (‰)	2006	-29.7 ± 0.5	3	-28.8 ± 0.08	10	-33.8 ± 1.2	5
$\delta^{13}C$ SPOM (‰)	2000-03	-27.1 ± 1.4	39	-26.5 ± 2.6	101	-29.7 ± 2.4	34
$\delta^{13}C$ Sediment (‰)	2000-03	-27.0 ± 0.09	3	-27.2 ± 0.41	3	-27.7 ± 0.2	3
	2006	-27.0	1	-28.4± 0.71	2	-27.5 ± 0.8	4
C/ N SPOM	2000-03	9.3 ± 2.6	40	11.4 ± 5.1	101	9.4 ± 2.6	34
C/ N Sediment	2000-03	15.1 ± 5	2	19.9 ± 2.3	3	9.9 ± 2.8	2

Table 2: ANOVA with 2 factors (watershed and species) for $\delta^{13}C$ and $\delta^{15}N$. A significant watershed*species interaction was observed. (*Significant at $p < 0.05$)

Source of Variation	df	MS	*F*-value	p
$\delta^{13}C$				
Watershed	2	220.56	264.65	<0.0001*
Species	1	0.64	0.77	0.3820
Watershed x Species	2	11.28	13.54	<0.0001*
Error	121	0.83	-	-
Total	126	-	-	-
$\delta^{15}N$				
Watershed	2	83.44	151.0	<0.0001*
Species	1	1.33	3.01	0.0848
Watershed x Species	2	9.27	9.27	<0.0001*
Error	163	0.44	-	-
Total	168	-	-	-

CHAPTER 3

VALVE GAPE RESPONSE OF TWO FRESHWATER BIVALVES (*CORBICULA FLUMINEA & LAMPSILIS RADIATA*) TO STORM EVENT TURBIDITY

Abstract

Freshwater bivalves have been utilized as biomonitors of water quality and early warning indicators of contaminants, because of their ubiquitous distribution, relatively non-mobile behavior, and the ability for bioaccumulation of a wide range of pollutants. The feeding responses of different species, however; are not known, especially during storm events when many non-point source (NPS) contaminates are mobilized into aquatic environments. This laboratory flume study simulated repeated storm events, and examined the effects of increased turbidity and decreased food quality on valve gape response of two freshwater bivalve species (*Corbicula fluminea* and *Lampsilis radiata*). *Corbicula fluminea* is a non-native species, while *Lampsilis radiata* is a threatened species native to the southeastern United States. Variations in valve gape are thought to be associated with changes in feeding response. The storm events can be separated into a baseline (pre-event), peak, and chronic (slowly decreasing) turbidity periods on the basis of changes in sedimentation. Results showed a significant difference ($p< 0.05$) between the species responses to increased discharge and turbidity. The non-native species (*C. fluminea*) opened more intensively during the peak turbidity period ($p < 0.01$) and had an extended valve closure response during the chronic period. *Lampsilis radiata* exhibited little change in gape response to increased turbidity during peak and chronic turbidity periods. This study suggests that feeding strategies of *C. fluminea* may be better adapted to frequent turbid

environments found in "disturbed" watersheds that are more urbanized or used for extensive agriculture. *L. radiata* may be better adapted to less turbid environments associated with less disturbed watersheds (i.e., forested) where food quality does not change significantly with discharge.

Introduction

The greatest diversity of freshwater mussels (Bivalvia: Unionidae) is found in the southcentral and southeastern United States (Williams et.al. 1993; Lydeard and Mayden 1995; Neves 1999). Freshwater mussels in North America have been in decline over the past century, and over 70% of unionid species are presently classified as endangered, threatened, or of special concern (Williams et al. 1993; Bogan 2002). Increased sedimentation, increased turbidity, and nutrient input associated with urbanization and the loss of fish host species for larval life stages have contributed to the decline in bivalve abundances and diversity in North Carolina Rivers and streams (NCDENR, 2002). Habitat fragmentation and an influx of non-native species that may be displacing native unionid populations (Bogan 1998; Raikow and Hamilton 2001; Lydeard et al. 2004) have heightened the decline. Urbanization is a pervasive global trend, and water quality generally decreases in expanding urban areas as hydrographs change, water residence times diminish, (Rose and Peters 2001; Old et al. 2006) and the local effects of storm events are amplified. The effects of these amplified storm events and changes in water quality on mussel populations in anthropogenically modified watersheds (urban and agriculture) are poorly understood.

Storm water runoff contains contaminants, such as sediment, suspended particulates, bacteria, excess nutrients (i.e., nitrogen), heavy metals, and pathogens, which pose a threat to benthic biota (Davis et al. 1995; Schueler 1997; USEPA 2002; Olapade and Leff, 2004).

Concentration of dissolved contaminants from point sources can occur during low flow conditions due to lack of dilution (Caruso 2002; Rogers et al. 2003). At the same time, the concentration of sediments and contaminants from non-point sources may decrease due to storm event discharge, which mobilizes these pollutants. As catchments are modified from forested to urban or agricultural use, the dynamics of runoff change and the hydrological relationships between discharge, suspended sediment, and solute concentrations also can change significantly (Old et al. 2006). Fine sediment associated with storm water runoff can impair the health of freshwater mussel populations by interfering with feeding, growth, and reproduction (Aldridge et al. 1987; McMahon 1991; Ward and Shumway 2004). Increased sediment loads from omnipresent urbanization can exacerbate future declines of threatened invertebrate species such as unionid mussels (Cooper 1993; Wood and Armitage 1997).

Freshwater bivalves gape open during suspension feeding (Gosling 2003). This feeding behavior has been explored as a potential bio-monitoring tool. For decades, various devices have been developed to measure gaping behavior and monitor nutrient pollution (Goldberg 1975; Gosling 1992). The relationship between valve gaping behavior and food quality directly affects the accumulation rate of pollutants and, hence, the sensitivity of bivalves as environmental monitors (Jorgensen et al. 1988; Markich et al. 2000). Particle food quality may vary with nutrients as well as with contaminant and sediment load (Fry 1999; Adams and Sterner 2000; Evgenidou and Valiela 2002; Ulseth and Hershey 2005). Also, particle food quality can vary with changes in turbidity and discharge (Kendall et al., 2001; Stoeckmann and Garton 2001). The ability of bivalves to accurately reflect pollutant levels is crucial to their role as biomonitors (van der Schalie et al. 2001; Miller et al. 2005; Liao et al. 2005). Traditional measures of water quality, such as excess nutrient levels of nitrogen or phosphorus and low dissolved oxygen levels are expensive, labor-intensive techniques that may miss episodic storm events and under report contaminant levels (Tanabe 2000). Suspended sediment can change bivalve food quality across a storm hydrograph (Old et al. 2006). Behavioral responses (e.g., valve gape) may falsely record contaminants. This could result in bioaccumulation during only a portion of the hydrograph. There is a need to better understand how freshwater bivalves may alter feeding strategies to changes in turbidity. Or, if different species use different feeding strategies, they may have different growth rates and bioaccumulate different pollutants in the same watershed.

The goal of this study was to measure the effects of turbidity levels during simulated storm events on the valve gape response of two extant species of freshwater bivalves found in North Carolina. We have measured how valve gap response changed during the rising, peak and falling discharge phases of the hydrograph, and have compared the response of the two bivalves to changes in turbidity and food quality.

Methods

Field Collections and Flume Design

In order to validate turbidity measurements made in the simulated storm event, turbidity and discharge samples were measured biweekly at field sites located in a North Carolina river basin in urban, agricultural, and forested watersheds from 2000 to 2003 (Chapter 1, Bucci doctoral dissertation). A YSI 6800 Series Sonde was used to measure turbidity over a 3-7 day period during storm events. The turbidity probe was calibrated with a 100 NTU APS polymer standard (YSI). Particle weight (mgL^{-1}) filtered on a 0.7µM GFF filter was related to NTU measured with the YSI Sonde turbidity probe by: (PW = 9.76 + 0.899 NTU) Where: PW = particle weight (mgL^{-1}); NTU = nephelometric turbidity units.

These data were used to determine the range of environmental variables that extant bivalves are exposed to during low and high flow conditions in watersheds with different land uses in the Neuse River Basin. Discrete water samples were analyzed for nutrient, particulate, isotopic, and elemental concentrations to determine particulate source and C/N ratio (Showers et al. 2005). Discrete water samples showed a maximum of ~130 mgL^{-1} suspended solids, while storm events in the field with turbidity measurements were made at 5 minute intervals, which had turbidity maximums of > 250 NTUs.

Study Animals

In the laboratory, groups of ten to fifteen adult *Corbicula fluminea* and *Lampsilis radiata* were monitored in three replicate experiments. *Lampsilis radiata* are found in headwater streams of the Neuse and Yadkin Pee Dee River Basins in the piedmont of NC. They are a state-listed threatened species in NC (NCDENR 2002). The native mussel *L. radiata* used in these experiments were raised in captivity in the College of Veterinary Medicine Mussel Barn, North Carolina State University in Raleigh, NC. The native mussels were randomly selected from a group of captive-reared animals derived from the glochidia of field-collected females. *Corbicula fluminea*, a non-native species, which is now relatively ubiquitous in the piedmont of NC and much of the continental US, were obtained from an urban freshwater habitat in the Neuse River basin (Lake Wheeler, 35.69° N; 78.70°W). In North Carolina, both species are found in a range of substrates, which range in character from muddy clays to coarse sand (Stiven and Alderman 1992). The animals were kept in holding tanks to acclimate to laboratory conditions (i.e., water temperature 20°C, turbidity < 20NTUs, and a 12 hour diurnal cycle) for approximately one month.

Experimental Flume Design

Turbidity and discharge were measured in urban, agricultural, and forested watersheds during storm events using a YSI-6920 Sonde to determine the range of environmental variables encountered by extant bivalve populations in the piedmont of NC. Water samples (500ml) were collected every 30 minutes at the field sites for nutrient, particulate, isotopic and elemental analysis with an ISCO autosampler over a 2-3 day storm event period. During experiments, water samples were collected from the flume every hour by hand in acid washed (10% HCL) Nalgene® containers. Particle samples were kept at 4^{o}C after collection, and then filtered on GFF 0.70 μm filters, precombusted at 500^{o}C for four hours before use. Filters were processed for C/N, $\delta^{15}N$ and $\delta^{13}C$ of suspended particulate organic matter (SPOM) using standard techniques with a Finnigan MAT Delta Plus® XL IRMS and an NC2500 Carlo Erba® CHNS elemental analyzer (Showers et al. 2005). The isotope ratio of each sample R_{sample} was compared to that of a standard $R_{standard}$[1] and expressed in delta notation, which has units of per mil (‰) (Peterson and Fry 1987). Dissolved nutrient (nitrogen) concentrations in the water samples were determined by standard flow injection techniques on a La Chat Quickchem 8000 (Showers et al. 2005).

The turbidity storm event curve that was measured at the field sites in the Neuse River Basin was replicated in the laboratory by adding sediment collected from the river bottom (estimated size range from 0.005-0.05mm) into a re-circulating flume. The closed freshwater recirculation system was designed and constructed of fiberglass and PVC® pipe (Fig. 1). A settling container was used to maintain turbid flow and to collect sediment

[1] $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample}-R_{standard})/R_{standard}] \times 10^{3}$

The differences in ratios are calculated in 'del' (δ) notation and have units of per mil (‰)

fallout. A bilge pump controlled with a Variac® power supply was used to circulate water in the system at different speeds. A magnetic gape sensor Clam Monitor® device, designed by the Nekton Corporation, was used to measure the valve gape response of each individual bivalve to changes in turbidity levels (Nekton 2006). The clam monitor was constructed to be part of a Biological Early Warning System and is on loan to North Carolina State University for use in bivalve behavioral research. The device has several Hall Effect Sensors® placed in a watertight plastic loop. The bivalves are glued to posts on the lower portion of the loop, and a small magnet is glued to the upper valve (Figure 1). The sensor then measures changes in electrical current created in the Hall probe (Hall 1879) as the bivalves open and close, which changes the position of the magnet relative to the Hall probe sensor. This device records real-time and continuous changes of mussel valve gape, which is related to feeding response changes during environmental conditions (Kramer et al. 1989; Borcherding and Jantz 1997). The micro-electronic currents generated by the Clam valve gape sensor were recorded on a laptop computer at a one second interval, and analyzed using the BioBay® software program developed by Nekton® Research.

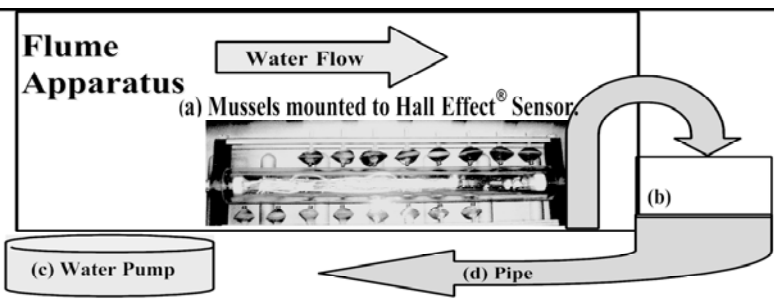

Figure 1: The diagram depicts water circulation in the flume for each experiment. The flume design was a closed system and used for measuring the valve gape response: (a) Mussel sensor connected to laptop computer, (b) additional reservoir, (c) water pump, (d) return PVC® pipe.

Tap water used in the flume was pre-treated with sodium thiosulfate for 24 hours to eliminate toxic chloride concentrations before submerging the live animals. Water temperature in the flume tank was controlled by thermostatically varying the air temperature with central heating and air-conditioning (~ 20°C or 68°F). Light in the laboratory was on a 12 hours on / off cycle, which started at 07:30 hours in the morning. Suspended particulate organic matter (SPOM) was collected and analyzed for isotopic nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) values to confirm the trophic composition (i.e., bacterial, terrestrial algae, and/or detritus) (DeNiro and Epstein 1978, 1981; Fry and Sherr 1984; Coffin et al. 1989). The C/N ratios were used as a proxy for food quality and have been shown to vary with discharge (Nichols and Garling 2000; Raikow and Hamilton 2001). The C/N ratio is a useful indicator of POM and sediment food quality (Nichols and Garling 2000; Adams and Sterner 2000; Kendall et al., 2001; Baker and Levinton 2003). Phytoplankton species have a relatively low C/N (5-8 by weight) ratio, while detritus, macrophytes, soil organic matter, and terrestrial plant debris tend to have higher C/N (10- >15) ratios (Valiela et al. 1992; Canuel et al. 1995; Iglesias et al. 1996; Kendall et al., 2001). Water samples were collected using a 500ml acid washed bottle at intervals across each experimental storm event.

Study Approach

A total of six simulated laboratory storm experiments (3 per species) were conducted between September 2004 and June 2005. The valve gape response of 39 *C. fluminea* and 29 native bivalves were tested in the flume apparatus. The experiments averaged 48 hours in duration. A baseline, peak, and chronic period were replicated for each experiment. The criterion for the baseline turbidity treatment period was 0 to 20NTUs (nephelometric turbidity units). The peak period was defined as 20 NTUs to 50% of the maximum NTUs for each of the three replicate experiments. The chronic treatment was defined as the period from 50% of maximum turbidity to the completion of the experiment. After a baseline period of time, a prescribed volume of fine sediment was mixed into the settling container at intervals to achieve the simulated storm event. Once a desired peak was achieved, additions of sediment stopped and the current flow was reduced to half flow. This method allowed the turbidity levels to decrease naturally within the flume circulation system. Similar to the falling hydrographs observed at field sites in the Neuse River Basin.

Statistical analyses

The dichotomous (bivariate, open/closed) valve gape response variable was the primary dependent variable used to analyze the six storm experiments (3 per species). Percent open valve gape was aggregated for each of the three experiments to reflect the mean of the individual responses. The bivariate data were calculated by counting the percent of individual specimens with an open gape for each 10-minute interval across the duration of each storm event. Each experiment contained a sample size of 9 to 16 bivalves (the holding limit of the gap response monitor). Because the gape response of each individual is variable in timing and duration, the response variable was normalized based on the maximum gape for each individual specimen and averaged across each experiment. Independent variables included turbidity (NTUs) and food quality. A Student's t-test was used to compare the average valve gape response of each species as independent groups across the chronic period. A generalized linear model (GLM) was used to examine the association between food quality (C/N) and turbidity across periods (Scheiner and Gurevich, 2001). The field data were analyzed using a multivariate GLM including watershed type as the independent and suspended solids as the dependent variable.

Repeated measures analyses of variance were used to assess the relationship between valve gape and turbidity, with a split plot design (2 species x 3 periods) over time using the

Statistical Analysis System software (SAS 2000). Interaction effects between species and turbidity periods were examined using mixed models with repeated measures (Littell et al. 2002). A partial least squares post hoc test was used to examine interaction effect among the two species groups for the mean valve gape response across periods (de Jong 1993). This method balances the two objectives of explaining response variation and explaining predictor variation.

Results

According to the results across all flume experiments, *Corbicula fluminea* were more frequently open in the peak period than the chronic or the baseline periods (Fig. 2; $p < 0.01$). *C. fluminea* displayed an extended valve closure response during chronic turbidity. Both species of bivalves had an average valve closure episode comprising 40% of the duration of two separate 10-hour blocks of time (Fig. 2, experiment 1). The mean valve gape values of *L. radiata* were less variable and there was no significant difference in valve gape activity between periods (Fig. 3). Thus, *L. radiata* valve gape activity was observed to be less responsive to changes in suspended solids in the flume than *C. fluminea* valve gape activity.

Turbidity period was significantly associated with the mean valve gape response for both species ($p < 0.01$). A significant difference ($p< 0.05$; $t_{stat} = -33$; df = 386) was observed in the value gape response of *C. fluminea* and *L. radiata* during periods of chronic turbidity (Student's t-test) (Fig. 4). The result was expressed by the minimum % valve gape open statistic (across each group of animals), which indicated that only 6.5% of *C. fluminea* compared to 26.7% of the *L. radiata* engaged in filter feeding during the chronic period (Table 1, Fig. 2). Although the bivariate proportion (% of open vs. closed valve gape) provided a meaningful indicator of early biological warning, repeated measures analyses confirmed a significant difference between species and period ($p < 0.01$). Least squares post hoc tests confirmed a statistically significant difference between turbidity periods for *C. fluminea* ($p < 0.001$), but not for *L. radiata* (Table 2).

The C/N ratio of SPOM (food quality) was measured against turbidity (NTUs), as the volume of sediment was introduced into the flume system. Results showed that during the peak turbidity, C/N for *C. fluminea* averaged 11.5 compared to 10.6 for the native (*L. radiata*) species (Table 3). When the relationship between C/N of SPOM and turbidity was examined using the GLM for both species combined, a significant association was found between food quality and turbidity ($p < 0.05$). Lower food quality was associated with the peak turbidity periods and higher food quality with the baseline and chronic periods (Table 3). The average C/N ratio of the test sediment was 11.8, which was comparable to the sediment found at the urban field site, where SPOM samples averaged greater than 10 at the peak period of a storm event (USGS 2005).

At the field sites, the turbidity and particle C/N relationships to discharge varied with watershed type. Turbidity increased significantly ($p < 0.01$) with discharge in the agricultural watershed, but showed little variation with increased discharge in the forested watershed (Chapter 2, Bucci doctoral dissertation). High turbidities (greater than 200 NTUs) were observed in the urban watershed during intermediate and high discharge levels with no significant relationship to discharge. SPOM C/N ratio increased significantly with discharge in the agricultural watershed ($p < 0.01$), but showed little change in the forested watershed. Suspended particulate organic matter (SPOM) C/N ratios reached maximum compositions (>25) in the urban watershed at intermediate discharge levels. This watershed also had the highest SPOM C/N composition of the three watersheds examined.

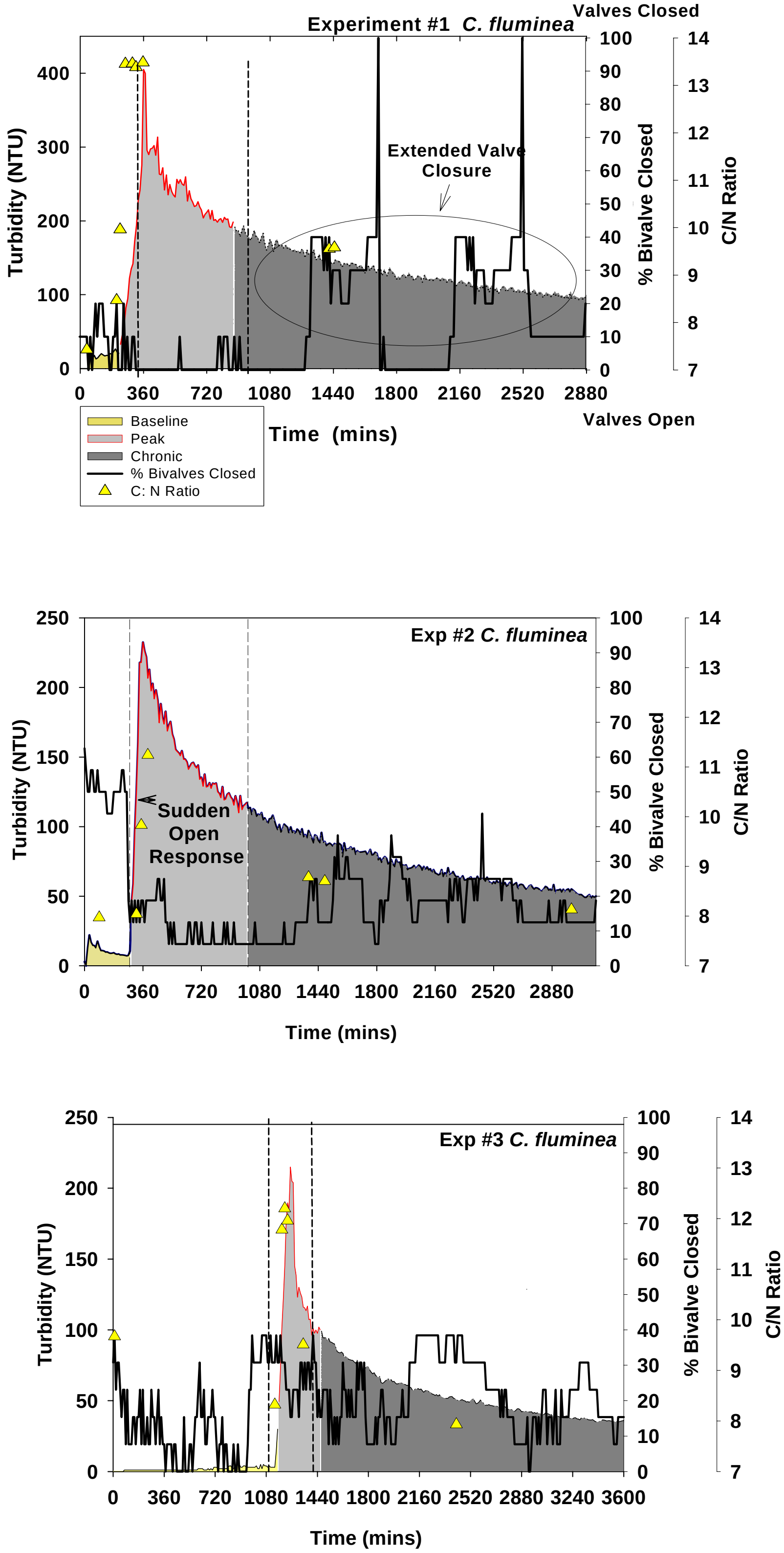

Figure 2: Turbidity vs. valve gape response for *C. fluminea*. The treatments were divided into baseline, peak and chronic periods. There was a significant interaction effect between the baseline and peak periods and between the peak and chronic periods for *C. fluminea* ($p < 0.01$). Sample sizes were 10 for #1, 16 for #2, and 13 for #3.

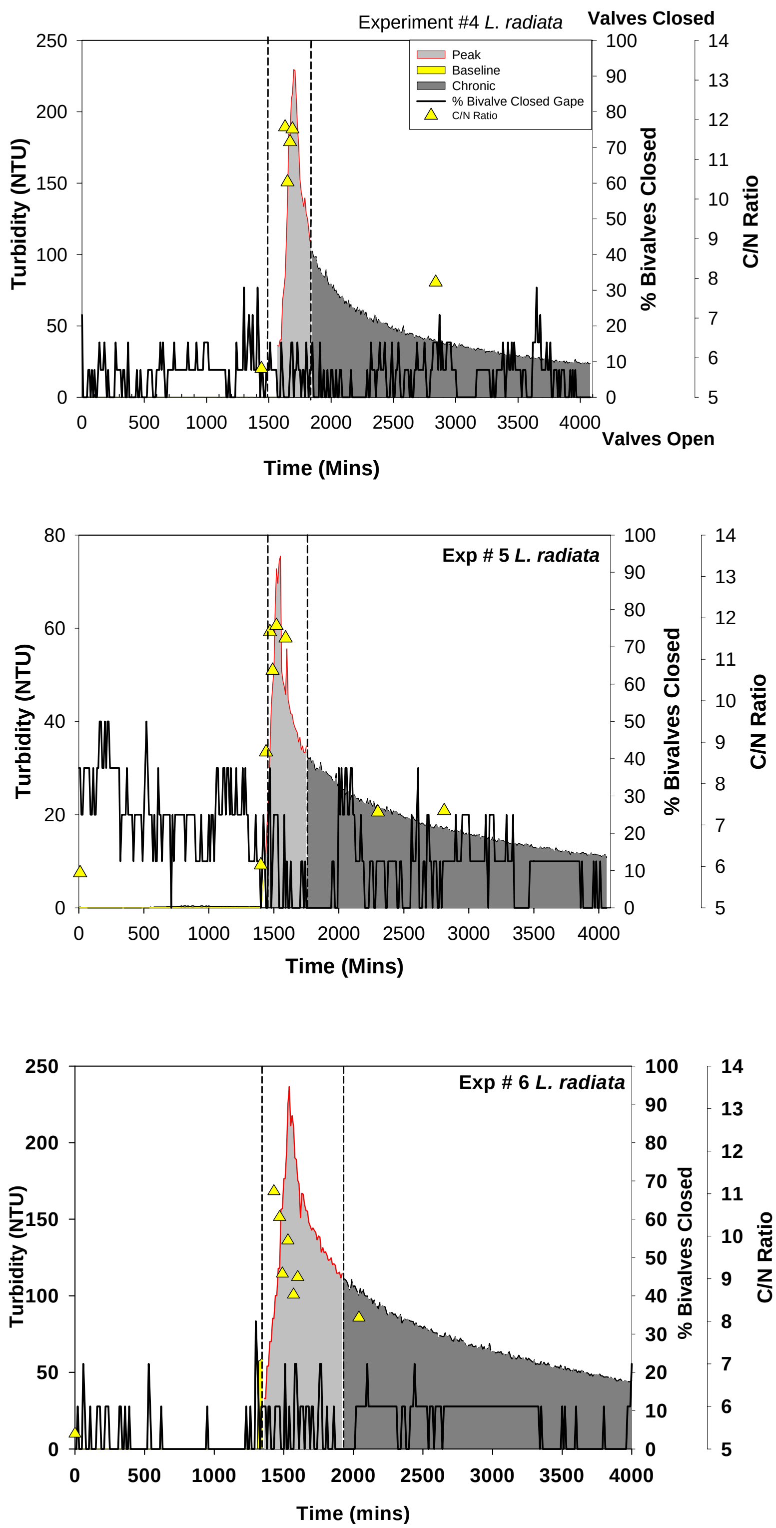

Figure 3: Turbidity vs. valve gape response for *L. radiata*. The native species valve gape response was less variable than the non-native species, particularly in experiments #4 and #6. There was not an extended valve closure response evident. Sample sizes were 13 for #4, 10 for #5, and 9 for #6.

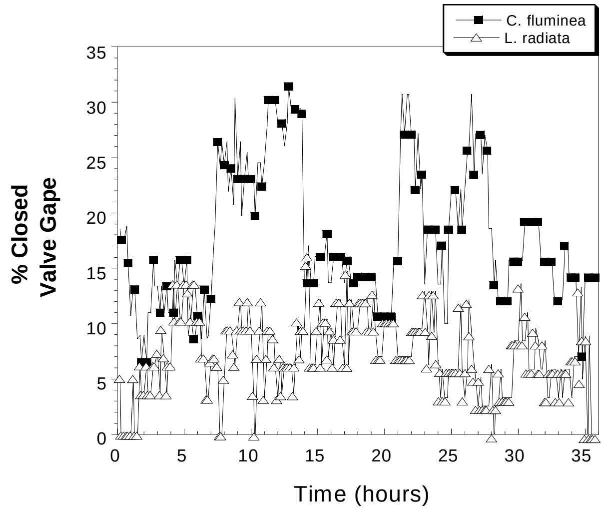

Figure 4: Effects of chronic turbidity on bivalve gape response. The average response for the chronic turbidity period was plotted for each species. There was a significant difference in mean valve gape response between non-native (*C. fluminea*) and native (*L. radiata*) species ($p < 0.05$; $t_{stat} =$ -33; df = 386).

Discussion

Storm events were simulated under controlled laboratory conditions to compare the valve gape response of two freshwater bivalves to changes in turbidity and food quality during different phases of a storm hydrograph, simulating an urban watershed event. In previous field analyses of storm event turbidity within the NRB, food quality and suspended solids concentrations varied significantly in agricultural and urban watershed types compared to forested watersheds (Chapter 1, Bucci doctoral dissertation). Urban watersheds showed the greatest variation in these parameters.

In the present study, *Corbicula fluminea* appeared better adapted to shorter time scale (i.e., peak to chronic) turbidity periods found in more developed watersheds. In contrast, the repeated measures analyses showed that the native species, *L. radiata* might be better adapted to lower turbidity environments found in less disturbed watersheds where food quality changes less with discharge. These dissimilar feeding strategies may represent a capability by non-native bivalves to assimilate more nutritious particles unique to urbanized stream hydrology.

Food quality, as measured by C/N ratios (Adams and Sterner 2000), varied significantly with turbidity period across storm events in the agricultural and urban watersheds. Food quality declined as turbidity increased during the peak periods of the simulated storm event (Table 3). As maximum flow was reduced, an extended, chronic turbidity period was observed and food quality improved. This relationship confirms previous work that food quality varies with suspended sediment concentrations during storm events (Buttman et al. 1994; Schneider et al. 1998). Since growth and energy metabolism are related to valve gape, it is reasonable to consider the open/close response in relation to food selectivity (Ortmann and Grieshaber 2003). *C. fluminea* engaged in feeding activity significantly more frequently than *L. radiata* when food quality was low (during peak periods), compared to the baseline and chronic periods (Fig. 2, Table 2). Although this result appears counter-intuitive, a possible explanation is that *C. fluminea* may increase their filter feeding behavior and clearance rate as higher volume, refractory material travels downstream during the peak period (Boltovskoy et al. 1995). During this period, *C. fluminea* may select out the more nutritive particles (Higgins 1980; Kadar et al. 2001; Baker and Levinton 2003). Thus, *C. fluminea* may effectively utilize labile, cellulosic detritus as an important food source (Crosby et al. 1989), compensating for the reduction in food quality (Hawkins et al. 1996; Navarro et al. 1996). This specialized response may be plausible as different sized algal particles are sorted to reduce turbidity (Newell and Ott 1999; Ward and Shumway 2004). Non-native species (Zebra mussel, *Dreissena polymorpha*) have shown higher efficiency for sorting large amounts of particles as a strategy for optimizing ingestion (Baker et al. 1998). The introduced mussel, *Corbicula gibba*, has demonstrated higher particle selection efficiency than native bivalve species (e.g., *Mya arenia*, *Mytilus edulis*, and *Crassostrea giga*), possibly related to cilia structure and labial palp size (Kiorboe and Mohlenberg 1981; Jorgenson et al. 1984). Conversely, the native species, *L. radiata* showed a variable pattern in valve gape response across a range of turbidity levels. Supporting this finding, Baker and Levinton (2003) found that native mussels (*Margaritifera margaritifera*) did not differentiate between nutritious (phytoplankton) and less nutritious (detritus) particles compared to *D. polymorpha*. A more comprehensive dose-response analysis of the filtration and particle rejection rates across turbidity treatments could confirm this feeding mechanism in future studies.

The significant differences observed in valve closure responses by *C. fluminea* may be indicative of a specialized feeding strategy. The combined response by *C. fluminea* at peak flow and during the chronic period may reveal a cost benefit strategy. One explanation may be that *C. fluminea* intrinsically reaches a threshold in terms of filter feeding activity as the storm attains chronic turbidity (Bayne and Newell 1983; Borcherding and Jantz 1997). Prolonged exposure of suspended solids overloads the gill's capacity to function (Morton 1971; Riisgaard and Randlov 1981) and may physically impede gas exchange across gill membranes (Aldridge et al., 1987; McMahon 1991). Previous studies have examined a potential adaptive response by non-native species (*D. polymorpha*) to a range of turbidity levels (Wisniewski 1990; Quigley et al. 1993; Alexander et al. 1994; Payne et al. 1995). Laboratory studies have shown evidence of a valve closure, acclimation response to infrequent levels of turbidity and an adjustment of metabolic rate in response to chronic exposure (Summers et al. 1996). Therefore, *C. fluminea*'s valve closure response during chronic turbidity may be advantageous for survival. Conversely, the native freshwater mussel, *L. radiata* has shown an acute sensitivity to high sediment load, experiencing high mortality and reduced fitness in laboratory experiments (Haag et al. 1993). Aldridge et al. (1987) found that when native freshwater mussels were exposed to high turbidity (> 400 NTUs) clearance rates decline, and the animals compensated by reducing metabolic demand.

Although *C. fluminea* populations tend to be shorter lived than native species (Bogan 1998), their ubiquitous nature and ability to dominate river ecosystems makes them ideal candidates as biomonitors (Doherty 1990; Tran et al. 2003; Liao et al. 2005). Evidence shows that the spread of non-native species is facilitated not only by their extraordinary reproductive capacity, but also through their tolerance for degraded habitats (Doherty et al.

1987; Bogan 1993; Ortmann and Greishaber 2003). Our results support previous studies, which suggest that *C. fluminea* may be more robust than native species in high turbidity environments (Kraemer 1979; Doherty 1990). Unlike native unionids, *C. fluminea* do not require a fish host to complete the metamorphosis of larvae to juveniles. They have a more direct life cycle, apparent broader tolerance to degraded habitats, and the feeding patterns in these trials suggest *C. fluminea* may be more tolerant to the turbidity changes associated with the urbanization of streams. Historically, *C. fluminea* originated on the Asian continent from drainage basins with a relatively high sediment load compared to the rivers in southeastern North America (Milliman and Meade 1983). Suspended sediments and water discharge in most watersheds are directly correlated to total precipitation, especially in urbanized watersheds, which tend to have rapid changes in flow rates over the event (Old et al. 2006). However, more study is needed regarding the behavioral and physiological factors, which may be among many inter-related contributors to the success of *C. fluminea* in urban watersheds (Ehrlich 1984; McMahon 2002).

As pollution in aquatic environments becomes more widespread, there is a need to improve our knowledge about the use of the clam monitor to facilitate environmental monitoring. A unique aspect of the present study was the use of a valve closure detection sensor to compare the response of two freshwater bivalves to turbidity levels. The efficacy of the Hall Effect® clam sensor to accurately detect valve gape in response to turbidity was demonstrated. Several valve gape meters have been developed to detect both physiological and behavioral responses, which include the Mussel Monitor® (Kramer et al. 1989) and the Dreissena Monitor (Borcherding and Jantz 1997). Recent studies have designed techniques using lightweight electrodes to monitor valve closure as well as threshold activity in freshwater bivalves, including *C. fluminea* (Curtis et al. 2000; Markich et al. 2000; Kadar et al. 2001; Tran et al. 2003). However, methods used to determine tolerable levels of turbidity to induce a significant change in valve behavior (i.e., valve gape, pumping activity and filtration rate) vary considerably (Sloff et al. 1983; Sluyts et al. 1996; Curtis et al. 2000). Quick adductions are associated with filter or pedal feeding and may function to enhance the flow of water across the gills (Foster-Smith 1976) or eject pseudofeces (Dame 1996). The problem of quantifying precise valve movement continues as extended periods of valve gape interrupted by rest periods (diurnal cycle); appear to give a continuous pattern of periodicity (Salanki 1966; Higgins 1980; Kontreczky et al. 1997; Ortmann and Grieshaber 2003). Cautious interpretation is warranted in laboratory investigations that evaluate behavioral factors, as there are inherent differences between and within a single species.

Evaluation of the valve gape response to intermittent high turbidity may improve our understanding of whether non-native bivalves possess a selective feeding advantage compared to native species in freshwater systems. During storm conditions, complex flow in the vadose zone (shallow portion between the land surface and the zone of saturation) can result in the concentration or dilution of solutes, which can result in complex discharge trends that can change from event to event (Lischeid et al. 2002). As the occurrence of event driven turbidity increases in agricultural and urbanized land use (Borsuk et al. 2004), *C. fluminea* may be more effective biomonitors in these habitats. Thus, the management practice of using non-native bivalves as sentinels in watersheds with predominantly non-point source contributions may be an effective strategy.

Table 1: Chronic turbidity analyses comparing the % valve gape for each species using a Student's t-test. The % open statistic was averaged across experiments. SD = Standard deviation; * $p < 0.05$.

Species	Chronic Turbidity Effects on Valve Gape				
	Sample Size	Mean % Open	SD	Maximum % Open	Minimum % Open
C. fluminea	39	17*	6.3	31.5	6.5
L. radiata	29	35	4.5	50.0	26.7*

Table 2: The interaction effect showed a significant difference* between the baseline and peak periods as well as between the peak and chronic periods for *C. fluminea*. The native species (*L. radiata)* did not show a significant difference. SE = Standard error.

Species	Mean % Open Valve Gape Species by Period Contrast (SE)				
	Baseline	Peak	Chronic	Pr > t	Sample Size
C. fluminea	0.4662 (0.04)	0.5426 (0.04)	0.4458 (0.03)	p < 0.0001*	39
L. radiata	0.4461 (0.03)	0.4368 (0.04)	0.4396 (0.04)	0.9036	29

Table 3: Mean values of C/N ratio and turbidity were calculated. Higher food quality (lower value) was observed during the baseline and chronic periods ($p < 0.05$). As turbidity increased, food quality decreased. Sample size means in parenthesis.

Turbidity Storm Periods	Non-native ***Corbicula fluminea***		Native ***Lampsilis radiata***	
	Mean C/ N	**Mean Turbidity (NTUs)**	**Mean C/ N**	**Mean Turbidity (NTUs)**
Baseline	8.3 (5)	5.16 (167)	5.7 (4)	0.5 (572)
Peak	11.5 (12)	173.3 (166)	10.6 (14)	142.7 (119)
Chronic	8.5 (8)	84.8 (631)	7.6 (4)	59.4 (662)

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EPILOGUE

THESIS SUMMARY

Chapter 1

This study raised new questions, regarding the food sources, available by watershed for native mussels across flow events. The problem of excess sediment and nutrient inputs associated with storm events to stream habitats is pervasive in the Neuse River Basin, North Carolina and may be related to the sharp and continuous decline of freshwater mussel populations in the Southeastern U.S. A complication to this issue is the need to better understand specific feeding processes in which to detect contaminant inputs with changes in stormwater flow. The consideration of water chemistry and the isotope composition of bivalve tissue provided insights into their feeding ecology. These relationships have not been previously examined sufficiently in this river basin with a range of watershed types.

Chapter 1 showed that the water chemistry and hydrology of each watershed type (i.e., agriculture, urban, and forested) might distinctly affect bivalve food sources. A generalized linear regression model (GLM) for the long-term (36 month) data showed that watershed land use was a predictor of mean $\delta^{15}N$ values of nitrate (NO_3^-; DIN). Also, mean $\delta^{15}N$ mussel tissue values from the agricultural site were significantly heavier than urban and forested watersheds, suggesting an assimilation of animal waste sources. Mean $\delta^{13}C$ mussel tissue values were significantly more depleted in the forested watershed, suggesting a greater availability of depleted $\delta^{13}C$ sources, such as bacterial sources. This material in the forested watershed may contain a larger portion of DOC available to bivalves, which is associated with a more negative $\delta^{13}C$ value of labile carbon. The short-term (3 to 5 day) storm event data indicated that the urban watershed showed greater changes with discharge in bivalve food sources than the forested watershed, which included both $\delta^{15}N$ and $\delta^{13}C$ and C/N of SPOM. Both the long and short-term data indicated that low food quality (C/N > 8) was associated with increased discharge in the agriculture and urban watersheds. Also, higher mean value of suspended solids during increased flow was associated with decreased food quality in the urban and agricultural watersheds.

Implications of this study suggest that native bivalve food sources vary among watershed type. Consequently, there may be connection between declines of native bivalve populations in more disturbed, higher turbidity watersheds. The combined isotopic and hydrologic data in the forested watershed suggests that native mussels (*E. complanata*) may consume SPOM sources predominantly in low flow conditions. Native mussels were not as well represented in the urban watershed (Table 1B), although this study provided important baseline data of the important food sources associated with different flow conditions. Since it is resource intensive to quantify both point and non-point nutrients and organic pollutants using traditional water quality monitoring technology, more knowledge of native bivalves as biomonitors is needed as they integrate pollution levels.

Chapter 2

In Chapter 2, this field survey showed a significant association between the isotopic composition bivalve tissue (*C. fluminea* and *E. complanata*) and watershed type. Routine monitoring of bivalve isotopic composition may be an effective way to detect long-term habitat changes. This knowledge may improve the current understanding of bivalves as biomonitors. The tissue analyses ($\delta^{15}N$) of both species examined reflected elevated nitrate in the agricultural watershed, which is indicative of an animal waste source. Also, both species reflected significantly negative $\delta^{13}C$ food sources in the forested watershed where more detailed examination of the organic matter in these watersheds is needed (e.g., microbial activity, dissolved material). Based on the watershed hydrology and water chemistry relationships examined in this study, *E. complanata* may consume food sources that are more readily available, during lower flow conditions. *C. fluminea* may be more adept at selecting nutritious particles associated with refractory (detritus) material at higher turbidity and flow conditions. The implications are that non-native (*C. fluminea*) bivalves may better tolerate poor water quality conditions and lower food quality associated with disturbed watersheds (i.e. urban, agriculture) where frequent discharge and non-point runoff increase suspended solids concentrations. Since food quality of suspended particles is related to habitat conditions, which affect bivalve communities, non-native bivalves may tolerate adverse factors and thus be better suited as sentinel species across a wide range of watershed types. The combined results support the growing evidence, which suggests that native mussel populations have been severely impacted in urban and agricultural watershed types.

Chapter 3

Chapter 3 was based on a laboratory study that utilized a Hall® Probe valve gape sensor. Results suggested that feeding strategies of the non-native bivalve (*C. fluminea*) may avoid the gill clogging phase of storm events associated with higher turbidity conditions. Conversely, the native bivalve (*L. radiata*) may not possess this adaptation and may be unable to distinguish between lower and higher turbidity conditions. This native species may have a disadvantage in turbid environments, and be better suited as a bio-monitor in undisturbed watersheds (i.e. forested) where average food quality is higher and does not change significantly with discharge. Also, this study highlighted a potential behavioral strategy in which *C. fluminea* may possess an advantage compared to the native mussel *L.*

radiata in terms of differential assimilation of more nutritive fine particulate matter associated with the peak period of the storm event. Although it was evident that SPOM food quality was lower during peak turbidity, highly nutritious smaller sized particles (e.g., bacteria) may be attached to detritus material and thus available to selective consumers.

This laboratory study was unique in that it evaluated feeding activity (valve gape response) across distinct periods of sedimentation across a storm event hydrograph. During storm conditions at field sites, complex flow in the stream's geomorphology can result in the concentration or dilution of solutes, which can result in complex discharge trends that can change from event to event. As land use changes with development, the occurrence of frequent event driven turbidity may increase in agricultural and urbanized watersheds and the widespread bivalve, *C. fluminea* may be more effective biomonitors in these habitats. Thus, the management practice of using non-native bivalves as sentinels in these watersheds may be an effective strategy. Conversely, the native species (*L. radiata*) may be more efficacious as biomonitors in less disturbed watersheds (i.e. forested). Ideally, this laboratory study would have utilized a different comparison native species (*E. complanata*), which was examined throughout the field studies in this dissertation. However, the valve gape sensor device was not designed to hold such a larger, rectangular shaped bivalve species. Consequently, the Unionid species (*L. radiata*) was used, which also co-resides in study sites with the non-native bivalve, *C. fluminea*.

FUTURE RELATED STUDIES

The results of this dissertation have shown that there is a need to better understand the hydrology in freshwater stream habitats across higher temporal variability scales such as storm events. A contribution of this dissertation research is the knowledge of how the food sources for different bivalve species reflect significantly different isotopic compositions across watershed types. This study has implications for the health of freshwater mussels and their effectiveness as biomonitors. This dissertation examined how two different bivalve species feed across different water chemistry conditions and watershed type. These factors relate to primary production and the food quality available to bivalves (primary consumers) and may shed light on how well they select and assimilate sources. The results of this dissertation will contribute to the body of literature that examines the feeding ecology of freshwater bivalves. Specifically, complex abiotic and biotic factors need to be better understood to improve the health of these ecosystems in terms of reducing the degradation of habitat, controlling sediment and nitrate concentration levels, understanding the impact of and non-native bivalves.

With respect to future studies, the range of environmental impacts on the NRB watersheds characterized in this dissertation offer an opportunity to examine chemical variation in shells to reconstruct natural seasonal conditions of the habitat. Such an assessment can be accomplished by comparing the chemistries of shells from undisturbed and disturbed watershed sites with shells from native and non-native bivalves to better understand their feeding ecology. The natural extension of this study is to further understand the effects of nutrients and hydrology on the bivalve feeding ecology across watershed types related to shell growth rates. Recent technological advances permit microsampling of carbonate to obtain records with monthly and thus seasonal resolution.

The results of this dissertation have prompted preliminary work in $\delta^{18}O$ bivalve shell carbonate studies designed to determine the approximate shell growth rates of specimens from different watershed types (Appendix C). The goals are to determine whether the shell growth rates of non-native and native bivalves vary with different land use in these watersheds. Implications would apply to the previously analyzed turbidity, discharge and nitrogen concentrations relative to the isotopic composition of food sources and tissue. Since flow, turbidity and nitrogen concentrations may affect food quality and thus feeding strategy, the goal would be to measure annual growth rates of *C. fluminea* compared to *E. complanata* across NRB watershed study sites. The methods used are based on the fact that aquatic organisms absorb O_2 in the form of CO_2 and carbonate ($CaCO_3$). This carbonate builds into calcareous shells, acquiring ^{16}O and ^{18}O in relative proportions to their abundance at a particular point in time. This is made possible by the fact that ^{18}O is preferentially removed from the water and incorporated into the calcite during the crystallization process. This effect diminishes as temperature increases, so that calcite precipitated at colder temperatures will have more ^{18}O (positive signal) than calcite precipitated at warmer temperatures (growth bands laid down). Therefore, micro-milling of accretionary biogenic carbonate yields ontogenetic variation in $\delta^{18}O$ and $\delta^{13}C$ values that can be used to characterize the annual growth rate and age of the $CaCO_3$ secreting organism.

Preliminary results suggest that non-native (*Corbicula fluminea*) bivalves may grow faster in the agricultural and urban watersheds and native (*Elliptio complanata*) mussels may grow faster in the less disturbed watersheds (i.e., forested, Fig. 1C). These data complement findings detailed in this dissertation research that show *C. fluminea* may preferentially select nutritive food particles in disturbed habitats with low food quality. If this relationship holds true, then it supports the idea that native mussels may show a lower physiological tolerance in disturbed watersheds.

A complementary study to the laboratory experiment (chapter 3) would be to conduct the valve gape sensor at an urban site in the NRB, which has a similar turbidity curve used in the laboratory experiment. The objective of this future field study would be to test the effects of turbidity across different levels of the storm event hydrograph (i.e., baseline, peak and chronic) on the valve gape response between the non-native and native bivalves. The Nekton Research Laboratory Inc. has designed a larger valve gape sensor as a result of the previous laboratory study to accommodate large sized mussels. This would facilitate a direct comparison between *C. fluminea* and *E. complanata* feeding strategy in response to field turbidity conditions. Native mussels may feed in intermediate and low flow conditions, as suggested in chapter 1, and their low digestive efficiency could make them vulnerable to poor assimilation of food particles in watersheds with lower food quality. The results of this future field study would either support or contradict the laboratory findings, which showed that the non-native bivalves: 1) feed significantly more in the peak turbidity period associated with lower food quality; and 2) avoid feeding during the chronic higher turbidity period of the storm. Considering the overall findings, the results of this dissertation work have provided valuable insights into native and non-native feeding ecology in diverse watershed types and have generated new directions in environmental bivalve biomonitoring research.

APPENDICES

APPENDIX A

Map 1A: Bivalve and water chemistry sampling sites located within each watershed.

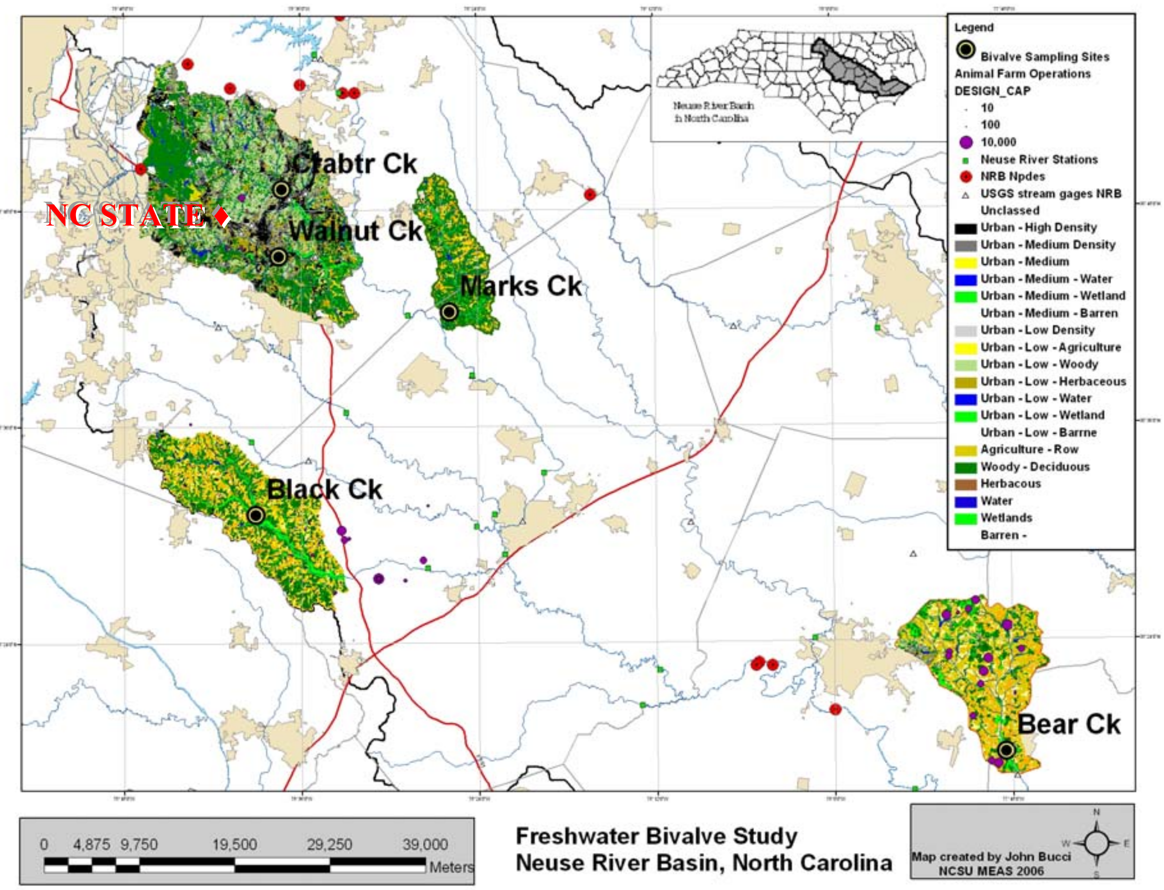

Table 1A: Percent land use land cover (LULC 2000) across watersheds. Watershed designations were based on both point source data and LULC calculated percentages.

	Forested	**Forested**	**Agriculture**	**Urban**	**Urban**
Watershed	Marks Creek	Black Creek	Bear Creek	Crabtree Creek	Walnut Creek
Size (sq. miles) **(sq. kilometers)**	**28.5** **73.8**	**105.7** **273.8**	**59.0** **152.8**	**92.8** **240.3**	**46.3** **119.9**
% LULC					
Urban	**2.0**	**3.4**	**3.4**	**29.7**	**26.8**
Agricultural	**28.7**	**45.7**	**66.7**	**1.0**	**4.1**
Woody	**63.1**	**40.3**	**23.9**	**55.4**	**50.2**
Herbaceous	**0.8**	**1.2**	**0.7**	**10.7**	**13.7**
Water	**0.8**	**1.6**	**0.7**	**0.9**	**0.9**
Wetlands	**4.1**	**7.2**	**4.6**	**1.1**	**3.3**

APPENDIX B

Abundance survey of bivalves across watersheds

A survey was conducted to determine the relative abundance of each bivalve species across study sites. In addition, archival data was utilized by the North Carolina Department of Fisheries (NCDENR, 2001). A 500m^2 area was surveyed at each site to determine the number of each species by physically counting live individuals in each sample area. Dead bivalve individuals were not used in the assessment. *Corbicula fluminea* were more widespread across urban and agricultural watershed sites (Table 1B).

Table 1B:

Date	SCI_NAME	Project	WATER WAY	COUNTY	NUM_OBS	Time (hrs)	LAT	LONG	SUBSTRATE
26-May-94	*Elliptio complanata*	DOT	Marks Creek	Wake	26	0.5	35.787	-78.437	sandy clay
26-May-94	*Elliptio complanata*	DOT	Marks Creek	Wake	65	1	35.752	-78.429	sandy clay
2-Aug-01	*Elliptio complanata*	DOT	Crabtree Creek	Wake	3	1.5	35.79	-78.586	Sandy with low woody debris
26-Sep-03	*Elliptio complanata*	DOT	Marks Creek	Wake	2	1	35.787	-78.437	sandy clay
15-Mar-06	*Elliptio complanata*	Thesis	Marks Creek	Johnston	5	1	35.787	-78.437	sandy to clay
15-Apr-06	*Elliptio complanata*	Thesis	Bear Creek	Johnston	0	1.5	35°17'45"	-77°47'89"	sandy to clay
7-Jun-06	*Elliptio complanata*	Thesis	Crabtree Creek	Wake	3	2	35°48'57"	-78°37'34"	sandy
15-Mar-06	*Corbicula fluminea*	Thesis	Marks Creek	Johnston	5	1	35.787	-78.437	sandy to clay
15-Apr-06	*Corbicula fluminea*	Thesis	Bear Creek	Johnston	25	2	35°17'45"	-77°47'89"	sandy to clay
7-Jun-06	*Corbicula fluminea*	Thesis	Crabtree Creek	Wake	52	1	35°48'57"	-78°37'34"	sandy

References

North Carolina Department of Environment and Natural Resources, (NCDENR) (2001) Standard operating procedures for benthic macro-invertebrates. 52-55 pp.

APPENDIX C

Shell Growth Rates

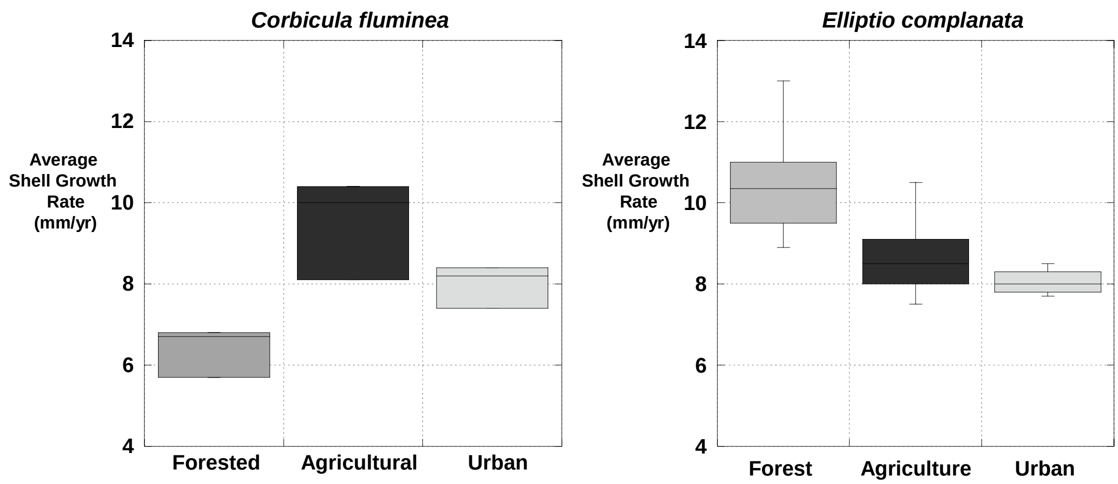

Figure 1C: *Corbicula fluminea* average annual growth rates (n = 3) across watershed type were measured from study sites in the Neuse River Basin, NC. *Elliptio complanata* sample sizes ranged from 4 to 9 specimens per watershed type.