ABSTRACT

Bucci, John P. Blue crab trophic dynamics: Stable isotope analyses in two North Carolina estuaries.

Eutrophication is increasing in estuaries as a result of anthropogenic activity along the land-sea margin. Human activities contribute large amounts of nitrogen and carbon compounds to watersheds, resulting in changes in resource availability through alteration of biogeochemical cycles and habitat destruction. Although the effects of poor water quality on lower trophic level biota is well understood, the impact of nutrient waste on upper trophic levels, such as blue crabs (*Callinectes sapidus*), has not been well studied. Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope ratios can provide time and space integrated information about feeding relationships and energy flow through food webs. An isotopic comparison of the trophic structure of two North Carolina estuaries was undertaken to understand the impacts of anthropogenic runoff on blue crab interactions and feeding habits. This study examined isotopic signatures of primary producers, as well as blue crab and their bivalve prey (*Rangia cuneata* & *Corbicula fluminea*) as indicators of potential changes in food web relationships in response to eutrophication. The Neuse River Estuary is an “impacted” system that experiences high nitrogen loading and drains areas of urban development, row crop agriculture, and concentrated animal operations. The Alligator River Estuary by comparison, is designated as a “less-impacted” system in this study. The Alligator River Estuary is classified as having “Outstanding Resource Waters” and low nutrient loading. In each estuary, samples were collected in the upper, middle and lower regions of the river. Bivalves collected from the Neuse River Estuary yielded a significant difference ($p<0.0001$) in mean nitrogen isotopic composition of tissue (10.4‰ ± 0.82; N=66) compared to the bivalves collected from the Alligator River Estuary (6.4‰ ± 0.63; N=45). Similarly, the
Neuse River Estuary blue crabs had a mean nitrogen isotopic composition of 11.41‰ (± 1.3, N=77), which was significantly different (p<0.001) than the less-impacted Alligator River blue crabs (9.65‰ ± 0.6; N=77). The mean nitrogen isotopic ratios between blue crabs and bivalves were significantly different (p < 0.0001) in the Neuse (1.01 ‰ ± 0.13) compared to the Alligator River Estuaries (3.2 ‰ ± 0.1). Linear regression analyses showed a significant inverse relationship between δ¹⁵Ν values of blue crab tissue and water quality for the Neuse River Estuary (R² = 0.7; p=0.01). A generalized linear model was conducted using blue crab tissue δ¹⁵Ν as the dependent variable and river estuary as the independent variable of interest. This analysis showed a significant difference between rivers (p<0.0001) controlling for size, site, and the river by site interaction. The results of this study indicate that: 1) A relationship exists between the uptake of anthropogenic nutrients by primary producers and the subsequent energy transfer to estuarine consumers, represented by δ¹⁵Ν and δ¹³C ratios, in two North Carolina estuaries; and 2) an inverse relationship exists between blue crab tissue enrichment and water quality in an impacted estuary.
BLUE CRAB TROPHIC DYNAMICS: STABLE ISOTOPE ANALYSES IN TWO NORTH CAROLINA ESTUARIES

by

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A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Master of Science

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DEDICATION

I dedicate this effort to my wife for her patience, encouragement and inspiration and to my family for their constant love and support. To the wisdom of naturalists like John Muir, Jane Goodall and Edward Wilson who inspire me to see the essence of a river not as flowing past us but through us.
BIOGRAPHY

As a native Rhode Islander, John Bucci grew up with a deep appreciation for marine organisms and oceanic processes. Summer sailing trips to Block Island and quahogging the mudflats of the intertidal zone 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 experimental psychology from the University of Hartford. After graduation, John worked with developmentally delayed children as a bio-behavioral psychology 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 volunteering at the Long Marine Laboratory and taking ecology courses at the University of California, Santa Cruz. His background in biology and health care has provided him with a unique perspective with which to pursue ecological research. In 2000, John moved from Dallas, Texas where he was taking oceanography courses and working as a research associate at the Southwestern Medical Center, to start a master’s program in the Marine, Earth and Atmospheric Sciences Department at North Carolina State University. This path has led him to his master’s research focusing on stable isotope ecology and estuarine processes. John plans to continue his research and will pursue his Ph.D. program at NCSU in the spring of 2004.
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I thank my good friend Brian Usry, who was my reliable field associate. His help made the collection process possible, especially on those stormy Neuse River expeditions. Stable isotope laboratory personnel, Bernie Genna (for his technical advice), Blaik Pulley, and Dr. Ed Noga (NCSU Veterinary School) have also been instrumental to this research. I also thank Hugh Porter (bivalve expert) at the UNC Marine Institute who was helpful in the proper identification of bivalve species. I owe many thanks to Dr. Kenneth Pollock (NCSU) for his statistical guidance and Dr. Thomas Kwak (NCSU) for his suggestions on isotope mixing model and gut content analyses. I also thank Dr. Jake Vander Zanden at the University of Wisconsin for encouraging me to search for those elusive bivalves.

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GLOSSARY OF TERMS

**Anthropogenic activities**: Human influences result in large additions of nutrients (nitrogen/carbon) to rivers and coastal waters.

**Carbon**: An element commonly found in the form of \((\text{CO}_2)\), utilized by plants and animals to manufacture energy rich organic compounds.

**Bottom-up forces**: Organisms on a trophic level are limited by physical and chemical factors (e.g., nutrient enrichment) available from the level below.

**Energy flow**: The transfer of energy from one organism to another through the trophic levels in an ecosystem.

**Energy system**: Nutrients available for growth to trophic level organisms. At each level of the food web, about 90% of the chemical energy is lost in the form of heat. The total energy passed from one level to the next is about one-tenth of the energy received from the previous organism.

**Eutrophic**: Water bodies or habitats having high concentrations of nutrients (e.g., \(<300-500 \text{ g C m}^{-2} \text{ y}^{-1}\)).

**Food chain**: The linear arrangement of a food web showing the transfer of energy and organic materials through various trophic levels of organisms.

**Food web**: A map of the feeding interactions in an area. It is a network (schematic depiction) of interconnecting food chains.

**Food web structure**: The order or arrangement of trophic levels in a food web. It provides a framework for understanding community interactions and comparison among communities.

**Nitrogen**: Required for protein synthesis, and is in the form of ammonium \((\text{NH}_4^+)\), nitrate \((\text{NO}_3^-)\), and nitrite \((\text{NO}_2^-)\).

**Nutrients**: Elements (e.g., nitrogen) in particulate and dissolved form required for growth by organisms.

**Oligotrophic**: Water of low productivity; nutrient-poor \((<100 \text{ g C m}^{-2} \text{ y}^{-1})\).

**Primary production**: The process in which dissolved inorganic carbon and nitrogen is converted to organic carbon and nitrogen.

**Top-down forces**: The removal or addition of predators (e.g., consumers) can exert affects (change abundance) on lower trophic levels.

**Trophic**: Relating to nutrition.

**Trophic level**: Stage in a food chain or web leading from primary producers (lowest trophic level) to primary and secondary consumers (highest level). Each organism obtains their energy in a similar manner.

**Trophodynamic studies**: Examine the factors that affect transfers of energy between trophic levels and the control of production.
Chapter 1 Introduction

Pollution of terrestrial and marine ecosystems is regarded as one of the highest priority problems facing the health of species that occupy these habitats (Vitousek et al. 1997). Despite their value to a variety of species, marine ecosystems are showing signs of deterioration such as chronic eutrophication (Group of Experts on the Scientific Aspects of Marine Pollution, GESAMP 1990, Peterson & Estes 2001). Human activities enhance nitrogen and carbon compounds to watersheds, destroy habitat, and change resource availability through alteration of biogeochemical cycles (Duarte 1995, Heip 1995). Alterations of food and resource availability, especially in highly productive systems, represent bottom-up perturbations of food web dynamics (Micheli et al. 2001). Similarly, removal or addition of top predators through exploitation, species introductions, and habitat alteration represents a top-down perturbation. Top-down (e.g., predation) and bottom-up (e.g., nutrient availability) shifts in trophic levels modify consumer interactions, impact community structure, and decrease biodiversity in aquatic habitats (Polis & Strong 1996, Micheli et al. 2001).

When wastewater, sediments, sewage, or fertilizers are introduced into a waterway, the concentration of available nutrients in that system will increase, resulting in a condition known as "eutrophication" (Heip 1995, Lalli 1997, Paerl et al. 1998). Eutrophication includes the occurrence of harmful algal blooms, low dissolved oxygen, and fish kills; all of these indicators have significantly increased since the 1980’s (Paerl et al. 1993, Burkholder & Glasgow 1997). Although primary consumers recycle a portion of phytoplankton carbon and nitrogen, algal bloom events triggered by increased nitrogen loading, together with strong vertical stratification, promote bottom water hypoxia (dissolved oxygen < 2-3 mg l⁻¹) in an estuarine system.
Despite the well-documented importance of estuaries to blue crab fisheries (McLusky 1989, Levinton 1995), few consistent and comprehensive studies exist that examine the trophic relationships of these organisms in their respective food webs. Stable isotopic composition of nitrogen and carbon ratios were used to assess blue crab (*Callinectes sapidus*) trophic structure in two North Carolina River estuaries: one impacted by anthropogenic runoff (nitrogen and carbon loading), and one less-impacted. An isotopic food web model was constructed and shown to be an effective tool for identifying the effects of anthropogenic nutrients on trophic level structure and blue crab feeding relationships.

One example of a nutrient impacted body of water is the Albemarle-Pamlico Estuarine (APE) ecosystem of North Carolina. The APE is the second largest estuary on the U.S. mainland (Epperley & Ross 1986). In a national water quality assessment study, four river basins (Chowan, Roanoke, Tar, and Neuse) in the Albemarle-Pamlico drainage area contained elevated concentrations of nitrogen. Of the large river basins, total nitrogen concentrations in 1990 were highest (1-3 mg l\(^{-1}\)) in the Neuse and Tar basins, and lowest (0.2-0.8 mg l\(^{-1}\)) in the Roanoke and Chowan basins (Harned & Davenport 1990). Water quality in the Albemarle-Pamlico drainage basins is influenced by an interrelated set of natural, cultural, and hydrologic factors. Natural factors include physiography and geology, cultural factors include land use and population distribution, and hydrologic factors include climate and the amount and distribution of surface-water runoff and ground water discharge (Skrobialowski 1996).

In eastern North Carolina, the Neuse River Estuary (NRE), which is a major tributary to the APE system, displays symptoms characteristic of eutrophication. In the past decade, the Neuse watershed has sustained a 17% increase in human population, and a 285% increase
in swine production (North Carolina Division of Water Quality, NCDWQ 2001). The NRE is considered "nutrient sensitive" and has been included as one of the nation’s 20 most threatened rivers (North Carolina Division of Environmental and Natural Resources, NCDENR 2002). The NRE has been heavily impacted by high intensity urban runoff, by-products of row crop agriculture, fertilizers, atmospheric deposition, concentrated animal feeding operations (CAFOs), as well as effluent from municipal wastewater treatment plants (NCDWQ 2001). Anthropogenic effects on estuarine habitats can adversely affect food-web interactions and are thought to be cumulative and synergistic (Peterson et al. 1985, Zaitsev 1992), thus negatively impacting commercially important estuarine species such as the blue crab.

1.2 Study Objectives

This study examined the food web consequences of nutrient loading using stable isotope analyses, with a focus on blue crab feeding relationships. The stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) provide powerful tools for estimating the trophic positions of and carbon flow to consumers in food webs. New research assessing the processes influencing community structure is necessary to detect potential food web alterations in response to anthropogenic nutrient loading (Micheli et al. 2001). Using a combination of stable isotope analyses, gut content analyses, and laboratory feeding experiments to detect food web responses to excess nutrient loading, this study will determine whether dietary differences exist among blue crabs collected in two river estuaries, one impacted by relatively high levels of eutrophication, and one less-impacted. Species such as the blue crab are an important part of these complex systems and altering their feeding relationships has ramifications for the entire community (Kennish 1992).
A unique aspect of this study is that it examines multiple trophic levels within a complex estuarine food web. Researchers have used stable isotope analysis to identify estuarine food web alterations by delineating consumer and prey pathways. Prior investigations in estuaries impacted by anthropogenic nutrients have found a link between the isotopic signatures of nitrogen and carbon sources and primary producers (Valiela 1992, McClelland & Valiela 1998b). An overall goal of the present study is to identify potential differences in the nitrogen isotopic ratios of blue crab and bivalve tissue in response to nutrient loaded sites. Specifically, this study examined blue crab and two species of bivalves (Rangia cuneata & Corbicula fluminea), as well as suspended particulate organic matter as indicators of changes in water quality. The primary objective of this study was to determine the utility of stable isotope analysis as a tool in understanding and predicting changes in blue crab trophic dynamics in response to anthropogenic inputs. Specifically, I investigated whether bottom-up forces are impacting trophic organisms such as primary producers (macrophytes and algae), primary consumers (bivalves), and secondary consumers (blue crabs). The second objective of this research was to develop a food web model for two river estuaries of varying water quality. A third objective was to determine which energy pathways (via diet sources) transfer nutrients through the food web for blue crab consumption.

Chapter 2 Literature Review

2.1 Food Web Theory

Stable isotopes of carbon and nitrogen provide an energy-based measure of food web relationships in estuarine systems. Isotope tracers reflect an integrated history of physical and metabolic processes within ecosystems (Peterson & Fry 1987, Michener & Schell 1994).
Since the publication of Hairston et al.’s (1960) initial proposition of food web theory, the effects of top-down and bottom-up forces on community structure have been studied (Pimm 1982, Polis & Winemiller 1996, Worm et al. 2000a). Bottom-up hypotheses follow the basic laws of thermodynamics: biomass of primary and secondary consumers is dependent on primary productivity and attenuates as energy is lost through transfer up the food chain (Lindeman 1942, Brett & Goldman 1997). Central to the mechanism of these energy system processes is the concept of trophic structure. Trophic structure is commonly viewed as the number of trophic levels or the length of a food chain (Lalli 1997). Food webs, unlike food chains, are more complex depictions of energy flow through groups of species. The aquatic food web is defined as an energy system with multiple and shifting interactions between organisms (Lalli 1997). A trophic level is composed of organisms at the same level in the food web in which one group of organisms serves as the source of nutrition for other groups of animals (Levinton 1995). The energy extracted from a trophic level and assimilated for growth by the next level is defined as transfer efficiency (Steele 1974)\(^1\).

The ecological theory of food webs has produced consumer-resource models for predicting how consumers respond to differences among individuals in the resource population (Briand & Cohen 1987, Winemiller 1990). Since food web models describe complex relationships, researchers have called for studies that quantify energy flow and recognize the importance of feeding interactions (Cohen et al. 1990). Changes in feeding habits may determine the fate of populations in an ecosystem (Hairston & Hairston 1993, 1993).

\(^1\) \(E_t = \frac{P_t}{P_{t-1}}\), \(P_t\) is the annual production (joules) at trophic level \(t\), and \(P_{t-1}\) is the annual production in preceding trophic level \(t-1\). \(E_t\) = Transfer Efficiency (15\% for estuarine systems containing 4 trophic levels).
Link 2002). Stable isotope analyses have emerged as a tool with the potential to test changes in food web relationships.

2.2 Trophic Dynamics

The estuarine food web begins with phytoplankton (primary producers) and rooted macrophytes (e.g., *Spartina alterniflora*) that utilize simple dissolved forms of nitrogen and carbon to produce the first trophic level (Paerl 1997). A simplified estuarine food web may contain four trophic levels (Levinton 1995). Bivalves feeding directly on marine algae make up the second trophic level; they are referred to as primary consumers. Subsequent trophic levels are formed by omnivores, such as blue crabs, that feed on smaller herbivores and carnivores (secondary consumers). Demersal fish species (tertiary consumers) form higher trophic levels and feed on larger herbivores and carnivores (bivalves and blue crabs) (Lalli 1997).

A research priority in the face of increasing flux of nitrogen into watersheds is determining how different types of discharges influence food-web interactions. Understanding bottom-up influences on consumers is an important part of the process. For example, Seitz & Lipcius (2001) compared the relative influence of top-down and bottom-up factors to population abundance of the infaunal clam *Macoma balthica* in two estuarine ecosystems exposed to varying degrees of nitrogen flux (5km to 50km spatial scale). Their results suggest that bottom-up control was responsible for differential clam densities between habitats at both spatial scales, rather than top-down control via predation (Seitz & Lipcius 2001). Particulate organic carbon (e.g., food for deposit-feeding clams) was significantly greater in the high clam-density habitat, which is consistent with the idea that bottom-up
factors principally dictate clam density, and that clam density drives predator (blue crab) density (Seitz & Lipcius 2001).

2.3 Blue Crab Ecology

The blue crab supports a valuable fishery to the state of North Carolina (NCDMF 2002). Significantly reduced landings of "hard" blue crabs were observed in 2000 and 2001, following the record high landings observed during 1996 to 1999, causing industry concern for the health of this valuable resource (NCDMF 2002). Recent landings in Albemarle and Pamlico Sound have decreased from 65,600 to 38,700 lbs from 1996 to 2000. A recent stock assessment indicated that adult blue crab harvest has been at or above maximum sustainable yield (MSY) during 1995-1999 (Eggleston et al. 2003), resulting in a stock status listed as "concern" (NCDMF 2002). The present study does not quantify the abundance of blue crabs; however, it is important to understand that a species decline exists in this ecosystem and that this outcome may be related to resource availability (high/low nutrient loading) and subsequent changes in feeding habits.

Blue crabs play an important role in the estuarine food web, providing prey for many species and, in turn, acting as a predator on other species (Eggleston et al. 1992, Dittel et al. 1997). Anthropogenic influences, which include eutrophication, habitat degradation and over-fishing, have the potential to impact blue crab populations because of the damaging role they have on prey populations and refuge zones. For example, once hypoxic events recede from shallow nearshore areas, adult male crabs migrate from deeper portions of an estuary and become crowded along the shallow flanks to take advantage of infaunal prey (Pihl et al. 1991, Bell et al. 2003). This crowding may increase rates of cannibalism on juveniles that normally seek refuge in shallow water habitats. Furthermore, infaunal bivalve prey would
become vulnerable, and potentially extinct, if hypoxic events force them to reside at shallower depths (Taylor & Eggleston 2000). Therefore, it is important to understand the trophic ecology of the blue crab in nutrient impacted areas.

The blue crab is a member of the swimming crab family, *Portunidae*. It is widely distributed along the coasts of North America and most abundant from Texas to Massachusetts (Gosner 1978). The maximum age is reported to range from 3 to 5 years (VIMS 2003). Blue crabs reach maturity at variable sizes in approximately 18 months. They are classified as general scavengers, bottom carnivores, detritivores, and omnivores (Dittel et al. 1997). It is well known that several species of bivalves (e.g., *Rangia cuneata*, *Macoma balthica*) and of oysters (e.g., *Crassostrea virginica*) are part of the blue crab’s diet (Baird & Ulanowicz 1989). However, older juveniles and adults sometimes incorporate some plant material such as planktonic algae, eelgrass (*Zostera marina*), and *Spartina alterniflora* into their diet (Orth & van Montfrans 1987). Predators on blue crabs include fish species such as the striped bass, (e.g. *Morone saxatilis*), red drum (e.g. *Scianops ocellata*), and the Atlantic croaker (e.g. *Micropogonias undulatus*) as well as other blue crabs (Orth et al. 1999).

In a study by Ebersole & Kennedy (1995), individual blue crabs were allowed to forage on 3 bivalve species (soft clam-*Mya arenaria*, Atlantic Rangia clam-*Rangia cuneata*, and the hooked mussel-*Ischadium recurvum*). Profitability curves predicted that the blue crab preferred the soft clam to the less energetically profitable *Rangia* clam. However, when the difference between prey profitabilities was not as great, profitability alone was not a clear predictor of blue crab preference. Thus, Ebersole & Kennedy (1995) concluded that blue crabs would eat *Rangia* bivalves when forced to do so. This finding may be relevant to the
current study where the Rangia clam is the only choice of bivalve species available in certain estuarine areas.

Migration and movement of blue crabs among various habitats is seasonal, depending on life stage, sex, maturity, and associated salinity preferences (Chesapeake Bay Program, CBP 2003). For example, in the spring and summer, male blue crabs move to low salinity and shallow water, and in the winter they move from the shallows to deeper waters (Judy & Dudley 1970, VIMS 2003). After mating, females move to the seaward zone, while males often remain in the mixed and upper reaches of the estuary. Of the sparse research available on blue crab migration, adult male blue crabs may be less mobile than females and tend to reside relatively close (range from 130m to 7km) to their feeding habitats (Scholar 1980, Wolcott & Hines 1990). Judy and Dudley (1970) recovered 80% of 5 thousand adult males within a 24km² area of where they were released in a large North Carolina estuary. The study suggests that males displayed limited movement compared to females. Therefore, male blue crabs provide a relatively valid indicator as a food web predator in local estuarine communities.

2.4 Bivalves

Bivalves such as unionids have been used to indicate the isotopic composition of the base of the food web because they utilize primary producers, have a long life-span, slow metabolism, and are less mobile than blue crabs (Cabana & Rasmussen 1996). Natural abundance of stable carbon and nitrogen isotope ratios (δ¹³C and δ¹⁵N) have increasingly been used to examine the relative importance of autotrophic sources in supporting food webs that include bivalves (Incze et al. 1982, Peterson et al. 1985). Thus, bivalves integrate short-term fluctuations in primary producer isotope signatures over time (Wang & Fisher 1996).
Though data are limited, certain species of bivalves have preferential rates of metabolic assimilation. Assimilation rates for different types of unionid mussels (*Pleurobema sintoxia* and *Sphaerium striatinum*) were assessed based on the isotopic composition of foot muscle tissue (Raikow & Hamilton 2001). A two-source mixing model suggested that unionids consumed 80% deposited and 20% suspended material. Alternatively, these bivalves favored the highly enriched living component of suspended and benthic organic matter rather than assimilating the bulk material.

Bivalve tissue has been utilized as a bio-indicator of anthropogenic pollution (Nichols & Garling 2000, McKinney et al. 2002). Stable isotope analyses of tissue can be a useful tool for illustrating shifts in trophic structure. Results from a similar study linked elevated $^{15}$N values of nitrogen derived from septic wastewater with those seen in Unionidae mussel (*Elliptio complanata*) tissue (McKinney et al. 2002). Regression models predicted $\delta^{15}$N enrichment in mussel tissue based on the fraction of land-use categories (e.g., agriculture, urban and residential development) in respective watersheds.

Bivalves represent an ideal study organism for identifying bioaccumulation of pollutants because of their capacity to process water at high rates. They play an important ecological role as well, especially in shallow water like that of the APE ecosystem, as prey for blue crabs and small fish. Bivalves often dominate the animal biomass of food webs and are widely used to assess the degree of environmental pollution of estuarine ecosystems (Pechenik 2000). Therefore, water quality parameters associated with habitats may be reflected in the nitrogen isotopic signatures of the tissue of these organisms.

The present study utilized two different species of bivalves based on availability in the study sites: 1) *Rangia cuneata* 2) *Corbicula fluminea*. These bivalves are common in
brackish-water habitats of mid-Atlantic coastal waters (Harrel 1993). *Rangia cuneata* have been found in the Neuse River and Pamlico Sound estuary system (NCDENR 2002). *Rangia cuneata* is in the family *Mactridae* and in North Carolina, adults’ range from 2.5 to 6.0 cm in length (Turgeon et al. 1988). *Rangia cuneata* move little after settling. Fairbanks (1963) observed little movement of bivalves in a laboratory setting. As a non-selective filter feeder, *Rangia cuneata* transform large quantities of plant detritus and phytoplankton into bivalve biomass (Hughes & Seed 1981).

The second species targeted in this study is the Asian clam, *Corbicula fluminea*. This species of bivalve first invaded the west coast of the U.S. in the 1930’s and continued its spread into southeastern rivers in the 1960’s (Williams et al. 1993). An abundance of *Corbicula fluminea* has been found in North Carolina in the Neuse River and adjacent estuaries. The Asian clam is reproductively prolific and occurs in dense quantities, up to thousands per square meter, and has been associated with the decline of native mussels in some areas (Sickel 1986). A prominent effect of the introduction of the Asian clam into the United States has been its ability to alter benthic substrate and compete with native species for limited resources.

**2.5 POM**

Particulate organic matter (POM) in estuaries is a mixture of terrestrial, fluvial and marine components, consisting of decomposed material from plant material and sediments, organic debris, and phytoplankton cells. Planktonic fragments, terrestrial plant organic matter and estuarine plant detritus predominantly comprise the estuarine particulate organic matter (POM) as represented in the present study. Primary producers are a major source of this material (McClelland & Valiela 1998a). Diagenesis affects $\delta^{15}N$ values of POM through
microbial mineralization, which reduces the amount of nitrogen and enriches the $^{15}$N content of organic substrate due to preferential utilization of the light isotopes $^{14}$N (Thornton & McManus 1994).

There are many possible sources of particulate organic matter within an estuary, each with its own signature. Particulate organic matter is made of particles that take up nitrogen loads from terrestrial and estuarine sources (Valiela 1992, McClelland & Valiela 1998a).

Carbon ratios ($\delta^{13}$C) offer a means of elucidating origins and fates of organic carbon and potential food sources for blue crabs in estuaries and nearshore marine waters. This process occurs because high riverine inputs of terrestrial carbon may produce a distinct $\delta^{13}$C depleted signature (average $> -25$‰) separable from enriched (average $< 20$‰), marine autochthonous carbon (Smith & Epstein 1970, Simenstad & Wissmar 1985). Furthermore, terrestrial and salt marsh C$_3$ plants can have depleted signatures ranging from $-23$‰ to $-33$‰ in comparison to phytoplankton, which are more enriched (Haines 1976, Lajtha & Michener 1994).

2.6 The Major Nutrient Contributions

2.6.1 Nitrogen

In the Neuse River Estuary there is concern that water quality has declined during the past 30 years as urban areas and agricultural farms have increased (Luettich et al. 2001). The relative isolation of the NRE from ocean inputs and the long flushing times of freshwater to saltwater provide an ecosystem, which promotes non-conservative mixing of nutrients such as nitrogen (Paerl 1997). According to a 4-year study, the pattern of nitrate measured within the NRE gradually declined down estuary from New Bern to the mouth (Christian et al. 1991). The US Environmental Protection Agency (EPA) has required North Carolina to establish a Total Maximum Daily Load (TMDL) for nitrogen entering the NRE.
Nitrogenous compounds from fertilizer, animal operations and wastewater sewage reach rivers and estuaries through overland flow, groundwater flow, and atmospheric discharge (Macko & Ostrom 1994, Nixon 1995). These sources can originate as either point sources (highly localized) or non-point sources (more diffuse areas such as urban runoff, farmlands, and the atmosphere). However, the largest single source of nitrogen in the APE may be non-point source (45% runoff) compared to 5% for point sources (Paerl et al. 1993). Coastal and estuarine waters are influenced by both exogenous (new) and endogenous (regenerated) nitrogen sources. Anthropogenic sources are considered new, since nitrogen is the primary limiting nutrient in most estuarine systems (McClelland & Valiela 1998a).

Nitrogen occurs as nitrate (NO$_3^-$) in oxygenated surface and shallow ground water, which declines non-conservatively toward the mouth under most flow conditions (Christian et al. 1991, Kendall et al. 1998).

Increased nitrate contributions to coastal areas result in nutrient wastewater loading, which can elevate the overall nitrate stable isotope signatures of POM and groundwater entering estuaries (Valiela 1992, McClelland & Valiela 1998b). In the present study, the nutrient load is the total amount of nitrogen contributed by terrestrial and atmospheric inputs (Hopkinson & Vallino 1995). In summer, when low flow conditions exist and winds are calm, the estuarine waters become highly stratified and new dissolved oxygen cannot circulate, This condition leads to low dissolved oxygen concentrations that may negatively stress estuarine organisms (Dauer et al. 1992).

Physical factors vary the uptake of nutrients by estuarine organisms (Christian et al. 1991). For example, riverine dissolved nutrients can have an impact on biota during the time of maximum hydrological flux, spring runoff (Mayer et al. 1998, Paerl et al. 1998). Also,
major climate cycles (e.g., El Niño) influence the hydrological cycle and vary the concentration of nitrogen delivered to estuarine ecosystems (Stapp et al. 1999). For example, the NRE had an unusually wet spring in 1998 that resulted in high discharge and high nitrogen loading from upstream sources. The resulting freshwater discharge may have pushed the estuarine salt wedge toward the mouth of the river, affecting the amount of nutrients available to organisms.

2.6.2 Carbon

Carbon sources come principally from tributaries and runoff from the land, although sewage input also occurs to a minor degree. Carbon enters the estuary and is taken up by bacteria, phytoplankton and plants (Levinton 1995). However, a portion of the carbon is respired and leaves the estuary as gas and a smaller amount is deposited into the sedimentary benthic layer. In the NRE, spring precipitation and nearly windless summers combine to trap large concentrations of organic nutrients, which fuel plankton blooms (Paerl 1983). The assimilation by consumers of carbon sources, such as vascular plants and algae can be determined on the basis of their $\delta^{13}$C characteristics (Fry & Sherr 1984).

2.7 Foundations of Stable Isotope Food Web Studies

Stable nitrogen and carbon isotope ratios are increasingly used to provide time-integrated information about feeding relationships and energy flow through food webs (Cabana & Rasmussen 1994, Vander Zanden & Rasmussen 1999). The isotope ratio of a sample $R_{\text{sample}}$ is compared to a standard $R_{\text{standard}}$. Higher values of the heavier isotope ($^{15}$N) indicate that a reservoir is more enriched in heavy nitrogen (Wada & Hattori 1978), and

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

The differences in ratios are calculated in 'del' ($\delta$) notation and have units of per mil (‰)
lower or negative values indicate a more depleted reservoir. The basis for the use of stable isotope analysis is that organisms retain the stable isotope signals of the foods they assimilate within the recent past (‘they are what they eat’), a phenomenon first demonstrated by DeNiro & Epstein (1978). In this landmark study, the authors demonstrated that carbon isotopic compositions of animals reflect those of their diet within 1‰. Subsequent studies have confirmed these results (Macko et al. 1982, Hughes & Sherr 1983, Peterson & Fry 1987).

The measurement of carbon and nitrogen stable isotope ratios involves the process of complete conversion of the sample to a gas by combustion and separation of pure gases (CO₂ and N₂) (Fry & Sherr 1984, Peterson & Fry 1987). The stable isotopes of nitrogen occur with an approximate number of ¹⁴N atoms for each ¹⁵N atom. The precise ratio of ¹⁵N to ¹⁴N differs among nitrogen pools in the environment (Clark & Fritz 1997). Biological materials contain carbon and nitrogen with various proportions of their naturally occurring stable isotopes ([¹³C]/ [¹²C] and [¹⁵N]/ [¹⁴N]). The lighter isotopes (¹²C & ¹⁴N) are preferentially utilized by living organisms.

To elucidate the origin and the fate of organic matter in an estuary, natural tracers such as δ¹³C and δ¹⁵N are used as source indicators (Lajtha & Michener 1994). Stable carbon (¹³C) isotopes can be measured as part of a two-source mixing model³ for studying diet sources in food webs (Peterson et al. 1985, Vander Zanden & Rasmussen 2001). Therefore, feeding linkages are identifiable because isotopic values of different sources vary spatially in estuarine ecosystems (Kwak & Zedler 1997). The conservative transfer of carbon isotopic compositions to the animal from the diet can be useful where there are food sources with large differences in δ¹³C values (Fry & Sherr 1984). Depending on the mobility of the

³ % preya = (δ¹³C pred - δ¹³C preyb ) / (δ¹³C preya - δ¹³C preyb ) X 100; % preyb = 100 - % preya
consumer and the temperature of the water, the tissue turnover rates for $\delta^{15}$N and $\delta^{13}$C values can be biased toward feeding patterns of the recent past. For example, studies of bivalve tissue show that assimilation of diet sources range from three to six months (Raikow & Hamilton 2001). The practice of using laboratory and field data to quantify isotope fractionations of organisms and plants and applying these fractionations to infer trophic relationships in natural systems is well established (DeNiro & Epstein 1981, Minagawa & Wada 1984).

Nitrogen signatures from different pools are quite distinct, making nitrogen sources identifiable and traceable within an ecosystem (McClelland & Valiela 1998b). The use of multiple isotopic ratios ($\delta^{13}$C and $\delta^{15}$N) provides more accurate resolution between several potential diet sources (Hobson 1999) than a single stable isotope method (Peterson et al. 1985). Nitrogen isotopic signatures range from approximately 28‰ for human and animal waste to 2‰ for fertilizers (Table 1).

Isotopic fractionation is a process that can result from an organism’s ability to discriminate nutrients during tissue assimilation or by the isotopic differences between assimilated and unassimilated nitrogen (referred to as assimilative fractionation) (Vander Zanden & Rasmussen 2001). Many factors can influence this process in organisms, such as gut passage time, digestive enzyme activity as well as the nature of the food particles (Peterson & Fry 1987). In a review of 22 studies and 20 different species in estuarine, lake and pelagic food web ecosystems, the error variance in trophic fractionation ($\delta^{15}$N and $\delta^{13}$C) and mixing models was negligible. These results were based on the assumption that primary consumers comprised the baseline of the food web and served as a conduit for bottom-up energy transfer.
2.8 Empirical Evidence using Stable Isotope Analyses

2.8.1 Aquatic Systems

Studies have shown stable isotope analysis to be an accurate and useful tool in the assessment of anthropogenic nutrient input on trophic level structure in freshwater ecosystems (Vander Zanden & Rasmussen 2001). Cabana & Rasmussen (1996) estimated the number of trophic levels in a food web by subtracting mean mussel $\delta^{15}N$ from fish $\delta^{15}N$ tissue and comparing these differences to the value expected for a single trophic level increment (3.4‰) (Minigawa & Wada 1984). Results suggested that $\delta^{15}N$ signatures of primary consumers (unionid mussels) provided less temporal variability than tissue of primary producers.

Research in a North Carolina riverine ecosystem suggested that primary consumers at the base of a food web can be used as indicators of terrestrial nutrient loading. In a highly nitrogen loaded basin, Showers et al. (in prep), documented differences in the Unionid mussel ($Elliptio complanata$) $^{15}N$ tissue from an agriculturally dominated watershed. Organisms in this watershed were thought to experience a shift toward elevated $\delta^{15}N$ values, which was suggested because of animal wastewater migration into the watershed through groundwater. The above studies illustrate that aquatic systems have served as testing sites for the efficacy of stable isotope analysis. As the successful utilization of stable isotope analysis develops, there has been a need to apply these techniques to estuarine ecosystems.

2.8.2 Estuarine Systems

Stable isotope analysis has been successfully applied in complex communities such as estuaries to monitor increasing food web organisms. Similar to fresh water ecosystems, salt
marsh embayments and estuaries serve as refuge areas for many species of flora and fauna such as the blue crab (Kennish 1992, Levinton 1995).

Spatial and temporal variability can influence the detection of trophic base alterations. For example, an estuary can experience rapid and frequent change because of tidal and upstream river flow as well as seasonal hydrological variation (Festa & Hansen 1978). Primary producer species respond with rapid growth to excess nitrogen inputs (Fry et al. 1992, Pinckney et al. 2001). The combination of phytoplankton and macroalgal photosynthesis creates oxygen; however, the increased oxygen demand (created by the microbial decay of the associated organic matter) can reduce oxygen availability, driving dissolved oxygen levels to a hypoxic range. The following studies highlight an association between anthropogenic nitrogen inputs and δ15N ratios of lower and upper level trophic organisms.

McClelland & Valiela 1998a showed a connection between δ15N tissue enrichment and percent anthropogenic wastewater from septic tanks with primary and secondary consumers. The average δ15N of wastewater increased from 0.5‰ to 9.5‰. These results correlated with increases in δ15N of eelgrass, macroalgae and particulate organic matter. Effects of anthropogenic sources on upper level estuarine flora and fauna have also been examined (Riera et al. 2000, Vizzini et al. 2002). A combination of multiple stable isotopes (15N, 13C, and 34S) was used to trace the flow of organic matter and identify trophic relationships (Peterson et al. 1985). Where there was a major river input of organic matter, multiple isotopes were used to discriminate between particulate organic matter derived from plankton and salt marsh plants (Spartina alterniflora).
Kwak & Zedler (1997) identified sewage derived organic matter sources (macroalgae, marsh microalgae, and *Spartina foliosa*) that supported consumers in different wetland habitats. Multiple stable isotopes were used to determine whether animals utilized these plant foods of two different flow systems. A two source-mixing model was used to approximate relative inputs of each source to each wetland trophic structure. Macroalgae and POM δ^{15}N ratios were substantially higher in the Tijuana Estuary (sewage influenced) compared to the less impacted San Dieguito Lagoon. The authors suggested that sewage-derived organic matter and macroalgae were major food sources for near shore fish.

A food web analysis of juvenile blue crabs using whole animals compared the importance of marsh-derived diets in supporting their development (Fantle et al. 1999). The blue crabs fed the high-protein diet received zooplankton and the low protein diet consisted of detritus. The high-protein diet was associated with a higher occurrence of rapid growth than juveniles fed a low-protein diet. Thus, a detritus based diet may be related to less nutritional value for blue crab development.

Riera et al. (2000) compared δ^{15}N values in benthic organisms collected from an estuarine site that drained a highly urbanized basin and an estuary that was isolated from these inputs by dams. In the low nitrogen input river, mean δ^{15}N values were 1.5 ± 1.6‰ for suspended particulate organic matter (POM) and 10.0 ± 1.7‰ for benthic invertebrates such as the green crab (*Carcinus maenas*). In contrast, in the high nitrogen input river, mean δ^{15}N values were 8.1 ± 0.1‰ for POM and 18.2 ± 1.2‰ for green crabs (a statistically significant difference). The authors concluded that δ^{15}N tissue enrichment in benthic estuarine organisms was associated with anthropogenic nitrogen.
Unlike traditional gut content analyses, stable isotope analyses integrate energy flow over space and time scales and offer insight to patterns of feeding habits (Michener & Schell 1994). Despite progress in stable isotope ecological research, limitations exist. First, data tend to be sparse (Table 2) (Riera et al. 2000, Dunton 2001) and second, relationships between diet and consumers are established on the basis of the means of these small data sets. The present study examines a larger group of trophic organisms exposed to a range of sites and water quality.

Chapter 3 Hypotheses

1) Ho: There is no difference in mean $\delta^{15}$N signature in the tissue of blue crabs collected from locations with varying water quality.

$H_1$: There is a relatively high mean $\delta^{15}$N signature in the tissue of blue crabs collected from locations with high nitrogen loading.

$H_2$: There is a relatively low mean $\delta^{15}$N signature in the tissue of blue crabs collected from locations with low nitrogen loading.

2) Ho: There is no difference in the proportion of bivalves in the diet of blue crabs at locations with varying water quality.

$H_1$: There are a lower proportion of bivalves in the diet of blue crabs at locations with high nitrogen concentrations.

$H_2$: There are a higher proportion of bivalves in the diet of blue crabs at locations with high nitrogen concentrations.

3) Ho: There is no difference in the proportion of POM and plants in the diet of blue crabs in locations with varying water quality.
H₁: There are a lower proportion of POM and plants in the diet of blue crabs in locations with high nitrogen concentrations.

H₂: There are a higher proportion of POM and plants in the diet of blue crabs in locations with high nitrogen concentrations.

**Chapter 4 Methods and Materials**

Data were based on POM, plant and animal sampling at six research stations in the Neuse River Estuary and six in the Alligator River Estuary during the summer of 2002. Carbon and nitrogen isotopic values were utilized to construct a trophic model. Results from a two source mixing model, gut content analyses and feeding experiments were also used to detect feeding relationships among blue crabs in impacted versus less-impacted sites.

**4.1 Study Area Selection**

The study sites were located in the Neuse and Alligator River Estuaries as part of the APE ecosystem in North Carolina (Figure 1). The Outer Banks protects this estuary from the harsh impacts of the ocean. The enclosed waters are shallow (2-4 meters in depth), wind-mixed with limited tidal effect, and poorly flushed, with an average residence time in major tributaries of 50-100 days on an annual cycle (Christian et al. 1991). These physical features make the Albemarle-Pamlico Estuary sensitive to impacts from excessive nutrients (Pinckney et al. 2001).

**Impacted Stations**

The Neuse River Estuary is a major tributary to the APE, emptying into the southwestern corner of Pamlico Sound. From Streets Ferry to Minnesott Beach, the Neuse River has experienced high chlorophyll a levels, associated with over production of algae, and subsequent low dissolved oxygen (DO), leading to fish kills (NCDWQ 2001). In this
study, the Neuse River is designated as “impacted.” Impacted is defined as having a high potential for the occurrence of eutrophication (an increase in the rate of supply of nutrients/organic matter to an ecosystem) (Pinckney et al. 2001). The Neuse River was chosen based on basin-wide assessment of water quality data presented by the North Carolina Department of Environment and Natural Resources (NCDWQ 2002). The entire Neuse River basin has one of the fastest growing animal populations in the United States. There are 2 million swine (20% of the state's annual total) and 15 million poultry (80% of the state's annual total) in this basin (Spruill et al. 1996).

The Neuse River sub-basin is part of the estuarine portion of the entire drainage basin. Land-use GIS® data indicates that the watershed is comprised of forest and wetland (56%), followed by surface water area (26%), agricultural croplands/animal operations (11.4%), with 6% being urban (Figure 2). This ecosystem is considered "nutrient sensitive" by the state's classification and experiences periodic harmful algal blooms in the summer (NCDENR 2002). The National Pollutant Discharge Elimination System (NPDES) program monitors and treats wastewater from urban areas. These discharges are a large source of nitrate inputs (NCDWQ 2001). There are 19 wastewater discharge sites in this sub-basin. A major point source in the Neuse River releases approximately 0.5 million gallons per day (mgd) of effluent into the river usually after secondary treatment. Average nitrate concentrations (1-3mg N l⁻¹) have been higher in the Neuse compared to other rivers in the APE (McMahon & Woodside 2000).

**Less-impacted Stations**

The Alligator River Estuary (ARE) is designated as the “less-impacted” or "control" river in this study. This river has better higher quality water than the Neuse River, therefore
less potential to stress estuarine organisms by anthropogenic eutrophication. The Alligator River is a major tributary to Albemarle Sound. It has higher water quality and drains neither major urban areas nor large agricultural farms (NCDENR 2001). In contrast to the Neuse, the Alligator River is part of the Pasquotank River Basin (intra-coastal waterway canal not included) and is classified as having "Outstanding Resource Waters" (NCDWQ 2001).

The Pasquotank River Basin encompasses 3,635 square miles of low-lying land located in the northeast coastal plain of North Carolina. Over 23,000 acres of freshwater lakes are contained in the basin in addition to 900,000 estuarine acres. Study stations are within this sub-basin. This sub-basin includes a mixture of public lands and the Alligator River National Wildlife Refuge (Figure 3). The river and its creeks are a nursery where young fish thrive and develop and it contains the state's best remaining example of a palustrine swamp forest (Lynch & Peacock 1982a). The ecosystem wetland adjacent to the river has abundant wildlife such as black bear, wolves and many breeding birds. Results from the NCDENR assessment report for 2002 shows that mean nitrate concentrations in the ARE were less than 0.1 mg N l\(^{-1}\) from 1995 to 2000 (McMahon & Woodside 2000). Also, dissolved oxygen was reported to be an average of 9 mg l\(^{-1}\) for this time period. This sub-basin contains the lowest human population density in the entire Pasquotank River basin.

4.1.1 Sample Site Criteria

Prior to the summer of 2002, six field stations were established for each of the two river estuaries from upper estuary, mid-estuary, to lower estuary (Figures 4 and 5). The study collection area included a 64km section of the NRE sub-basin compared to a 42km section of the ARE sub-basin. Latitude and longitude locations were recorded for each station using the geographical positioning system (GPS). Sampling occurred in 2 to 5 meters
of water. Average daily dissolved oxygen ranged from 0 to 9 mg l\(^{-1}\) and the benthic substrate type ranged from organic mud in the upper estuary to lower estuary.

4.2 Blue Crab Collections and Analyses

Sample Size

Adult male blue crabs were sampled (N=10 per station) at sites located at 13-21 kilometer intervals from the beginning of each river estuary (Figures 4 and 5). Collection was achieved using crab pots, which were placed at GPS designated stations. The length of time between baiting and retrieval of crab pots was limited to 6 days to prevent possible starvation effects in the tissue (Hobson 1993). Individual crabs were placed on ice immediately following collection and then placed in a freezer to prevent decomposition (Macko & Ostrom 1994). Also, this study has larger sample sizes per site relative to previous studies. The present study used approximately 77 blue crabs per river estuary for comparison of each food web structure. Since nitrogen and carbon isotopic enrichment has been shown to be positively correlated with crab size in benthic food webs (France 1998), blue crab samples were measured and separated into two categories (small < 114 mm and large > 114 mm) based on limits set by NC Division of Marine Fisheries.

Blue crab tissue analyses

Extraction of walking leg muscle tissue (Figure 6) was the method consistent with previous literature (Riera et al. 2002) and the most feasible for isotope analysis given the sample size in this study. Also, the issue of isotopic heterogeneity is overcome by sampling the same tissue in the same location of each animal. Tissue from different parts of the organism will have different turnover rates (Monteiro et al. 1991). For instance, muscle tissue compared to gills reflects a longer-term dietary history and thus integrates nutrient
sources more consistently (Hughes & Sherr 1983, Tieszen et al. 1983, Fantle et al. 1999). To assess intra-individual tissue differences, paired t-tests were performed to detect significant differences among pairs of leg and gill samples of blue crab tissue. Preliminary results showed that there was not a significant difference (p>0.05) between the right and left leg and the right and left gill within individual crabs. Leg tissue samples were eventually the only tissue type used for $\delta^{15}$N and $\delta^{13}$C analyses.

Two milliliters of solid material were stored frozen in glass micro-vials. After dissection from the cuticles, samples were freeze-dried, then washed with 10% HCl (to remove carbonates) and rinsed in de-ionized water (Showers & Angle 1986). Tissue samples were freeze-dried. Because of possible sample heterogeneity, samples were ground and mixed. The samples were then stored in a cool, dry area in closed containers (such as scintillation vials) prior to isotope analysis.

4.3 Bivalves

Approximately 10 to 15 bivalves were collected using a clam rake, from each station. Bivalve foot muscle tissue was isotopically analyzed for $\delta^{15}$N and $\delta^{13}$C from all collection sites. Muscle tissue was extracted because it has a relatively longer term (>90 days) dietary history than stomach lining or blood, which is metabolized faster (Tieszen et al. 1983, Raikow & Hamilton 2001, McKinney et al. 2002). Upon collection, the bivalves were placed on ice for 24 hours and then placed in a freezer to prevent decomposition. The foot muscle area sampled to be consistent anatomically and to reduce sample heterogeneity (Figure 7). Bivalve species were identified at each site. The species, Atlantic Rangia ($Rangia cuneata$) and the Asian Clam ($Corbicula fluminea$) were discovered in several locations in both the Neuse and Alligator River estuaries.
4.4 POM and Plant Material

Sampling sites for POM and vascular plant material (e.g., terrestrial organics, *Spartina alterniflora*, and freshwater macrophytes) corresponded to blue crab and bivalve collection sites. Sites were chosen to enable construction of trophic food webs across an upper to lower estuary transect. Depth at sampling stations ranged from 1 to 5 m. Samples were processed for isotopic nitrogen ($\delta^{15}$N) and carbon ($\delta^{13}$C) signatures. Water samples were collected from each of the six stations along a river estuary and refrigerated to prevent algal growth. Particulate matter samples were obtained by filtering 500ml of estuarine water taken from a depth of 0.5 m below the surface. Particulate organic matter was removed from water samples onto pre-combusted (4h, 500ºC) glass fiber filters with a nominal pore size of 0.7 microns. The membranes were acidified in 10% HCl to remove carbonates, rinsed with DI water and kept frozen. POM and plant material samples were dried at 60ºC and ground to guarantee homogeneity. POM sample filters were wrapped in pre-combusted aluminum foil and placed in polypropylene vials. The vials were stored in a dry place until isotopically analyzed.

4.5 Gut Content Analyses

Gut content analyses of 83 blue crabs were conducted in combination with isotope analyses to examine the relationship between consumption of plant versus animal material. Stomachs were extracted approximately 24 hours after collection and placed on ice. They were stored in 70% ethyl alcohol until analysis.

Analysis of invertebrate gut contents has been successfully implemented to track organic matter across trophic levels in freshwater ecosystems (Benke et al. 2001). However, this method has not been well documented in blue crabs (Kwak & Zedler 1997, Fantle et al.)
A potential limitation of this approach is that blue crab food content is often fragmented and unidentifiable because blue crabs possess a gastric mill stomach, which grinds up food items before digestion. Thus, not all of the contents are assimilated and gut content analyses can lead to an overestimation of a particular diet source (Hughes & Sherr 1983, Rosenfeld & Mackay 1987).

In the laboratory, gut contents were analyzed under a light microscope at magnifications from 10X to 60X within two months of capture. The percent fullness in each gut was determined visually by observation of settling layers of source material created while stored in a test tube (Cannicci et al. 2002). The percent composition for each gut was divided into animal and plant material. Average percent composition (measured in multiples of 5%) was visually assessed and used for further analysis and verification. For example, the percent assigned to each category was based on the observed fraction of plant or animal material present in each stomach. Gut material was further identified and placed into the following mutually exclusive categories: a) mollusk tissue and shell, b) shrimp c) polychaetes d) blue crab tissue and shell material, e) fish tissue, f) zooplankton, g) algae and detrital material, h) and mucus. This methodology is based on the percentage point procedure used by Wear & Haddon, (1987) and Rosas et al. (1994).

4.6 Laboratory Blue Crab Feeding Trials

Crab feeding experiments were conducted as an exploratory approach to determine whether short-term carbon and nitrogen enrichment is reflected isotopically in blue crab muscle tissue. An additional goal was to verify whether blue crab diet includes the types of bivalves collected for the model food web. Laboratory-feeding studies were conducted at the NCSU Veterinary Medicine fish laboratory in Raleigh, North Carolina under the supervision
of Dr. Edward Noga. A 14-day pilot study was conducted examining three different types of diets, fed to three adult male blue crabs. Prior to the experiment, three blue crabs were randomly sampled from less-impacted river estuary sites. Isotopic signatures were obtained from the walking leg muscle and served as a baseline prior to feeding. Subsequent isotopic analysis of leg muscle on the baseline individual was not performed. The three diets consisted of bivalve foot tissue collected at study stations in the Neuse and Alligator River Estuaries (Figures 4 and 5).

Diet 1: Neuse River Estuary bivalves (*Rangia cuneata and Corbicula fluminea*)

Diet 2: Alligator River Estuary bivalves (*Rangia cuneata*)

Diet 3: Combination of Diet 1 and 2

The crabs were fed daily at the same time and the amount of consumption was recorded. Each crab was placed in a 151 liter tank. Estuarine water conditions were approximated and controlled for salinity, dissolved oxygen, nitrate and temperature on a daily basis.

Blue crabs were sacrificed at the completion of the two-week trial. Tissue samples were extracted as in the original procedure. This trial was conducted to determine whether carbon (\(\delta^{13}C\)) and nitrogen (\(\delta^{15}N\)) ratios were reflected in the isotopic signatures of the bivalve tissue.

4.7 Water Quality & Nutrient Data

In the NRE, water quality parameters (e.g., DO mg l\(^{-1}\), NO\(_3^-\)) were averaged monthly (15 minute intervals for the 3 month study period) and used in regression analyses based on data from the United States Geological Society and NC State’s Center for Aquatic Ecology research stations (Figure 4). Water samples, using a 500 ml bottle, were taken at each station
at the time of animal collection. Nitrate (NO$_3^-$) concentrations (mg N l$^{-1}$) of the water collected were kept on ice and measured in the laboratory within 24 hours.

In the ARE, water quality parameters using the North Carolina Department of Natural Resources water quality station data (Route 64) located proximal to the lower estuary sites (Figure 5) were averaged (N=72) across the three month study period. Discrete measurements (50 water quality, DO mg l$^{-1}$ samples per site) at the upper and mid-estuary sites were taken using a Sonde instrument (YSI® Multi-parameter water quality logger). The water quality loggers were calibrated with standard procedures. These values were within range of seasonal averages (NCDENR 2002).

4.8 Stable Isotope Analyses

Isotopic analysis of tissue, POM and dissolved nitrate were completed in the NCSU Stable Isotope Laboratory. A Delta XL, CF-IRMS (isotope ratio mass spectrometer) connected to a Carlo Erba® Elemental Analyzer; NA 2500 Series determined the $\delta^{13}$C and $\delta^{15}$N on all the samples. The samples were placed on tin boats, combusted in the EA and the resultant gas (CO$_2$ and N$_2$) were separated in a gas chromatograph. The separated gas is carried to the mass spectrometer in a helium stream where sample gas peaks are compared to standard gases. The isotopic composition of a sample (e.g., organism solid tissue) is quantified relative to an international standard reference material (Pee Dee Belemnite limestone for carbon and air for nitrogen). Ratios of $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N are expressed as the relative per mil differences between the sample and conventional gas standards (Fry & Sherr 1984, Peterson & Fry 1987).
4.9 Two Source Mixing Model

The use of stable isotopes for examining food web relationships or for tracing the fate of nutrient sources relies on the assumption that producers and/or different sources of nutrients have distinct ratios of the naturally occurring isotopes of a particular element. For example, Hughes and Sherr (1983) showed that blue crab diet consisted of a large proportion (81%) of algae and (19%) of marsh grass, *Spartina alterniflora*. The current model was designed to identify diet sources that support blue crab consumers in two different river estuaries, and to seek trophic linkages between habitats. The relative contribution of two sources of nutrients, assimilated by an organism, was estimated by calculating the weighted average of the stable isotope signature of the sample with respect to two sources or end members (Van Dover et al 1992). The diet sources chosen were within the standard 1‰ assumption of a given consumer (DeNiro & Epstein 1978). The simple two-source $\delta^{13}C$ mixing model is used to quantify contributions of two potential food sources (prey ‘a’ and prey ‘b’) to a predator population (pred) (Figure 8) (Fry & Sherr 1984). The mean $\delta^{13}C_{\text{prey } b}$ for POM at each station as one end-member in the mixing model was utilized. The mean bivalve tissue collected at each station represented the second end-member source, $\delta^{13}C_{\text{prey } a}$. The predator in this model was represented using the mean $\delta^{13}C_{\text{pred}}$ of blue crab tissue. When diet sources have distinct isotope ratios, the signature of the sample reflects the relative contribution from each source to the consumer (Fry & Sherr 1984). The results are expressed as a percent of body tissue carbon derived from these diet sources. This approach combined with the gut content analyses and dual isotope analyses offers an understanding of the major feeding pathways exhibited by blue crabs in this system.
Chapter 5 Statistical Methods

The objectives of this study included a) determining the relationship between nitrogen tissue enrichment and indicators related to water quality, and b) measuring differences in blue crab feeding habits at impacted versus less-impacted sites. SAS® statistical software (version 8.2) was used for statistical modeling. A test for normality was performed (using the “W” statistic as part of Proc Univariate) to ensure that the data were normally distributed (Schlotzhauer & Littell 1991).

A Generalized Linear Model (GLM) was used to examine differences in blue crab $\delta^{15}$N tissue enrichment with respect to river estuary (NRE and ARE), site (upper, mid, or lower), and crab body size (small < 114mm and large > 114mm). Because of significant river by site interactions, separate models were developed for each river.

To determine whether mean $\delta^{15}$N varied by site (upper, mid, and lower estuary), generalized linear models were run for each river using $\delta^{15}$N enrichment of crab and bivalve as the dependent variables (Cody & Smith 1991). Tukey’s studentized range test was used to test post-hoc differences in mean $\delta^{15}$N between upper, mid, and lower estuary sites. For blue crab models, body size was added as a covariate to generalized linear models for each river, using blue crab tissue $\delta^{15}$N as the dependent variable and site as the independent variable of interest. Student’s t-test was used to determine whether $\delta^{15}$N enrichment of blue crabs and bivalves differed between the impacted and less-impacted rivers. To address the first hypothesis which considers whether the blue crab tissue is significantly more enriched at the impacted than at the less impacted sites, a GLM was conducted using tissue $\delta^{15}$N as the dependent variable and river estuary as the independent variable, including site, body size, and river*site interaction terms as covariates.
Student’s t-test were conducted to determine whether POM $\delta^{15}$N was significantly different in the NRE compared to the ARE. Simple linear regression analyses were used to test the relationship between tissue enrichment and water quality. Specifically, the relationship between isotopic composition of invertebrates and water quality parameters by site was evaluated. Regression plots from the NRE and ARE were compared qualitatively. The model used an estimated best-fit line ($y=b_0 + b_1x$) produced by least squares regression. The regression analyses were weighted by sample size per site since there were unequal sample sizes. The weighted average test places more emphasis on larger sample sizes. The response variable was blue crab $\delta^{15}$N leg muscle tissue versus the independent variable mean POM $\delta^{15}$N ($\text{Leg}\delta^{15}\text{N} = b_0 + b_{\text{POM}^{15}\text{N}}$). Variables such as POM $\delta^{15}$N and dissolved oxygen were chosen as representative of water quality at sites in each river estuary.

The second hypothesis tested was whether a lower proportion of bivalves were measured in blue crab diet associated with impacted (higher nutrient) loaded sites. A regression analysis (% Bivalve in blue crab stomach $= b_0 + b_{\text{POM}^{15}\text{N} \& \text{DO}}$) was performed to measure the strength of the relationship between the percent bivalves consumed at sites of varying water quality. An additional method to assess the second hypothesis used a two-source mixing model (Hughes & Sherr 1983). It was constructed to determine the proportion of diet assimilated in blue crab tissue in the impacted river. Carbon isotopic signatures were evaluated based on the assumption that a consumer (e.g., blue crabs) and its potential diet were separated by $1\% \pm 0.5$ (DeNiro & Epstein 1978). Distinct (end-member) carbon isotopic diet sources were a necessary part of this analysis (Peterson & Howarth 1987).

Finally, the third hypothesis tested whether there was a higher proportion of POM and plant material in blue crab diet associated with impacted sites. Carbon isotopic signatures
were also evaluated from the dual isotopic trophic model to infer feeding relationships.

Similar to the analysis for the second hypothesis, a regression analysis (% Detritus in blue crab stomach = b0 + b1POM $^{15}$N & DO) was performed to measure the strength of the relationship between the percent detritus consumed at sites of varying water quality.

Chapter 6 Results

$\delta^{15}$N of POM and plants

In the Alligator River estuary, POM showed mean $\delta^{15}$N values of 4.56‰ (SD ± 0.5; N=12) and terrestrial plants (C3) had $\delta^{15}$N signatures of 5.81‰ (SD ± 2.1; N=3). *Spartina alterniflora* was not found in the ARE. In the NRE, mean $\delta^{15}$N for POM was 6.74 ‰ (SD ± 1.34; N=18), which was significantly different (p<0.0001) than the POM $\delta^{15}$N values for the ARE. In the NRE, *Spartina alterniflora* had $\delta^{15}$N values of 3.8‰ (SD ± 0.21; N=3) and terrestrial plants (C3) had $\delta^{15}$N signatures of 5.74‰ (SD ± 0.13; N=2).

$\delta^{15}$N of invertebrates and demersal fish

The *Corbicula sp.* bivalves were found primarily in upper estuary areas with relatively low average salinity compared to the lower estuary stations. They were not found at sites in the ARE. This type of bivalve is a North Carolina invasive species and was sampled as part of the trophic model. Primary consumers had the least $\delta^{15}$N variation (SD=0.72) compared to primary producers (SD=0.86) and secondary consumers (SD=0.95) among river estuaries, suggesting a greater consistency as a bio-indicator of nutrient sources.

The bivalves collected from the NRE yielded a significant difference (p<0.0001) in mean nitrogen isotopic composition of 10.4‰ (SD ± 0.82; N=66) compared to the ARE bivalves 6.4‰ (SD ± 0.63; N=45) (Figure 9) using a two sample t-test. Within the ARE, a generalized linear model (GLM) showed that $\delta^{15}$N values varied significantly by site.
(F=25.22, df=2,44 p<0.0001). A Tukey’s studentized range (p=0.05) test showed there were significant differences (p<0.05) in bivalve δ15N values between upper and mid and between upper and lower estuary (Figure 10). Similarly, a GLM within the NRE showed that δ15N varied significantly by site (F=58.59, df=2,79 p<0.0001). There were significant differences (p<0.05) in bivalve δ15N values between mid and upper, mid and lower and upper and lower estuary (Figure 10).

The NRE blue crabs sampled at all locations had mean δ15N values of 11.41‰ (SD ± 1.3; N=77). Isotopic data from the ARE revealed that blue crabs had δ15N values of 9.65‰ (SD ± 0.6; N=77). There was a significant difference (p<0.001) in mean δ15N values between blue crab tissues sampled in the impacted versus the less-impacted river estuary (Figure 11) using a two sample t-test. Generalized linear models were then used to test whether blue crab tissue δ15N differed by site within each river. For the ARE, the F-value for site was 12.45 (df=2,80, p<0.0001). A Tukey’s studentized range test showed statistically significant differences (p<0.05) between upper and mid-estuary sites, and between lower and mid-estuary sites (Figure 12). For the NRE, the F-value for site was 79.83 (df=2,95, p<0.0001). A Tukey’s studentized range test showed statistically significant differences (p<0.05) between upper and mid, upper and lower, and between mid and lower estuary sites (Figure 12).

In the NRE, demersal fish species (*Paralichthys dentatus*) had a δ15N value of 15.16‰, N=1, which was higher than similar species collected in the ARE. The combined mean δ15N values of demersal fish (*Paralichthys dentatus* and *Pylodictis olivaris*) sampled from the ARE were 12.35‰ (SD ± 0.49; N=3).
\( ^{\delta^{13}}C \) of POM and plants

In the Alligator River Estuary, the use of carbon isotopic signatures had two main purposes: construction of a trophic model and determining the feeding habits of blue crabs.

In the ARE, \( \delta^{13}C \) values of POM were -27.73‰ (SD ± 2.01; N=12). In the NRE, \( \delta^{13}C \) values of POM were slightly more variable than the ARE POM samples at -26.88‰ (SD ± 2.96; N=18). Terrestrial (C\(_3\)) plants showed \( \delta^{13}C \) values of -26.56‰ (SD ± 0.90; N=3) for the ARE compared to -25.10‰ (SD ± 0.73; N=2) for the NRE.

A sample of C\(_4\) plants (\textit{Spartina alterniflora}) was collected in the NRE (\( \delta^{13}C \) values of -12.79‰; SD ± 0.06; N=3) but not found in the ARE. These results were averaged with the terrestrial plant \( \delta^{13}C \) values and shown in the trophic model (Figure 13B) for comparison.

\( \delta^{13}C \) of invertebrates and demersal fish

The ARE bivalves had mean \( \delta^{13}C \) values of -24.9‰ ± 3.12, (N=45) compared to the NRE bivalves, which had mean \( \delta^{13}C \) values of -25.84‰ (SD ± 2.35; N=66). The ARE blue crabs showed \( \delta^{13}C \) values of -24.66‰ (SD ± 2.75; N=77), which were different (2.8‰) than the NRE blue crabs (-21.8‰; SD ± 1.9; N=77). The \( \delta^{13}C \) values (- 24.46‰; SD ± 0.53; N=3) of the NRE fish tissue were slightly higher than the ARE sample with values of -24.79‰ (SD ± 0.52; N=3).

6.1 Trophic Dynamics Model

The trophic dynamic model estimated a total of four trophic levels for each estuary examined. These levels included representatives of primary producers (terrestrial organics freshwater macrophytes, estuarine plankton and POM), primary consumers (bivalves), secondary consumers (blue crabs) and tertiary consumers (fish). The trophic level separation with regard to nitrogen ratios for both rivers showed an average difference of 2.7‰ (Table
3). The mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic values were calculated for all trophic levels. Since both isotopes are utilized to determine enrichment and feeding relationships, trophic comparisons were evaluated.

The mean nitrogen isotopic ratios between blue crabs and bivalves were significantly different ($p < 0.0001$) in the Neuse (1.01 $\pm$ 0.13) compared to the Alligator River Estuaries (3.2 $\pm$ 0.1) using a two-sample t-test. In both river estuaries, crabs collected from upper estuary sites had larger carapace widths relative to crabs collected from mid to lower estuary sites (Table 4). For example, in the ARE, the largest individuals had the highest $\delta^{15}\text{N}$ signatures (120.8mm and 9.95‰). Similarly, this relationship was observed in the NRE (118.9mm and 12.62‰).

6.2 Regression Analyses by Sites

A series of linear regression analyses were conducted to examine the relationship between water quality and nitrogen enrichment of blue crab and bivalve tissue. There was a significant association between mean blue crab tissue and POM $\delta^{15}\text{N}$ at NRE sites ($F = 11.9$; $R^2 = 0.7$; $p = 0.01$) (Figure 14). The ARE did not yield a significant association ($F = 0.8$; $R^2 = 0.17$; $p = 0.41$); however, the slope was in the same direction as the NRE. An inverse relationship was observed between blue crab $\delta^{15}\text{N}$ tissue enrichment and DO (mg l$^{-1}$) for the NRE ($F = 35.2$; $R^2 = 0.9$; $p = 0.002$), whereas in the ARE the relationship was not significant ($F = 3.2$; $R^2 = 0.44$; $p = 0.14$) (Figure 15). Regression plots were created for each river estuary to examine the relationship between blue crab tissue $\delta^{15}\text{N}$ and POM $\delta^{15}\text{N}$ by body size by site (Figures 16 and 17). These analyses support a trend of increasing crab $\delta^{15}\text{N}$ tissue enrichment with decreasing water quality in the NRE.
Primary consumers represented by $\delta^{15}$N values of bivalve tissue showed positive slopes in regression analyses in both rivers, although the relationships were not statistically significant (Figure 18). The relationship was strongest using POM $\delta^{15}$N in the ARE ($F = 15.1; R^2 = 0.88; p = 0.06$) compared to the NRE ($F = 0.9; R^2 = 0.24; p = 0.39$). To test the association between blue crab diets in impacted versus less-impacted sites; the percent of bivalves and detritus in stomachs was measured against POM $\delta^{15}$N and DO mg l$^{-1}$.

Regression models revealed that consumption of detritus by blue crabs at less-impacted sites was not related to a significant decrease in water quality ($F = 2.9; R^2 = 0.42; p = 0.16$) (Figure 19). Similarly, there was no clear trend in the data ($F = 0.3; R^2 = 0.08; p = 0.63$) for the impacted sites. Also, there was not a relationship observed between the percent of bivalves consumed by blue crabs and decreased water quality in either river estuary (Figure 20). However in the ARE, blue crabs showed a borderline significant relationship ($F = 5.8; R^2 = 0.59; p=0.07$) implying that blue crabs may consume a lower percentage of bivalves as water quality decreases.

Student’s t-tests were run comparing the slopes of both river estuary regressions (Table 5). These tests showed a significant difference ($t=3.1; p< 0.05$) between the ARE and NRE when the bivalve tissue and mean POM $\delta^{15}$N were compared. Conversely, the data did not reveal a significant relationship between bivalve tissue $\delta^{15}$N and dissolved oxygen ($t = 1.8; 0.05 < p < 0.1$).

**6.2.1 Multivariate Blue Crab Analyses**

A Generalized Linear Model (GLM) was used to examine differences in blue crab tissue $\delta^{15}$N with respect to river (NRE and ARE), site (upper, mid, or lower estuary), and crab body size. Interactions were assessed between river and body size, river by site, and site...
by body size. These analyses showed a borderline-significant interaction for river by size (p = 0.0542), a very strong interaction for river by site (p<0.0001), and no interaction for site by body size (p = 0.3705). Based on these interactions, separate models were developed for each river. Generalized linear models were run for the NRE and the ARE, with blue crab tissue $\delta^{15}N$ as the dependent variable and site and body size as covariates.

These analyses showed that for both rivers, site was significantly associated with $\delta^{15}N$ (p<0.001) even after controlling for body size. In the NRE, the mean $\delta^{15}N$ values were 12.62‰ ± 0.81 for the upper estuary, 11.46‰ ± 0.84 for the mid estuary, and 10.11‰ ± 0.75 for the lower estuary. In the ARE, the mean $\delta^{15}N$ values were 9.94‰ ± 0.85 for the upper estuary, 8.89‰ ± 0.45 for the mid estuary, and 9.74‰ ± 0.58 for the lower estuary. Body size remained significantly associated with tissue $\delta^{15}N$ in the NRE (p=0.0048), but not in the ARE (p=0.9429), controlling for site.

To address the first hypothesis (H1) which considers whether blue crab tissue $\delta^{15}N$ is significantly more enriched in the impacted than the less impacted river estuary, a GLM was conducted using blue crab tissue $\delta^{15}N$ as the dependent variable and river estuary as the independent variable, including site, body size, and river*site interaction terms as covariates. This analysis showed a significant difference between rivers (p<0.0001) even after controlling for size, site, and the river*site interaction.

6.3 Gut Content Analyses

As a complementary approach, gut content analyses were conducted among 83 blue crabs selected randomly during the summer of 2002. The sampling included a cross-section of approximately half (53%) of the total blue crab sample from the 12 designated stations. Four main categories were distinguished in blue crab gut content analyses (Figure 21). These
categories were indicative of a general estuarine trophic structure, which included primary producers, primary and secondary consumers. The plant category included C₃ and C₄ vascular plants, algae and detritus material. Impacted sites showed less animal material (19% vs. 28%) and more plant (40% vs. 33%) compared to the less-impacted sites (Figure 21). Uncertainty in this observational technique is inevitable and was considered in the final conclusions.

6.4 Two Source Mixing Model

A simple two-source mixing model was constructed to examine percent relative ingestion of all possible diet sources sampled (e.g., POM, terrestrial organics, estuarine plankton, *Spartina alterniflora* and bivalve tissue). It is known that the detritus pathway of most vascular plants serves as a food source for estuarine consumers such as bivalves and blue crabs (Fry & Sherr 1984, Peterson & Howarth 1987). Only NRE blue crabs were utilized because the diet sources collected possessed distinct δ¹³C values within a detectable range (DeNiro & Epstein 1978, Peterson & Howarth 1987). Approximations using this blue crab diet assimilation model suggested variations in relative contributions of POM, vascular plant material and bivalves. Three scenarios were tested based on diet sources that were previously isotopically analyzed (Table 6). In the first scenario (POM versus *Spartina*), data revealed that POM (64%) was preferentially ingested relative to *Spartina* (36%). In the second scenario (bivalve versus *Spartina*), bivalve tissue comprised of 69% of the blue crab diet compared to 31% for *Spartina*. In the third scenario, a combination of plant material yielded a greater proportion (94%) of blue crab diet assimilated in tissue compared to a bivalve-based diet (6%). Although other possible combinations exist, these results imply that
a mixture of estuarine POM, terrestrial plant material and Spartina sp. may be a dominant
diet source for the NRE blue crabs.

6.5 Diet Feeding Laboratory Trials

A controlled feeding experiment was conducted using two isotopically distinct diet
sources to determine the time required for a diet shift to be reflected in the isotopic
composition of a consumer (blue crab muscle tissue). The diets consisted of study bivalves
collected from the ARE and NRE. Water quality parameters such as dissolved oxygen,
nitrate concentration, salinity and temperature were maintained to approximate estuarine
conditions. Three blue crabs selected randomly from estuarine waters outside the study
rivers were isotopically analyzed for carbon and nitrogen ratios in muscle tissue (mean values
$\delta^{15}N = 10.01\%o \pm 1.5; \delta^{13}C = 19.93\%o \pm 2.25; N=3$). Isotopic signatures ($\delta^{15}N$ and $\delta^{13}C$) from
blue crabs fed different diets for a time period of 14 days did not reveal a substantial
difference from baseline data. Statistical tests of significance were not performed because of
the small sample size. Evidence of tissue turnover in blue crab muscle in the two-week
period was not observed. The blue crabs that were fed the ARE bivalves showed $\delta^{15}N$
signatures of $11.09\%o \pm 0.16$ and the blue crabs fed the NRE bivalves showed signatures of
$9.8\%o \pm 0.06$. This result was surprising given the $\delta^{15}N$ ratios found for bivalves ($10.4\%o =$
NRE; $6.4\%o = ARE$) in the isotopic trophic model (Figures 13A and B).

Chapter 7 Discussion

7.1 Trophic Dynamic Model and Interactions

Both $\delta^{15}N$ and $\delta^{13}C$ enrichment of tissue were within the range of previously
examined stable isotope food web analyses. An average trophic level separation of $2.7\%o \pm$
0.5 was observed for the $\delta^{15}N$ ratios (Table 3). This finding is consistent with field and
laboratory studies, which show average $3.4 \pm 1.1\%$ enrichment in animal $\delta^{15}N$ versus diet (DeNiro & Epstein 1981, Minigawa & Wada 1984, Peterson & Fry 1987). Also, an average trophic level separation for $\delta^{13}C$ values of $1.8\% \pm 0.4$ (Table 3) was found, which is consistent with the accepted standard value for carbon isotopes of $1\% \pm 1.1\%$ (DeNiro & Epstein 1978, Peterson & Howarth 1987).

The average trophic level separation of $\delta^{15}N$ values between blue crabs and bivalves were significantly different ($p<0.0001$) in the ARE ($3.2\% \pm 0.09$) compared to the NRE ($1.01\% \pm 0.13$) (Figures 13A and B). As previous research suggests, such a result is possible when comparing nitrogen isotopes in two river estuaries with different nutrient loading rates (Riera et al. 2000).

7.2 $\delta^{15}N$: Effect of Anthropogenic Inflow

7.2.1 Representatives of Estuarine Primary Producers

The range of isotopic $\delta^{15}N$ and $\delta^{13}C$ data from POM, plants and animals observed in this study was consistent with values reported previously (Hughes & Sherr 1983, Peterson & Howarth 1987, McClelland et al. 1997, Fantle et al. 1999, Riera et al. 2000, and Hayase et al. 2001). POM $\delta^{15}N$ values were significantly higher ($p<0.0001$) in the impacted compared to the less impacted river estuary. Previous studies have reported that primary producers utilized nitrate and showed significantly higher $\delta^{15}N$ values from sewage inflows compared to less impacted sites (McClelland & Valiela 1998a, Gartner et al. 2002). This process is known as the conversion of sewage (human or animal) to nitrate through nitrification (Heaton 1982). Since there were point source dischargers proximal to NRE stations in the present study, it is conceivable that anthropogenic inflow enriched the $^{15}N$ nitrate concentrations available to primary producers.

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7.2.2 $\delta^{15}N$ Rich Estuarine Invertebrates

Primary consumers showed a large difference (4‰) in mean $\delta^{15}N$ values between impacted (Figure 13B) and less-impacted (Figure 13A) sites. This result confirms previous research that reflects an association between anthropogenic nutrients and lower trophic levels. In a study by Hobbie et al. (1990), trophic $\delta^{15}N$ enrichment of macroalgae (*Fucus vesiculosa*) occurred as a result of the incorporation of enriched nitrate delivered by wastewater discharge. Similarly, a study in a Baltic Estuary examined the effects of a municipal sewage on invertebrates and showed significantly higher $\delta^{15}N$ tissue values ($R^2=0.96; p<0.01$) closer to a nutrient point source (Hansson et al. 1997).

A study in the middle estuary of Plum Island Sound, Massachusetts (Deegan & Garrit 1997) reported a range of $\delta^{15}N$ values for primary consumers from 6.2‰ (*Nereis sp.*) to 0.7‰ (*L. saxatilis*). Supporting these data, a Cape Cod study showed $\delta^{15}N$ values for (*M. arenaria*) bivalves to be 5.8‰ (McClelland et al. 1997). A two-river estuary comparison by Riera et al. (2000) reported average $\delta^{15}N$ values for *Carcinus maenas* to be 10.0 ± 1.7‰, (N=2) in a low nitrogen loaded watershed and 18.2 ± 1.2‰, (N=2) in an impacted part of the estuary. These values were relatively consistent with the range of $\delta^{15}N$ values observed for blue crabs and bivalves found in the NRE and the ARE (Figures 13A and B).

7.3. Bivalve and Blue Crab Tissue Enrichment

Although causal relationships cannot be inferred, the data from this study suggested that blue crab and bivalve tissue enrichment were associated with areas of poor water quality. In the NRE, high $\delta^{15}N$ values were measured in bivalves, blue crabs and demersal fish (Figures 13A and B). Further evidence showed that the upper NRE stations, which included Glenburnie and Sandy Point (Figure 22), showed significantly different (p<0.05) blue crab
and bivalve $\delta^{15}$N values compared to lower estuary sites (Figures 10 and 12). The isotopic data support the first hypothesis ($H_1$) that higher blue crab tissue $\delta^{15}$N signatures are associated with locations with high nitrogen loading. For example, the higher $\delta^{15}$N values for blue crabs (11.41‰) and bivalves (10.40‰) collected from the NRE mid to upper estuary suggested that municipal sewage (+12 to +14‰) and animal waste (+16 to +20‰) may have influenced tissue enrichment. This finding is consistent with a study that measured nitrogen loading effects on upper trophic level benthic consumers. McClelland & Valiela (1998b) reported elevated $\delta^{15}$N values for primary (10‰) and secondary consumers (12‰) in an estuary with substantial wastewater contributions. In the present study, regression analyses showed an inverse relationship between $\delta^{15}$N values of blue crab tissue and poor water quality (Figures 14, 15 and 16), which lends support to the first hypothesis ($H_1$).

Although the $\delta^{15}$N values of blue crabs in the NRE decrease from the upper (12.6‰) to the lower estuarine environment (10‰), this result may be due to consumption of POM (and nitrate) by plankton toward the river mouth. Furthermore, non-point sources of nitrogen may be contributed laterally from terrestrial areas to study sites along the estuary. In addition, estuarine circulation parameters such as variation in river depth and shape as well as mixing of salt and fresh water may affect water quality and nutrient enrichment. Finally, the location of research sites which were primarily on the sides (1km from the shoreline) of each river estuary may have affected isotopic values as circulation may be limited in these areas.

The regression analyses in both river estuaries showed a positive relationship between tissue enrichment and water quality (Figure 14). However, only the NRE showed a statistically significant relationship ($p=0.01$) between $\delta^{15}$N values of blue crab tissue
enrichment and POM. Similarly, both the ARE and NRE showed an inverse relationship between tissue enrichment and water quality using DO mg l⁻¹ (Figure 15). In this model, the NRE also showed a significant association (R²=0.88; p=0.002).

Within each estuary, relationships comparing bivalve tissue and water quality were mixed (Figure 18). The stronger association (slope=2.8; p=0.06; R² = 0.88) observed in the less-impacted river suggested that this habitat might respond more sharply to environmental perturbations than the impacted river (slope=0.33; p=0.39; R² = 0.24). However, the bivalve δ¹⁵N tissue enrichment comparison within each river estuary showed a significant difference (p<0.0001) between upper, mid and lower NRE estuarine sites, suggesting a possible integration of point source and anthropogenic nutrients (Figure 10).

7.4 Inference of Feeding Habits

7.4.1 Isotopic Evidence

Dual isotope plots (δ¹⁵N and δ¹³C) revealed possible feeding associations (Figures 13A and B) (Peterson et al. 1985, Peterson & Fry 1987). In the regression analyses, results showed slopes in the same direction for the percentage of bivalves consumed by blue crabs in both river estuaries (Figure 20). However, since a significant relationship was not found (Neuse; p= 0.36; and Alligator; p = 0.07), the 2nd hypothesis (H₁) that a lower proportion of bivalve diet is associated with impacted sites was not supported. Furthermore, based on the margin of error associated with gut content analyses, the fractional material attributed to bivalves in the ARE (19%) and NRE (16%) does not support this hypothesis (Figure 23).

The regression analyses of percent detritus of blue crab diet (Figure 19) showed no clear trend (R² = 0.08; p=0.63) for both estuaries. This data did not support the 3rd
hypothesis (H2) that the impacted blue crabs consumed a more plant-based diet as water quality decreased.

7.4.2 Blue Crab Feeding Relationships

The present study analyzed POM and vascular plants to determine the proportion of plant material in blue crabs’ diet. Previous research has shown that *Spartina alterniflora* (e.g., live and detrital) served as an important food source to benthic invertebrates (Peterson et al. 1985; Deegan & Garritt 1997; Kwak et al. 1997).

In a study by Vizzini et al. (2002), δ_{13}C values of plant detritus (-13‰) and POM (phytoplankton dominated) (-21‰) were markedly distinct. The research suggested that carbon uptake of detritus dominated plant material may be more important for benthic organisms and POM may be an alternative food source for planktonic invertebrates and demersal fish. Hackney & Haines (1980) studied two marshes in St. Louis Bay, Mississippi. One marsh was a C_4 dominated system with *Spartina alterniflora* (δ_{13}C = -12.2‰), the other was a terrestrial plant (C_3) dominated system of *Juncus roemerianus* (δ_{13}C = -26.2‰). The invertebrate consumers sampled from locations proximal to these primary producers had carbon isotopic compositions ranging from -20 to -26‰, indicating a possible mixing of carbon material from both marshes. Consistent with these findings, the NRE blue crabs may have consumed a combination of POM, C_3 and C_4 plants as shown by the relatively high proportion (94%) of plant material in the two-source mixing model (Table 6, scenario 3).

Also, in the isotopic trophic model (Figure 13B) the NRE blue crabs showed an average δ_{13}C value of -21.8‰, which may be a result of a combination of values (-13‰ to -26‰) representative of POM, vascular plants and bivalves. In sum, the isotopic data by itself did not show convincing evidence that the NRE blue crab diet was associated with a particular
diet source. However, this study has shown that several methods are needed to identify such feeding trends.

7.4.3 Two Source Mixing Model

While the origins of organic matter sources are difficult to determine, various carbon forms including detritus are detectable using a two-source mixing model (Hughes & Sherr 1983). There is evidence of assimilation of macrophytes (S. alterniflora) by consumers in two less-impacted estuarine systems (Sapelo Island, Georgia and Sippewiset Marsh, Massachusetts, Haines 1976, Peterson et al. 1985). A linear regression in the Haines study showed that the $\delta^{13}$C value of a crab (Uca pugnax) was strongly associated with a combination of diet sources (C3 and C4 plants). Complimenting this finding, a two source mixing model revealed that C4 plants accounted for 87% of crab diet compared to 13% from C3 plants.

In the present study, the two-source mixing model utilized the 1‰ (consumer – diet) standard assumption of $\delta^{13}$C values (DeNiro & Epstein 1978) to determine whether blue crabs from the impacted sites consumed more plants than animal material. Since the impacted sites experience more eutrophication, it is possible that blue crabs relied more upon a steady source of plant material. Further evidence showed that blue crabs may have had a limited bivalve diet compared to a more abundant plant-based diet. According to scenario three (Table 6), 6% of bivalve tissue compared to 94% of a combination of terrestrial and Spartina sp. plant material was a comprised of the blue crab diet. This result complemented the gut contents data that showed 16% of bivalve compared to 40% of detritus and algae material in the NRE blue crab stomachs (Figure 23).
7.5 Gut Content Analyses

This approach identified four main trophic categories. The categories of interest in the present study included animal (e.g., bivalve) material as well as POM, vascular plant and detritus material. Similar diet sources have been found in previous blue crab stomach content analyses (Rosas et al. 1994). Although site and seasonal variation were not controlled for, gut content analyses (Figure 21) suggested that a combination of primary producer material may be the dominant material ingested by blue crabs collected from the NRE (40%) compared to the ARE (33%). This trend, although not tested statistically, was consistent with the 3rd hypothesis (H2), that plant material comprised a higher proportion of blue crab diet at locations impacted by high nutrient loading. Previous research has suggested that blue crabs collected from nutrient impacted sites contained mostly (60%) algae and detritus (Alexander 1986). In support of the 2nd hypothesis (H1), the blue crab gut content analyses at the NRE sites showed less animal material (19%) compared to the ARE sites (28%, Figure 21). Although more plant and less animal material was observed among the stomachs of blue crabs collected across NRE sites (Figure 21), gut content identification has intrinsic uncertainties and more investigation may be required.

7.6 Diet Feeding Trial

The blue crab diet feeding trial was conducted to verify the assimilation of a diet of bivalves collected from impacted versus less-impacted sites. However, statistical tests were not conducted because of the small sample size (N=3). There was no difference in tissue enrichment between the blue crabs fed three distinct diets. One limitation was that the baseline crab tissue was not isotopically measured. Baseline measurements would have allowed for a before and after comparison of tissue assimilation. The more optimal design
would have been to sample the legs of the baseline crabs as the diet switch was taking place. Previous tissue enrichment studies have produced variable results. Larger benthic invertebrates such as blue crabs assimilate nutrients at different rates, depending on the life stage of the organism (Fry & Arnold 1982, Tieszen et al. 1983). Therefore, a time period greater than two weeks would have been more appropriate.

7.7 Limitations

Several limitations should be considered in the present study. The estuaries are dynamic and are associated with spatial and temporal variations that are difficult to measure. For example, the isotopic and ecological differences between the Neuse and Alligator ecosystems may be associated with natural differences in nutrient production. Also, nutrients become less concentrated downriver and measurements of dissolved oxygen may have been more indicative of photosynthesis than water quality. Therefore, variables such as $\delta^{15}N$ POM and dissolved oxygen used in the regression analyses may not have been meaningful indicators of water quality. Similarly, when human and animal wastewater derived nitrogen is added to a system, the $\delta^{15}N$ value will increase. However, nitrate can be added from fertilizer to a system (producing low water quality) without the $\delta^{15}N$ value showing much of a positive change. These factors can vary levels of exposure to organisms.

Gut content analyses were conducted on approximately half of the total blue crab sample for both estuaries. More complete analyses would include more individuals exposed to a larger pool of potential diet sources and would have allowed statistical analyses of gut contents by site. Furthermore, a factor unaccounted for is inefficient conversion of food ingested or not converted into growth. Assimilation efficiencies (bivalve versus detritus) may not be the same for all of the materials analyzed in the blue crab guts. Migration of blue
crabs may have caused under or overestimation of the isotopic results. However, restricting the study sample to less mobile, adult males and using larger sample sizes served to minimize migratory effects. Given these limitations, consistent trophic level fractionation is thought to mitigate these factors.

Another factor not accounted for in the present study was seasonal variation. Enrichment effects may be more or less pronounced during higher fresh water flow (spring and winter seasons), which may dilute the nitrate and carbon available for uptake by primary and secondary consumers (Cifuentes et al. 1988). However in the present study, during a low flow season, major point sources combined with increased nitrate concentrations from the upper estuary may have elevated enrichment effects.

Chapter 8 Conclusion

The results from this study indicate that: 1) a relationship exists between the uptake of anthropogenic nutrients by primary producers and the subsequent energy transfer to estuarine consumers in two North Carolina Estuaries, 2) an inverse relationship exists between blue crab tissue enrichment and water quality in an impacted estuary, and 3) although plant and bivalve consumption by blue crabs was not significantly different in the impacted versus the less-impacted estuary, further study is warranted based on the patterns of invertebrate consumption found in previous research.

The dual $\delta^{15}$N and $\delta^{13}$C isotopic model combined with a two-source mixing model and gut content analyses proved to be a useful means of evaluating trophic relationships in this study. Despite the limitations in this study, the advantages of stable isotope analyses outweigh the disadvantages and make it a valuable research tool. The ability to accurately and efficiently measure a large sample size across a wide range of water quality sites was a
major contribution of this study. The majority of previous research utilizing isotopic values to construct food web models had much smaller sample sizes.

Future research should include a study of multiple years and control for seasonal variation with a more rigorous water quality monitoring method. Also, to control for enrichment and integration time differences, the effects of organism size should be more fully explored. A comparison of a food web model using an additional isotope tracer ($^{34}$S) and an additional control river may further clarify feeding relationships associated with water quality. Also, a larger sampling scheme of primary producers may resolve the mixed results found in the present study. Future investigation should focus on upper estuary Neuse River sites since the results of this study indicate a clear association between water quality and nutrient enrichment of estuarine plants and animals.

Finally, this research supports the theory that increased nutrient pollution may affect upper trophic organisms sampled in estuarine ecosystems (Lotze et al. 2001, Micheli et al. 2001). Although the debate continues, chronic eutrophication may promote an increase in phytoplankton size, which in turn forces a shift to fewer but larger consumers (Skei et al. 2000). This alteration may affect species diversity, thus reducing the food availability and potentially affecting the health of consumers such as blue crabs and bivalves (Post et al. 2000). The information provided by the present study offers managers an opportunity to consider solutions pertaining to issues of nutrient pollution on estuarine food webs.
Literature Cited


Chesapeake Bay Program (CBP) 2003. Blue Crab Information and Resources. <http://www.chesapeakebay.net/blue_crab.htm>


Table 1: Stable nitrogen isotope ($\delta^{15}$N) values (ranges shown) for dominant "new" and "regenerated" nitrogen sources in estuarine waters. From Velinski et al. 1989 and Fogel & Cifuentes 1993.

<table>
<thead>
<tr>
<th>N Source</th>
<th>$\delta^{15}$N</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_2$ in Atmosphere</td>
<td>0.0</td>
</tr>
<tr>
<td>Municipal Sewage: NO$_3^-$</td>
<td>+12.0 to 14.0</td>
</tr>
<tr>
<td>Animal Waste (swine, dairy poultry): NH$_4^+$</td>
<td>+16.0 to +28.0</td>
</tr>
<tr>
<td>Agricultural Land Runoff</td>
<td>+6.0 to +10.0</td>
</tr>
<tr>
<td>Fertilizers (synthetic)</td>
<td>-2.0 to +4.0</td>
</tr>
<tr>
<td>Atmospheric Deposition: NH$_4^+$</td>
<td>-20.0 to -10.0</td>
</tr>
<tr>
<td>Fossil fuel NO$_x$</td>
<td>-13.0 to +7.0</td>
</tr>
<tr>
<td>Fossil fuel NH$_4^+$</td>
<td>-10.0 to +2.0</td>
</tr>
</tbody>
</table>
Table 2: Summary of previous stable isotope ecological research. N/A signifies ratio not tested.
* Delta (δ) values are expressed as per mil (‰).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Sample Size</th>
<th>Organism/Organics</th>
<th>Sample/ Tissue</th>
<th>Type</th>
<th>δ¹³C ‰</th>
<th>δ¹⁵N ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayase et al. 2001</td>
<td>4</td>
<td>Croaker (Johnius vogleri)</td>
<td>White muscle</td>
<td>Fish Riverine</td>
<td>-22.9</td>
<td>12.4</td>
</tr>
<tr>
<td>Hughes &amp; Sherr 1983</td>
<td>2</td>
<td>Summer Flounder (Paralichthys dentatus)</td>
<td>White muscle</td>
<td>Fish Freshwater</td>
<td>-20.2</td>
<td>n/a</td>
</tr>
<tr>
<td>Riera et al. 2000</td>
<td>2</td>
<td>Green crab Carcinus maenas* (Less-Impacted)</td>
<td>Leg muscle</td>
<td>Crab Estuarine</td>
<td>N/A</td>
<td>8.3</td>
</tr>
<tr>
<td>Riera et al. 2000</td>
<td>2</td>
<td>Green crab Carcinus maenas* (Impacted)</td>
<td>Leg muscle</td>
<td>Crab Estuarine</td>
<td>N/A</td>
<td>11.7</td>
</tr>
<tr>
<td>Fantle et al. 1999</td>
<td>22</td>
<td>Juvenile Blue crab</td>
<td>Whole organism</td>
<td>Crab Estuarine</td>
<td>-15.9</td>
<td>11.1</td>
</tr>
<tr>
<td>Hayase et al. 2001</td>
<td>1</td>
<td>Mangrove crab (Seylla serrata) Lg</td>
<td>Muscle</td>
<td>Crab Marsh</td>
<td>-23.5</td>
<td>10.6</td>
</tr>
<tr>
<td>Hayase et al. 2001</td>
<td>1</td>
<td>Mangrove crab (Seylla serrata) Med</td>
<td>Muscle</td>
<td>Crab Marsh</td>
<td>-23.5</td>
<td>9.8</td>
</tr>
<tr>
<td>Hayase et al. 2001</td>
<td>2</td>
<td>Mangrove crab (Seylla serrata) Sm</td>
<td>Muscle</td>
<td>Crab Marsh</td>
<td>-24.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Kwak &amp; Zedler 1997</td>
<td>1</td>
<td>Yellow shore crab (Hemigrapsus oregonensis)</td>
<td>Muscle</td>
<td>Crab Channel Marsh</td>
<td>-17.4</td>
<td>13.8</td>
</tr>
<tr>
<td>McClelland et al. 1997</td>
<td>15</td>
<td>(Mya arenaria) Less-Impacted</td>
<td>Whole organism</td>
<td>Bivalve Estuarine</td>
<td>N/A</td>
<td>5.8</td>
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<tr>
<td>McClelland et al. 1997</td>
<td>15</td>
<td>(Mya arenaria) Impacted</td>
<td>Whole organism</td>
<td>Bivalve Estuarine</td>
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<td>8.3</td>
</tr>
<tr>
<td>Fantle et al. 1999</td>
<td>5</td>
<td>Mytilus edulis (Randomly selected)</td>
<td>Foot muscle</td>
<td>Bivalve Estuarine</td>
<td>-20.2</td>
<td>9.1</td>
</tr>
<tr>
<td>Riera et al. 2000</td>
<td>5</td>
<td>Mytilus edulis (Less-Impacted)</td>
<td>Foot muscle</td>
<td>Bivalve Estuarine</td>
<td>N/A</td>
<td>10.9</td>
</tr>
<tr>
<td>Raikow &amp; Hamilton 2001</td>
<td>20</td>
<td>Unionids</td>
<td>Foot muscle</td>
<td>Bivalve Freshwater</td>
<td>N/A</td>
<td>5.0</td>
</tr>
<tr>
<td>Peterson &amp; Howarth 1987</td>
<td>2</td>
<td>Spartina alterniflora</td>
<td>Cordgrass</td>
<td>Vegetation Marsh</td>
<td>-12.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Peterson &amp; Howarth 1987</td>
<td>4</td>
<td>Upland C, Plants</td>
<td>Plant Fragments</td>
<td>Vegetation Marsh</td>
<td>-29.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Fantle et al. 1999</td>
<td>3</td>
<td>POM</td>
<td>Surface Water</td>
<td>Marsh</td>
<td>-22.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Gartner et al. 2002</td>
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<td>POM</td>
<td>Surface Water</td>
<td>Oceanic</td>
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<td>7.1</td>
</tr>
<tr>
<td>Peterson &amp; Howarth 1987</td>
<td>10</td>
<td>POM</td>
<td>Surface Water</td>
<td>Estuarine</td>
<td>8.5</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Average trophic level enrichment for the Neuse and Alligator River Estuaries.

<table>
<thead>
<tr>
<th>Material</th>
<th>$^{13}$C</th>
<th>$^{15}$N</th>
<th>Trophic Levels</th>
<th>$\delta^{13}$C‰ Separation</th>
<th>$\delta^{15}$N‰ Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>POM</td>
<td>-27.30</td>
<td>5.65</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bivalve foot</td>
<td>-25.37</td>
<td>8.40</td>
<td>2</td>
<td>1.93</td>
<td>2.75</td>
</tr>
<tr>
<td>Blue Crab leg</td>
<td>-23.25</td>
<td>10.53</td>
<td>3</td>
<td>2.12</td>
<td>2.13</td>
</tr>
<tr>
<td>Demersal fish</td>
<td>-24.62</td>
<td>13.75</td>
<td>4</td>
<td>1.37</td>
<td>3.22</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td>1.8</td>
<td>2.7</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td>0.4</td>
<td>0.5</td>
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</table>

Table 4: Quantity of blue crabs collected, $\delta^{15}$N values of tissue and size of organism by location.

<table>
<thead>
<tr>
<th>Alligator River Estuary Location</th>
<th>Blue Crabs Collected</th>
<th>Leg $\delta^{15}$N (%)</th>
<th>Mean Carapace Width (Inches)</th>
<th>Mean Carapace Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up</td>
<td>29</td>
<td>9.9</td>
<td>4.7</td>
<td>120.7</td>
</tr>
<tr>
<td>Mid</td>
<td>15</td>
<td>8.8</td>
<td>4.4</td>
<td>112.6</td>
</tr>
<tr>
<td>Lower</td>
<td>33</td>
<td>9.7</td>
<td>4.5</td>
<td>114.2</td>
</tr>
<tr>
<td>Total/Mean</td>
<td>77</td>
<td>9.6</td>
<td>4.5</td>
<td>115.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neuse River Estuary Location</th>
<th>Blue Crabs Collected</th>
<th>Leg $\delta^{15}$N (%)</th>
<th>Carapace Width (Inches)</th>
<th>Carapace Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up</td>
<td>22</td>
<td>12.6</td>
<td>4.6</td>
<td>118.9</td>
</tr>
<tr>
<td>Mid</td>
<td>30</td>
<td>11.4</td>
<td>4.2</td>
<td>108.3</td>
</tr>
<tr>
<td>Lower</td>
<td>25</td>
<td>10.1</td>
<td>4.3</td>
<td>110.3</td>
</tr>
<tr>
<td>Total/Mean</td>
<td>77</td>
<td>11.4</td>
<td>4.6</td>
<td>116.8</td>
</tr>
</tbody>
</table>
Table 5: Two sample t-test data comparing slopes of both river estuary regressions.

<table>
<thead>
<tr>
<th>Slope T-test</th>
<th>Neuse River Slope</th>
<th>Alligator River Slope</th>
<th>T-value</th>
<th>Significance (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue crab leg $\delta^{15}\text{N}$ vs. POM ($\delta^{15}\text{N}$) (Figure 14)</td>
<td>0.95</td>
<td>0.69</td>
<td>0.33</td>
<td>N</td>
</tr>
<tr>
<td>Blue crab leg $\delta^{15}\text{N}$ vs. DO (mg/l$^1$) (Figure 15)</td>
<td>0.48</td>
<td>0.53</td>
<td>-0.17</td>
<td>N</td>
</tr>
<tr>
<td>Bivalve foot $\delta^{15}\text{N}$ vs. POM ($\delta^{15}\text{N}$) (Figure 18)</td>
<td>0.33</td>
<td>2.80</td>
<td>-3.10</td>
<td>Y</td>
</tr>
<tr>
<td>Percent detritus vs. POM ($\delta^{15}\text{N}$) (Figure 19)</td>
<td>3.77</td>
<td>27.84</td>
<td>-1.34</td>
<td>N</td>
</tr>
<tr>
<td>Percent bivalve vs. POM ($\delta^{15}\text{N}$) (Figure 20)</td>
<td>2.61</td>
<td>13.31</td>
<td>-1.76</td>
<td>N</td>
</tr>
</tbody>
</table>

Table 6: Two source mixing-model results using $\delta^{13}\text{C}$ ratios from the Neuse River trophic isotope model (Figure 13B). Vascular plants represent terrestrial ($C_3$) and *Spartina sp.* plants.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Consumer</th>
<th>Prey a (‰)</th>
<th>Prey b (‰)</th>
<th>Prey a (%)</th>
<th>Prey b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Blue Crab</td>
<td>POM</td>
<td>$-21.8$</td>
<td>$-26.9$</td>
<td>64</td>
<td>36</td>
</tr>
<tr>
<td>2) Blue Crab</td>
<td>Bivalve</td>
<td>$-21.8$</td>
<td>$-25.8$</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>3) Blue Crab</td>
<td>Bivalve</td>
<td>$-21.8$</td>
<td>$-21.59$</td>
<td>6</td>
<td>94</td>
</tr>
</tbody>
</table>
Figure 1: River estuary locations in coastal North Carolina
Figure 2: Proportion of land use/land cover types for the Neuse River sub-basin.

Figure 3: Proportion of land use/land cover types for the Pasquotank River basin.
Figure 4: Neuse River Estuary study sites marked by red flags. Yellow markers signify water quality monitoring stations.
Figure 5: Alligator River Estuary study sites marked by red flags. Yellow markers signify water quality monitoring stations.
% prey_a = \( \frac{\delta^{13}C_{\text{pred}} - \delta^{13}C_{\text{prey b}}}{\delta^{13}C_{\text{prey a}} - \delta^{13}C_{\text{prey b}}} \times 100 \)

% prey_b = 100 - % prey_a

Figure 8: Two-source mixing model equation. The term “pred” equals predator (blue crab) and “prey” equals either POM, vascular plant material or bivalve tissue.
Figure 9: Mean δ¹⁵N ratios for bivalve tissue were significantly different between each river estuary (p<0.0001; Neuse N=66; Alligator N=45).

Figure 10: A Tukey’s studentized range test showed significant differences (p<0.05) in bivalve δ¹⁵N values for the ARE between upper and mid and between upper and lower estuary. Within the NRE, there were significant differences (p<0.05) in δ¹⁵N values between mid and upper, mid and lower and upper and lower estuary.
Figure 11: Mean $\delta^{15}$N ratios for blue crab tissue were significantly different between each river estuary ($p<0.001$; Neuse N=77; Alligator N=77).

Figure 12: Mean $\delta^{15}$N ratios for blue crab tissue were compared by site location. A Tukey's studentized range test showed statistically significant differences between upper and mid-estuary sites, and between lower and mid-estuary sites ($p<0.05$) for the ARE. For the NRE, significant differences between upper and mid-estuary, upper and lower estuary, and between mid and lower estuary sites ($p<0.05$) were observed.
Figure 13: δ¹³C versus δ¹⁵N isotopic food web models for each estuary. * Vascular plants were comprised of terrestrial (δ¹³C; SD=0.06) and Spartina sp. (δ¹³C; SD=0.73)
Figure 14: Regression of Blue Crab Tissue Enrichment & POM by Sites

Figure 15: Regression of Blue Crab Tissue Enrichment & Dissolved Oxygen by Sites
Figure 16: Regression of Blue Crab Tissue $^{15}\text{N}$‰ versus POM $^{15}\text{N}$‰ by Site and Size

- Neuse River Large: $y = 1.11x + 4.40$, $R^2=0.80; p=0.02$
- Neuse River Small: $y = 1.01x + 4.68$, $R^2=0.95; p=0.001$

Figure 17: Regression of Blue Crab Tissue $^{15}\text{N}$‰ versus POM $^{15}\text{N}$‰ by Site and Size

- Alligator River Large: $y = 1.09x + 4.74$, $R^2=0.50; p=0.12$
- Alligator River Small: $y = 1.32x + 3.66$, $R^2=0.37; p=0.20$
Alligator River
$y = 2.80x - 6.05$
$R^2 = 0.88$
p = 0.06

Neuse River
$y = 0.33x + 8.14$
$R^2 = 0.24$
p = 0.39

Figure 18: Regression of Bivalve Tissue Enrichment & POM by Sites

Alligator River
$y = 27.84x - 108.3$
$R^2 = 0.42$
p = 0.16

Neuse River
$y = -3.77x + 42.21$
$R^2 = 0.08$
p = 0.63

Figure 19: Regression of Detritus Diet & POM by Sites
Figure 20: Regression of Bivalve Diet & POM by Sites

For the Alligator River:
- Equation: \( y = -13.31x + 70.16 \)
- \( R^2 = 0.59 \)
- \( p = 0.07 \)

For the Neuse River:
- Equation: \( y = -2.60x + 23.09 \)
- \( R^2 = 0.27 \)
- \( p = 0.36 \)

Figure 21: River estuary comparison of blue crab gut content analyses including several diet sources.
Figure 22: A digital-ortho photo of the upper Neuse River Estuary study sites. Red markers represent point source discharge in millions of gallons per day.
Figure 23: A detailed account of Figure 21. Gut content analyses of 83 blue crabs collected at stations from two North Carolina River estuaries.