STABLE ISOTOPE ANALYSIS: RESOLVING A COMPLEX GEORGIA SALT MARSH FOOD WEB; AND, IMPLICATIONS FOR THE MANAGEMENT OF AN INVASIVE SPECIES

by

CHARLES NICHOLAS DECURTIS, JR.

(Under the direction of Richard G. Wiegert)

ABSTRACT

Natural abundance stable isotope values and their distribution reflect physical and metabolic processes within ecological communities. Our knowledge of ecology in the Southeastern United States has increased significantly over the past few decades but lack of critical, such as detailed trophic structure/relationship data, information needed to understand, conserve, and protect natural resources has hampered our ability to effectively manage and conserve ecosystems. My research focused on utilizing stable isotope analysis to describe trophic connections in a Georgia salt marsh ecosystem and to apply the technique by combining statistical models with stable isotope analyses to track and determine area of origin for an invasive species in Georgia, the flathead catfish, *Pylodictis olivaris*.

I examine the food web of a saltwater marsh creek on Sapelo Island. Using natural abundance of stable carbon and nitrogen isotope ratios, I attempt to describe food web interactions from both a connectance viewpoint as well as a detailed trophic organization viewpoint. Also, I amended the creek over a 40 day period with an enriched nitrogen salt (Ammonium chloride) to examine trophic linkages that have been problematic due to small, sometimes indiscernible shifts from one trophic level to the next.

I investigated the applicability of natural abundance values of carbon and nitrogen isotopic ratios in muscle tissue to identify the river of origin of Flathead Catfish, *Pylodictis olivaris,* from the southeastern United States. Catfish were sampled by electrofishing in the Altamaha and Satilla Rivers during the summer of 1998 and 1999. Based on a cluster analysis of isotope values and length, and biology (feeding biology and gape limitation and reproductive maturation), the fish were grouped into 2 size classes within each river (< 550mm and >= 550 mm). This study demonstrates the utility of using isotopic ratios of tissue samples taken from creel specimens to accurately classify fish to their river of origin.

INDEX WORDS: Salt marsh, Food webs, Stable Isotopes, Flathead Catfish

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To Jane, Emily, Sophia and Charlie.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Our knowledge of ecology in the Southeastern United States has increased significantly over the past few decades but lack of critical, such as detailed trophic structure/relationship data, information needed to understand, conserve, and protect natural resources has hampered our ability to effectively manage and conserve ecosystems. It is especially important to better understand how changes to community structure affects biodiversity and ecosystem function. Changes in nutrient inputs, population dominance shifts, and invasive species can permanently alter systems to a point of system disequilibria with fatal consequences to a given ecological community. Biologists and ecologists need to develop methods utilizing current state-of-the-art-technology, such as DNA analysis and stable isotope analysis, that provide tools that natural resource managers and conservation biologists can use to understand, manage, and preserve natural systems. Natural abundance stable isotope values and their distribution reflect physical and metabolic processes within ecological communities. This allows scientists to examine flows of materials and connections within ecosystems (Peterson and Fry 1987).

My research focuses on utilizing stable isotope analysis to describe trophic connections in a Georgia salt marsh ecosystem. I apply the technique by combining statistical models with stable isotope analyses to track and determine area of origin for an invasive species in Georgia, the flathead catfish, *Pylodictis olivaris*.

Use of stable isotope analysis in ecology

Stable isotope analysis provides process and tracer information useful for examining food web structure (Peterson and Fry 1987). The justification for using isotopic ratios to examine food web mechanisms is that the isotopic ratio of a specific element in an organism is associated with dietary (or elemental assimilation) isotopic intake and reflects patterns of isotopic assimilation with increasing trophic position in the food web. Animal tissues reflect the isotopic composition of ingested organic matter, regardless of trophic level (Peterson and Fry 1987, Tieszen et al. 1983, Fry and Arnold 1982). The ratio of isotopes is expressed as a del value and is calculated as

$$‰ X = (X_{sample} – X_{standard} – 1) * 1000.$$

Isotopic ratios change and are predictable as elements are processed through the food chain (Peterson and Fry 1987). General trends in natural abundance values of isotopes are typically characterized by an increase from the primary consumers to the top predators of the food web because of selective isotopic fractionation. This relationship is useful for segregation of trophic components within a given community or ecosystem food web and is helpful in elucidating the stepwise transfer of organic matter (in the form of carbon and nitrogen compounds) throughout the food web (Peterson and Fry 1987, Fry 1988, Simenstad and Wissmar 1985, Montoya et al. 1990, Peterson et al. 1986).

Food web and community analysis

Food web research, as it may apply to natural resource management, is useful for qualitatively describing food web patterns and interactions across

vastly different ecosystem communities (Pimm 1982, Pimm et al. 1991, Polis 1994). Various quantitative methods have been utilized to identify connectivity between different organisms (or components) found within a food web (i.e., cycles, linkages, energy flow, etc.). Classification of nutrient sources of estuarine and marsh biotic compartments (benthic and pelagic) poses a major problem for researchers. Traditionally, studies of food web structure have relied on gut content analysis to delineate trophic structure and food web pathways (Odum 1968, Tagatz 1968, Odum and Heald 1972, Schoenly and Cohen 1991). One common method, gut content analysis, is difficult to execute, is confounded by material that has been partially digested, and only provides a "guess" at food components consumed by an individual (Hughes and Shen 1983). Also, gut content analyses only give an instantaneous image of material ingested. They do not provide a measure of food sources or allow examination of food source integration over time. Although it provokes useful discussion in ecology, food web theory has had little impact on conservation policy or management. This is because until recently no techniques allowed a simple, comprehensive examination of a given community. Stable isotope analysis provides process and tracer information essential for studying food web structure of large communities within reasonable time and financial constraints (Peterson and Fry 1987).

Invasive species

Invasive species are recognized by conservation biologists and natural resource managers as major threats to the flora and fauna of natural

ecosystems. Many non-native plants and animals have been intentionally introduced to provide food, recreation, or as control mechanisms. It has been estimated that approximately 4,500 non-native plants and animals have been introduced into North America (Barnes and Riggert 2000). High profile species such as the zebra mussel (Rosenberg and Ludyankski 1994, U.S. Congress Office of Technology Assessment 1993, Pimentel et al. 2000) have acquired public reputations and attention because of the devastating effects they are having on ecosystems, as well as economies.

Illegal fish introductions have had profound repercussions for ecosystems throughout North America. Endemic species may be negatively affected by predation and competition for food and space resources. Invasive species are responsible for nearly $97 billion in damages over the last 100 years (Cochran 1992). Pimentel et al. (2000) predict an annual cost of $138 billion from introduced and invasive species. Of the 958 threatened or endangered species 400 are at risk, primarily because of increased competition and predation by non-native, invasive species (The Nature Conservancy 1996, Wilcove et al. 1998). Hence, this dramatic impact on endemic populations compels us to understand, track, and manage exotic species. Understanding movements,origins, and impacts on food webs will provide managers with information critical for predicting population movements and dispersal.

Scope of this study

In this dissertation I address two distinct problems. In chapter 2, I consider the application of stable isotope analyses to a resource management problem.

Since the 1970's, *Pylodictis olivaris* has established large populations in the Altamaha and Satilla Rivers via illegal introductions. This species is popular as a game and food fish. But, increases in *P. olivaris* populations correlate with decreasing populations of *Lepomis spp.*, *Ameirurus spp.*, and *Moxostoma spp.* (Deener 2000, Quinn 1988, Bart et al. 1984, Guire et al. 1984, Ashley and Buff 1986). What is not certain is whether or not people interested in establishing populations for sport and food purposes are moving fish from the Altamaha River to the Satilla River. I employ analysis of stable isotope ratios of carbon and nitrogen from tissue samples to differentiate fish from the Satilla and Altamaha Rivers. Previous research, see Chamberlain et al. 1997, 2000, Hobson and Wassenaar 1997, Kelly and Finch 1998, Kelly et al. 2002, Meehan et al. 2001, on the utilization of stable isotopes to identify populations or origin have done much to further our knowledge and expand possibilities of stable isotope analysis for conservation and management. Previous research focused on "stable" materials (i.e. otolith carbonate, feather keratin) as the basis of analysis and comparison. Because of the difficulty in sampling bone and otoliths of *P. olivaris*, I chose to examine muscle tissue. Because it is technically impossible to differentiate fish in the field by direct examination, I combined stable isotope analysis with predictive discriminant function analysis to create a simple model useful for grouping fish by river. This methodology provides a means to take small samples from creel fish and determine river of origin for flathead catfish.

In chapter 3, I examine the food web of a saltwater marsh creek on Sapelo Island. Salt marsh communities are considered to be some of the most

productive in the world (Teal 1962, Reimold et al. 1973, Wiegert and Pomeroy 1981, Wiegert and Freeman 1990). Salt marsh ecosystems support rivers, creeks, and estuaries as well as adjacent terrestrial systems (Kraeuter and Wolf 1973). Salt marshes, usually characterized by detritus-based food webs, are dominated by bacterial production with secondary utilization facilitated by benthic-pelagic coupling agents such as diatoms, harpacticoid copepods, polychaetes, amphipods and grazers/shredders in the form of crab and shrimp species as well as various fish species (Wiegert and Pomeroy 1981, Wiegert and Freeman 1990). Previous isotopic research of trophic structure, community organization, and organic matter utilization has been limited to the examination of natural abundance levels of carbon isotope ratios in various community compartments (Fry and Sherr 1984, Haines 1976a, Haines 1976b, Haines 1977, Haines and Montague 1979, Hughes and Sherr 1983, Knieb and Stiven 1980, Sherr 1982).

Using natural abundance of stable carbon and nitrogen isotope ratios, I attempt to describe food web interactions from both a connectance viewpoint as well as a detailed trophic organization viewpoint. Also, I amended the creek over a 40 day period with an enriched nitrogen salt (Ammonium chloride) to examine trophic linkages that have been problematic due to small, sometimes indiscernible shifts from one trophic level to the next.

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CHAPTER 2

CLASSIFICATION OF FLATHEAD CATFISH *PYLODICTIS OLIVARIS* TO RIVER OF ORIGIN BASED ON CARBON AND NITROGEN ISOTOPIC RATIOS: MANAGEMENT IMPLICATIONS

Flathead catfish, *Pylodictis olivaris,* have been introduced into Georgia Rivers since the 1950's (Quinn 1988). These intentional stockings have coincided with rapid and disastrous declines in populations of native fish species such as the Sunfish *Lepomis spp*., Bullhead catfish *Ameiurus spp*., and Redhorse *Moxostoma spp.* (Bart et al. 1994, Jenkins and Burkhead 1994). This may be due to a combination of factors, including competition for resources and direct predation. *P. olivaris* rapidly becomes a dominant predator in most waters where it has been introduced, and may make up as much as 65% of the total catch fish biomass within a given river system (Guire et al. 1984).

Efforts to manage this fishery are hampered by the popularity of the fish as a food and recreation species. Its popularity has led to illegal introductions into rivers where established populations do not exist. In most cases where this invasive species becomes established it is impossible then eradicate it because of practical and economic constraints. It is important to limit environmental impacts, if possible, of invasive species by attempting to prevent or limit range expansion of these potentially damaging species. Efforts to control the range expansion of this species by the Georgia Department of Natural Resources (GADNR) in Georgia Rivers are limited to electrofishing and removal. Successful natural resource conservation and management is wholly dependent on accurate information describing the distribution and response dynamics of populations of plants and animals that can and do change dramatically over time and space. Unfortunately, this information is either difficult to collect or requires budgets beyond the resources of most natural resource managers. To most effectively

manage or combat invasive species, it is necessary to have access to relevant information about population movements. At present, there is no practical management tool available to track movements and introductions of species which are inherently difficult to observe (e.g. Fish in murky water systems). While GADNR personnel are aware of specific instances of illegal introductions into a given river, they have no way to enforce state and federal laws (see Lacey Act 1981, NEPA 1970, ESA 1972, NANPCA 1990, ASPEA 1992, NISA 1996, Georgia Code 17-5-1) regarding these introductions. This is because there is no inexpensive and effective procedure to identify the origin of a given fish.

Genetic information at the population/species level can provide important data for management and tracking programs (Avise and Hambrick 1996). But the acquisition of genetic information is expensive, requires a large investment in training and equipment, and the science required is still developing and maturing. Furthermore, while genetic data may indicate a common lineage for separate and distinct populations, it does not provide information useful for understanding and tracking movements at any but large scales. Stable isotope data provides a current snapshot of a given animal or plants nutrient source isotope ratio. These ratios can vary greatly across very small spatial scales depending on factors such as soil type, forest cover, degree of urbanization, farming ,etc (Peterson and Fry 1988). This difference in “background” isotope ratios makes the utilization of ratio data ideally suited for tracking over small or large spatial scales.

In this study, I investigated the ability of natural abundance values of carbon and nitrogen ratios in tissue samples (specifically, muscle tissue) to act as natural tags of river of origin. The specific objectives were to quantify geographical variation in the stable carbon and nitrogen isotope chemistry and then to determine if these differences were sufficient to allow the accurate classification to river of origin based on these isotopic ratios.

Methods

Study sites

I examined flathead catfish tissue samples from 2 major rivers (Figure 1) in coastal Georgia. While the study rivers are close in proximity, the Atlantic Ocean acts as a barrier to movement between the rivers. These rivers vary in respect to pH, pollution, total flow, and origin. There also is no freshwater exchange between the rivers. Although the intra-coastal waterway connects the estuaries, saltwater serves as a barrier to movement of freshwater species between the these rivers.

The **Satilla River** (Figure 2) is a 'black water' river formed in a watershed (9,143 km^2) that lies entirely in the coastal plain of Georgia. It is characterized by low pH (3.0 – 4.0) and clear water stained by tannins leaching from Cypress (Taxodium spp.) and Black Gum (Nyssa sylvatica) swamps in the riparian zone. There is very little pollution from industrial or municipal sources and insignificant agricultural runoff. River flow averages 80 m^3/s. The river flows directly into an estuary between Cumberland and Jekyll Island.

The **Altamaha River** (Figure 3), the largest river in Georgia, has headwaters that form in the Appalachian Mountains. The rivers drainage area is approximately 37,600 km^2. River flow averages 400 m^3/s. Altamaha water is typically silt-laden with a high degree of runoff from agricultural, rural housing, and industrial development. The river flows directly into an estuary between Sapelo and St. Simons Island.

Both the Altamaha and Satilla River are relatively unaltered by channelization and dams. The Oconee River, a tributary of the Altamaha River, has numerous dams and reservoirs. Both the Altamaha and Satilla rivers have had channel maintenance/dredging near the coast to maintain navigable water for the intra-coastal waterway and major sounds or channels.

Field methods and sampling procedures

Georgia Department of Natural Resources personnel using electro-shocking equipment collected flathead catfish on the Satilla and Altamaha Rivers. Redbreast sunfish, Redear sunfish, and Bluegill were collected with hook and line. Particulate organic matter (POM) samples were collected from each river in a transect running between the extremes of the electro-shocking sites. POM samples were collected on ashed Whatman GF/F filters. All samples were iced in coolers and transported to a lab for dissection. Muscle and liver samples were collected and frozen in individual sample bags bearing the sex, weight, length, date and location of the collected specimen. I analyzed 74 Flathead samples, 12 POM samples and 13 sunfish/bream samples.

Laboratory Methods

Tissue samples were dried in a vacuum oven at 60°C for 48 hours and then ground in a Spex ball mill for 5 minutes. POM samples were dried and scraped into a scintillation vial for storage. One-milligram samples were analyzed on a Finnigan Delta C mass spectrometer connected to a Carlo-Erba NA1500 carbon-nitrogen analyzer via Finnigan's Conflo II interface in the University of Georgia Institute of Ecology Analytical Laboratory. The carbon and nitrogen ratios were recorded as the difference between the isotopic ratio of a given sample relative to the Pee Dee Belemnite standard and atmospheric nitrogen (Peterson and Fry 1987). The $\delta^{15}N$ value for nitrogen is calculated by:

$$\delta\ ^{15}N = [(R_{sample}/R_{atmospheric\ N}) - 1] \times 1000$$

where R is the $^{15}N/^{14}N$ ratio. The $\delta^{13}C$ value for carbon is calculated by:

$$\delta^{13}C = [(R_{sample}/R_{PDB}) - 1] \times 1000$$

where R is the $^{13}C/^{12}C$ ratio. An increase in the $del^{13}C$ or $del^{15}N$ value indicates an increase for each respective element, ^{13}C or ^{15}N.

Data analysis

To test the use of carbon and nitrogen stable isotopes as indicators of river of origin for flathead catfish, I used a discriminant function analysis to classify to river of origin based on measured isotopic ratios of muscle tissue and length for a given sample. Discriminant analysis, a multivariate statistical procedure, is useful for examining ecological problems concerning group differences (index variable) with associated vectors of measurement(s) of pre-identified groups. The use of discriminant function analysis for prediction and/or

classification is described in most multivariate analysis statistics texts (Johnson and Wichern 1982, Stevens 1986, Huberty 2000). For a multivariate data set comprised of 2 or more groups a discriminant function analysis is used to examine linear combinations of variables (first discriminant function) to determine which maximize the mean difference between the groups. For unknown samples from a group of interest, the calculated value of the discriminant function is called the discriminant score. The unknown sample is assigned to a specific group from the pool of groups of interest based on which mean discriminant score is "nearest" to the samples discriminant score. The discriminant function analysis was used to identify linear combinations of the sample variables that efficiently characterize the differences among the rivers. Quantified differences in isotopic measurements and morphometric measurements of the individual specimens from either the Altamaha or Satilla Rivers are used to discriminate between the separate populations within each river. Discriminant function analysis can act as a predictive tool by classifying individual cases (fish) or samples according to the probability of membership in the pre-defined groups. Detailed discussions of the application and associated limitations of discriminant function analysis can be found in Williams (1981), McGarigal et al. (2000), and Huberty 1994. I use the predictive discriminant analysis formulation to predict the river of origin using data for stable carbon and nitrogen isotopes from muscle tissue and total body length. A stepwise analysis was utilized to examine the relevance of the measured variables.

The main goal of the discriminant analysis approach was to quantify the level of separation of each rivers' populations with respect to isotopic values, and to identify the variables useful for objective quantification and differentiation of the flathead catfish populations in the two study rivers. The stepwise and predictive discriminant analyses conducted for each rivers population of flathead catfish using carbon and nitrogen isotopic values in combination, contrasted with examining these variables singularly, resulted in consistently precise differentiation between these separate populations. This accuracy is reflected in the Wilk's lambda statistic. The data model was cross validated by removing each sample point (one at a time) from the main data set, re-estimating the discriminant function from the remaining samples and then using the parameters from this run to classify the sample.

Results

Flathead catfish collected ranged in size from 124 – 1075 mm (Table 2.1). Fish from both rivers had similar length/weight relationships (Figures 1 and 2). Since length is more easily measured in the field, and because length and weight are highly correlated, I choose to use length as a discriminating variable. Using both length and weight as discriminating variables would result in a multicollinearity and a nonintuitive matrix making discrimination and interpretation of the canonical functions difficult (Williams 1981). Discriminant analysis is analogous to a multiple regression analysis. Correlation coefficients between carbon and nitrogen isotopes and length were calculated for each river (Table

2.2). There also is significant correlation between isotopic values for carbon and nitrogen with length. Isotopic data from muscle samples show significant variation by size class within each river (Figures 3 – 6). Isotopic values were highly variable across all sizes of sample fish but groups segregated into functional breeding size classes, fish less than 550 mm being classified as sexually immature and fish greater than 550 mm being classified as sexually mature, exhibited very little variability (Table 2.1). This classification is based on breeding biology data (see Lee et al. 1981, Jenkins and Burkhead 1984, and Etnier and Starnes 1993), and the fact that in the field, DNR personnel were unable to sex fish accurately under the 550 mm cutoff point.

Descriptive Statistics for Altamaha River Samples

Analysis of POM samples indicated that carbon and nitrogen varied little over the transect from the upstream and downstream sampling endpoints (del C mean = -28.85 sd = .023; del N mean = 6.82 sd = 1.07). Flathead catfish length and weight ranged from 142 to 972 mm, and 50 to 13,000 g., respectively. Carbon isotope values ranged from –27.97 to –23.90, while nitrogen isotope values ranged from 10.47 to 13.81.

Descriptive Statistics for Satilla River Samples

Analysis of POM samples indicated that carbon and nitrogen varied little over a transect from the upstream and downstream sampling endpoints (del C mean = -27.28 sd = 0.962; del N mean = 7.31 sd = 0.077). Flathead catfish length and weight ranged from 124 to 1075 mm, and 21 to 19150 g.,

respectively. Carbon isotope values ranged from –25.94 to –22.18, while nitrogen isotope values ranged from 11.75 to 13.04.

Discriminant Function Analysis

General statistics are summarized in Table 2.2. A linear discriminant analysis was performed on nitrogen and carbon isotopes and length using the SPSS statistical software package (Version 10.0.5). The null hypothesis was that flathead populations in the Satilla and Altamaha Rivers do not differ with respect to stable carbon and nitrogen isotopes, that is, the mean discriminant scores do not differ between these geographically separated populations. I sampled a total of 56 flathead catfish (n=30 for the Altamaha River and n=26 for the Satilla River). The populations within each river formed two distinctive groups based on feeding biology and maturity. I use discriminant analysis to analyze a function that includes length, carbon isotope value, and nitrogen isotope value to distinguish between the Altamaha and Satilla Rivers.

Discriminant analysis, for each size class and pooled data, began with a examination of the null hypothesis of homogeneity of with-class variance-covariance matrices. The significance of each discriminant function was determined according to whether it contributed to the separation between the two populations from the Altamaha and Satilla Rivers.

Discriminant Function Analysis for the < 550 mm Class

Examining the < 500 mm size class data (Table 2.3), it can be seen from the nitrogen and carbon isotope Wilks' lambda and F-statistic scores that significant differences exist between the rivers for this size class. The sample

means are different between the rivers. Nitrogen isotopes measurements in the Satilla have a mean of 11.43 while in the Altamaha the mean is 12.50. Likewise, for carbon isotopes, mean values were –26.86 and –25.26 for the Altamaha and Satilla respectively. Since the standard deviation for these means is small the assumption of equal variances is met. In a test of equality of group means, significant differences exist between carbon and nitrogen variables but not length. Length is dropped when using a stepwise approach, as discussed later, from each size class analysis but when comparing the populations between rivers and making no size class delineation, because of the strong correlation between length and isotopic signatures, length is included in the analysis. Classification function coefficients (Table 2.4) are used to classify the individual catfish into groups where a fish is designated as a member of a river in which the value of its classification is greatest. These coefficients maximize the differences between fish from the two rivers. Since there are only two populations being considered, these classification function coefficients can be used to determine a linear discriminant function. By taking the differences of the coefficients I obtain a Fishers linear discriminant function useful for scoring unknown samples and classifying them by river. Fishers function for this size class is

RiverOrigin = -0.00218*length + 7.736*nitrogen – 5.549 * carbon – 51.503.

The Wilks' lambda statistic provides a measure of the variance in the discriminant scores that is not explained by differences among the populations. For the flathead catfish size class < 550 mm, only 0.177 % of the total variation is not explained by differences in the population when both nitrogen and carbon are

included in the discriminant function analysis. The eigenvalue for this analysis is 4.656 and is the ratio of the between-groups sum of squares and within-groups sum of squares. Classification results are summarized in Tables 2.3 and 2.4. From the structure matrix you can see that carbon and nitrogen are equally important to the canonical discriminant function coefficients but that length is insignificant. All of the original grouped cases were correctly classified and cross-validated.

Discriminant Function Analysis for > 550 mm Class

This size class, > 550 mm, is characterized by patterns similar to the smaller size class. Nitrogen isotopes from the samples had means of 13.33 in the Altamaha and 12.37 in the Satilla. Similarly, carbon isotopes had means of –25.36 and –23.67 in the Altamaha and Satilla Rivers, respectively. There is very little variability in these mean values within river populations. Length, as with the smaller size class, is considered an insignificant factor. The Wilks' lambda statistic of 0.173 % is consistent with the smaller size class. The eigenvalue for this analysis is 4.773. All samples were correctly classified and cross-validated to river of origin

Discriminant Function Analysis for Pooled Size Data

When the data was pooled, no size class delineation, the predictive value of the discriminant function analysis is less accurate. As would be expected, the standard deviation for length of a given river population is very large, 269.93 mm for the Altamaha and 267.30 mm for the Satilla. Also, carbon and nitrogen statistics are more highly variable than the separate class analyses. Off the

Altamaha samples 90% were correctly predicted and cross-validated to river of origin while 76.9% of the Satilla samples were correctly predicted with 73.1% cross-validated.

Discussion and Conclusions

Organic isotopes, such as carbon or nitrogen, of a tissue sample reflect the organic or nutrient sources being consumed or utilized in a given area (Peterson and Fry) and are a function of the trophic level of a given species. I anticipated that differences in carbon and nitrogen isotope signatures from muscle samples would be useful for classifying Flathead Catfish from different rivers to their river of origin. Statistically examining the data illustrates that isotopic differences between the Satilla and Altamaha rivers are consistent with this hypothesis. Obviously, as can be seen from the data in either river, if fish were moving between these rivers via the intracoastal waterway, isotopic signatures would be averaged out over time and discriminant function analysis would be ineffective in separating these populations. Since these populations remain completely separated, without human intervention, tissue carbon and nitrogen signatures for the different regions are different.

The isotopic data presented when combined with effective classification by discriminant function analysis provide empirical relationships effective at separating flathead catfish populations when sampling locations are unknown. The clustering of isotopic signatures by size and sexual maturity require a 2-way analysis for the most effective and accurate utilization of this technique. To best

utilize these methods, it is important to understand and have a thorough knowledge of the natural history of the species being studied.

Our ability to link individual animals or plants to area of origin is limited. DNA analyses can be very informative but are expensive and technically demanding. Stable isotope analysis while relatively inexpensive has not been effectively utilized to help solve management problems such as population origin or animal movement tracking. Stable isotopes can be used to differentiate among nutrient sources contributing to a consumers isotopic signature. It seems intuitive that the isotopic signature from a given population of animals would reflect the chemical composition of the base of the food chain with different regions probably having differences in nutrient sources that are reflected in different carbon and nitrogen isotopes, among others. Because of possible uncertainties about isotopic turnover rates in different species of animals must be considered before isotopic analyses become part of natural resource management techniques (Fry and Arnold 1982, Hesslein et al. 1993, Herzka and Holt 2000). If not taken into consideration, turnover in sample tissues for a given population of animals can make it difficult to utilize natural abundance levels of isotopes to solve management problems such as illegally introduced species tracking. But, the combination of stable isotope analysis and multivariate cluster or classification methods such as discriminant function analysis provide an effective tool if utilized correctly. Creel checks are one example where this technique would be effective and accurate at determining origin of live or dead fish from small tissue samples. Large-scale mapping of organic matter isotopic

signatures for a given ecoregion or state may provide researchers and managers an effective base for applying these methods to solve management problems.

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Table 2.1. Correlation Coefficients. Bold numbers indicate correlation is significant at the 0.01 level (2-tailed).

Group	Variable	Length	Weight	Nitrogen	Carbon
Altamaha (Pooled)	**Length**	1.00	**0.914**	**0.849**	**0.807**
	Weight	**0.914**	1.00	0.630	**0.705**
	Nitrogen	**0.849**	0.630	1.00	0.689
	Carbon	**0.807**	**0.705**	0.689	1.00
Satilla (Pooled)	**Length**	1.00	**0.897**	-0.314	0.586
	Weight	**0.897**	1.00	-0.223	0.435
	Nitrogen	-0.314	-0.223	1.00	-0.053
	Carbon	0.586	0.435	-0.053	1.00

Table 2.2. Summary Statistics. The sample range for each variable is followed by the mean and standard deviation (in parenthesis).

Group	Length (mm)	Weight (g)	$\delta^{15}N$	$\delta^{13}C$
Altamaha Pooled	142 – 972 504.4 (269)	50 – 13,000 3099 (3896.7)	10.47 – 13.90 12.54 (1.04)	-27.97 - -23.90 -26.01 (1.03)
Altamaha < 550 mm (n = 13)	142 – 420 238.5 (76.9)	50 – 850 194.9 (216)	10.47 – 11.91 11.43 (0.38)	-27.97 - -26.29 -26.86 (0.47)
Altamaha > 550 mm (n = 17)	518 – 972 707.6 (162.1)	1472 – 13,000 5319.9 (3923)	12.9 – 13.9 13.39 (0.31)	-26.33 - -23.90 -25.35 (0.856)
Satilla Pooled	124 – 1075 517 (267)	21 – 19,150 3490 (4724)	11.75 – 13.04 12.43 (0.26)	-25.94 - -22.18 -24.46 (1.04)
Satilla < 550 mm (n = 13)	124 – 470 290.8 (108.9)	21 – 1271 367.6 (364.7)	12.09 – 13.04 12.49 (0.27)	-25.94 - -23.99 -25.26 (0.503)
Satilla > 550 mm (n = 13)	576 – 1075 743.5 (161.2)	1517 – 19,150 6612.4 (5023)	11.75 – 12.61 12.37 (0.24)	-24.49 - -22.18 -23.66 (0.78)
Pooled < 550 mm	124 –470 264.65 (96.2)	21 –1271 281.3 (306.7)	10.47 – 13.04 11.96 (0.63)	-27.97 - -23.99 -26.06 (0.95)
Pooled > 550 mm	518 – 1075 723.2 (159.80	1472 – 19,150 5879.9 (4400.1)	11.75 – 13.90 12.95 (0.58)	-26.33 - -22.18 -24.62 (1.18)

Table 2.3a. Summary of discriminant function analysis for < 550 mm class.

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	df3
Length	0.923	1.993	1	24	0.171
N	0.265	66.423	1	24	0.000
C	0.255	70.152	1	24	0.000

Covariance Matrices

River		Length	N	C
Altamaha	Length	5924.6	15.884	24.885
	N	15.884	0.146	0.092
	C	24.885	0.092	0.224
Satilla	Length	11868.02	-8.712	-23.065
	N	-8.712	0.075	-0.02
	C	-23.065	-0.02	0.253
All	Length	9249.8	17.87	22.65
	N	17.87	0.40	0.48
	C	22.654	0.48	0.90

a. The total covariance matrix has 25 degrees of freedom

Box's Test of Equality of Covariance Matrices

River	Rank	Log Determinant
Altamaha	3	4.243
Satilla	3	5.027
Pooled within-groups	3	5.392

Test Results

Box's M	18.165
F (Approx)	2.613
F (df1)	6
F (df2)	4173.3
F (Sig.)	0.016

Table 2.3b. Summary of discriminant function analysis for < 550 mm class.

Summary of Canonical Discriminant Functions

Eigenvalues

Function	Eigenvalue	% of variance	Cumulative %	Canonical Correlation
1	4.667	100.0	100.0	0.907

Wilks' Lambda

Test of Fn (s)	Wilks' Lambda	Chi-square	df	Sig.
1	0.176	39.03	3	0.000

Standardized Canonical Discriminant Function Coefficients

Length	0.050
N	0.619
C	0.653

Structure Matrix

Length	0.791
N	0.770
C	0.133

Variables ordered by absolute size of correlation within function

Canonical Discriminant Function Coefficients

Length	0.001
N	1.863
C	1.337
(Constant)	12.407

Table 2.4. Classification results for the < 550 mm class.

Classification Function Coefficients

	Altamaha	Satilla
Length	-0.019	-0.017
N	148.28	156.02
C	-135.09	-129.53
(Constant)	-2660.15	-2608.64

Classification Results

Original River	Predicted (Altamaha)	Predicted (Satilla)	Total
Altamaha	13	0	13
Satilla	0	13	13
Altamaha (%)	100.0	0.0	100.0
Satilla (%)	0.0	100.0	100.0

Table 2.5a. Summary of discriminant function analysis for > 550 mm class.

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	df3
Length	0.987	0.360	1	28	0.553
N	0.237	90.0	1	28	0.000
C	0.476	30.883	1	28	0.000

Covariance Matrices

River		Length	N	C
Altamaha	Length	26,268.471	9.750	65.073
	N	9.750	0.087	-0.029
	C	65.073	-0.029	0.733
Satilla	Length	25972.44	-6.783	-28.923
	N	-6.783	0.058	0.095
	C	-28.923	0.095	0.611
All	Length	25564.92	-6.120	39.3
	N	-6.120	0.305	-0.387
	C	39.3	-0.387	1.382

b. The total covariance matrix has 29 degrees of freedom

Box's Test of Equality of Covariance Matrices

River	Rank	Log Determinant
Altamaha	3	7.079
Satilla	3	6.473
Pooled within-groups	3	7.148

Test Results

Box's M	9.197
F (Approx)	1.348
F (df1)	6
F (df2)	4631.79
F (Sig.)	0.232

Table 2.5b. Summary of discriminant function analysis for > 550 mm class.

Summary of Canonical Discriminant Functions

Eigenvalues

Function	Eigenvalue	% of variance	Cumulative %	Canonical Correlation
1	4.773	100.0	100.0	0.909

Wilks' Lambda

Test of Fn (s)	Wilks' Lambda	Chi-square	df	Sig.
1	0.173	46.46	3	0.000

Standardized Canonical Discriminant Function Coefficients

Length	0.002
N	0.882
C	-0.575

Structure Matrix

Length	0.821
N	-0.481
C	-0.052

Variables ordered by absolute size of correlation within function

Canonical Discriminant Function Coefficients

Length	0.000
N	3.22
C	-0.697
(Constant)	-58.761

Table 2.6. Classification results for the > 550 mm class.

Classification Function Coefficients

	Altamaha	Satilla
Length	0.051	0.051
N	190.69	176.97
C	-45.85	-42.88
(Constant)	-1870.95	-1621.88

Classification Results

Original River	Predicted (Altamaha)	Predicted (Satilla)	Total
Altamaha	17	0	17
Satilla	0	13	13
Altamaha (%)	100.0	0.0	100.0
Satilla (%)	0.0	100.0	100.0

Figure 2.1. Altamaha River. Length vs. weight, all data.

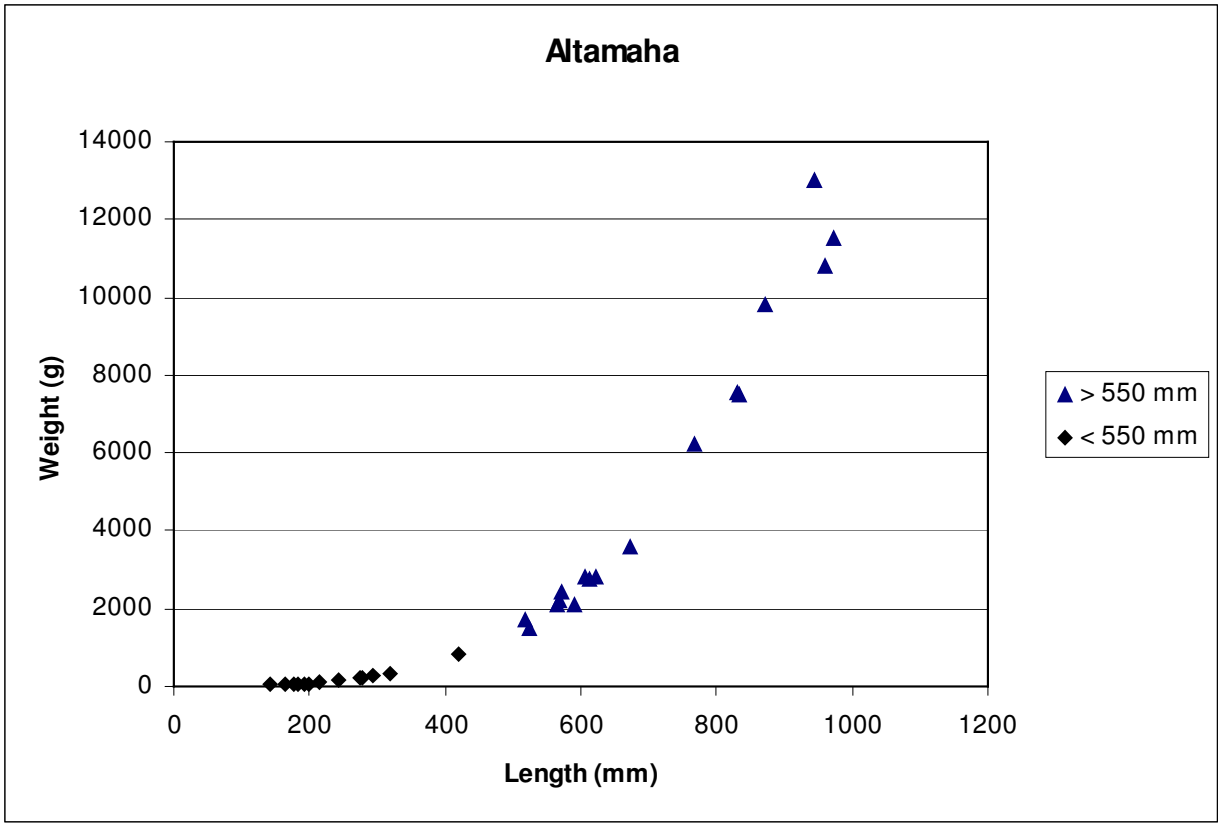

Altamaha
14000
12000
10000
8000
6000
4000
2000
0
Weight (g)
0
200
400
600
800
1000
1200
Length (mm)
> 550 mm
< 550 mm

Figure 2.2. Satilla River. Length vs. weight, all data.

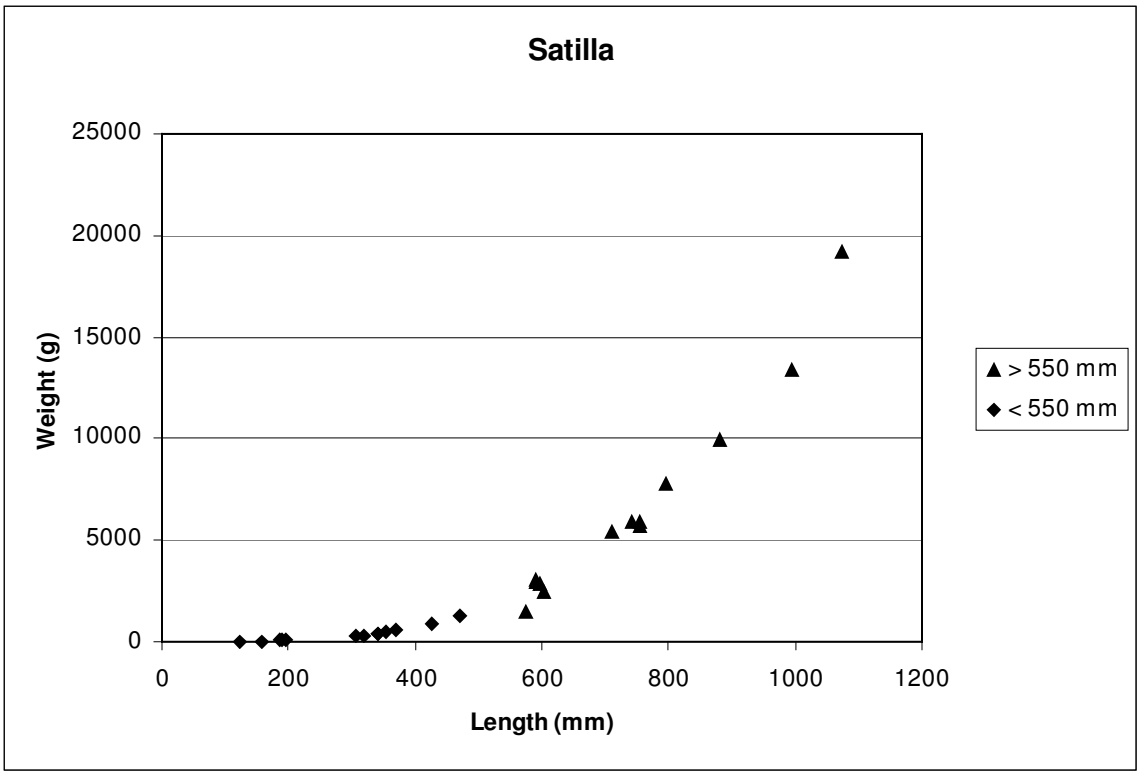
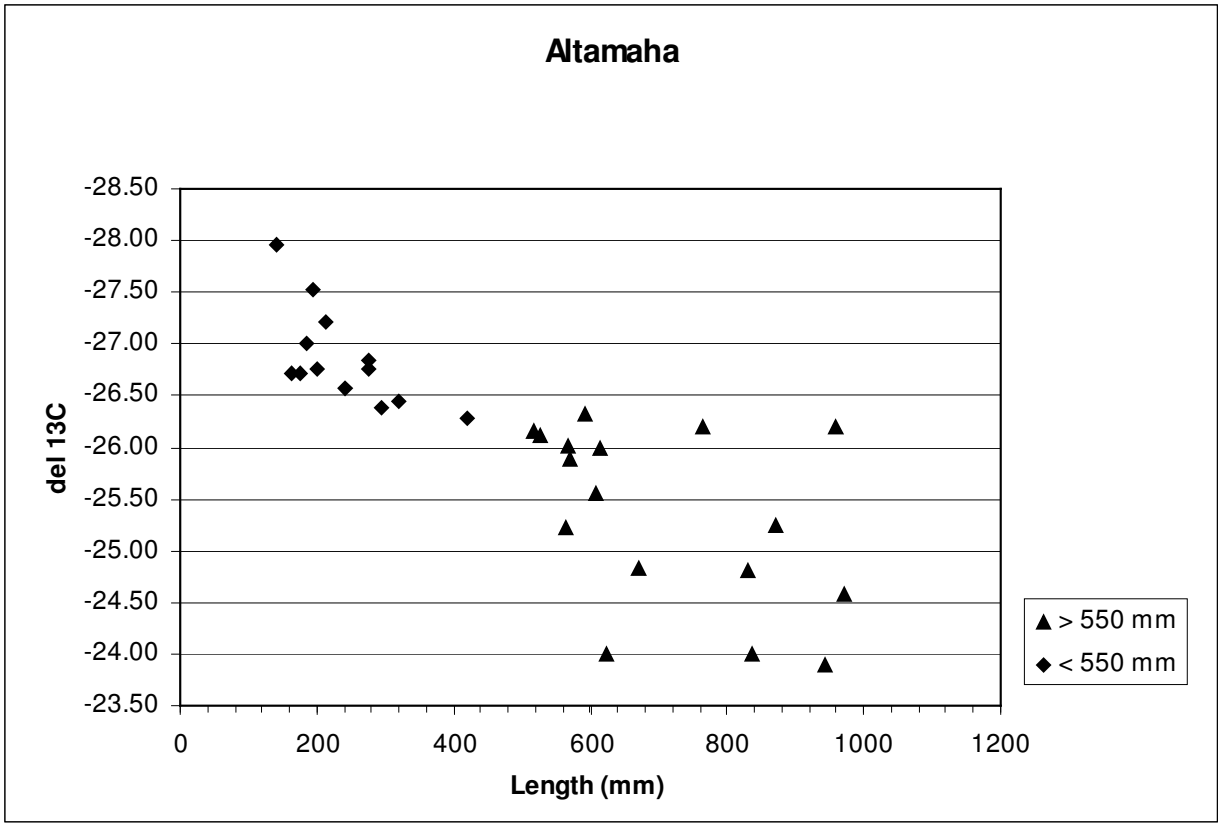

Satilla
25000
20000
15000
10000
5000
0
Weight (g)
0
200
400
600
800
1000
1200
Length (mm)
▲ > 550 mm
◆ < 550 mm

Figure 2.3. $\delta^{13}C$ values vs. length. Altamaha and Satilla Rivers.


Altamaha
-28.50
-28.00
-27.50
-27.00
-26.50
-26.00
-25.50
-25.00
-24.50
-24.00
-23.50
del 13C
0
200
400
600
800
1000
1200
Length (mm)
> 550 mm
< 550 mm

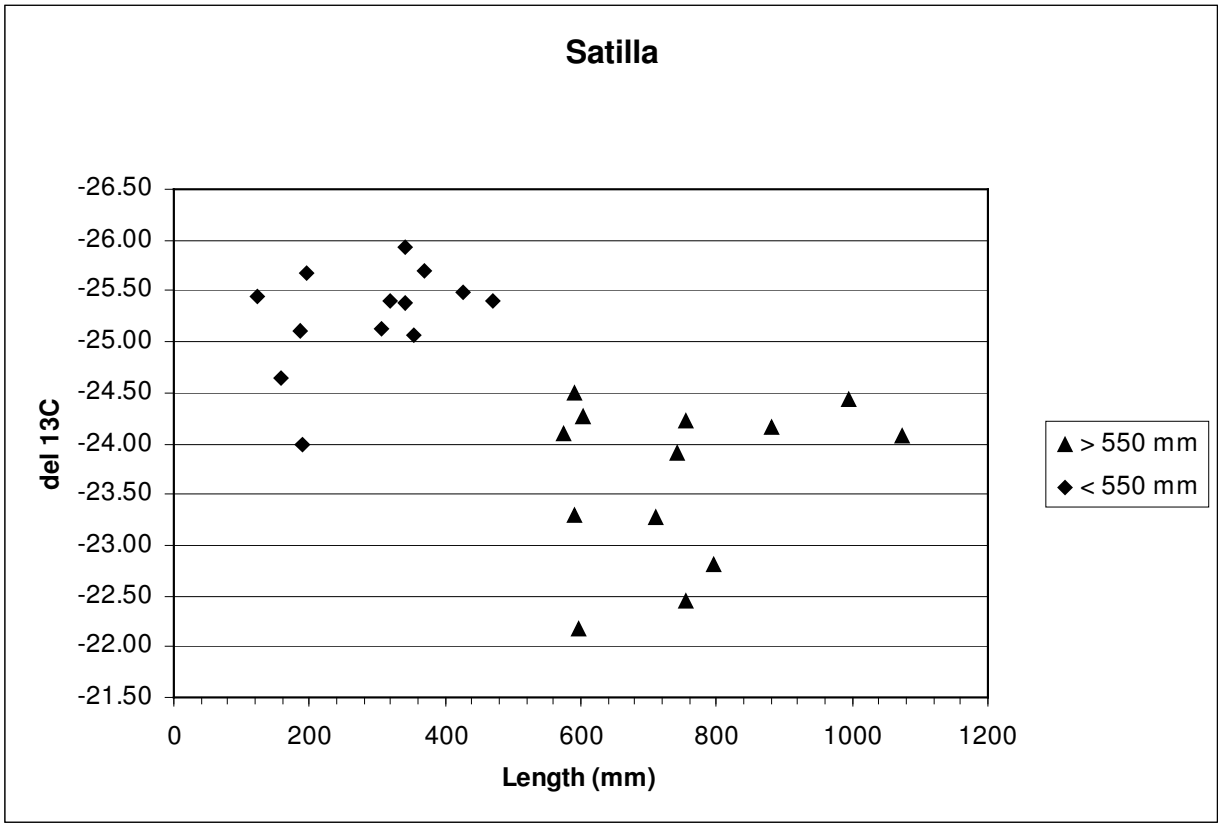
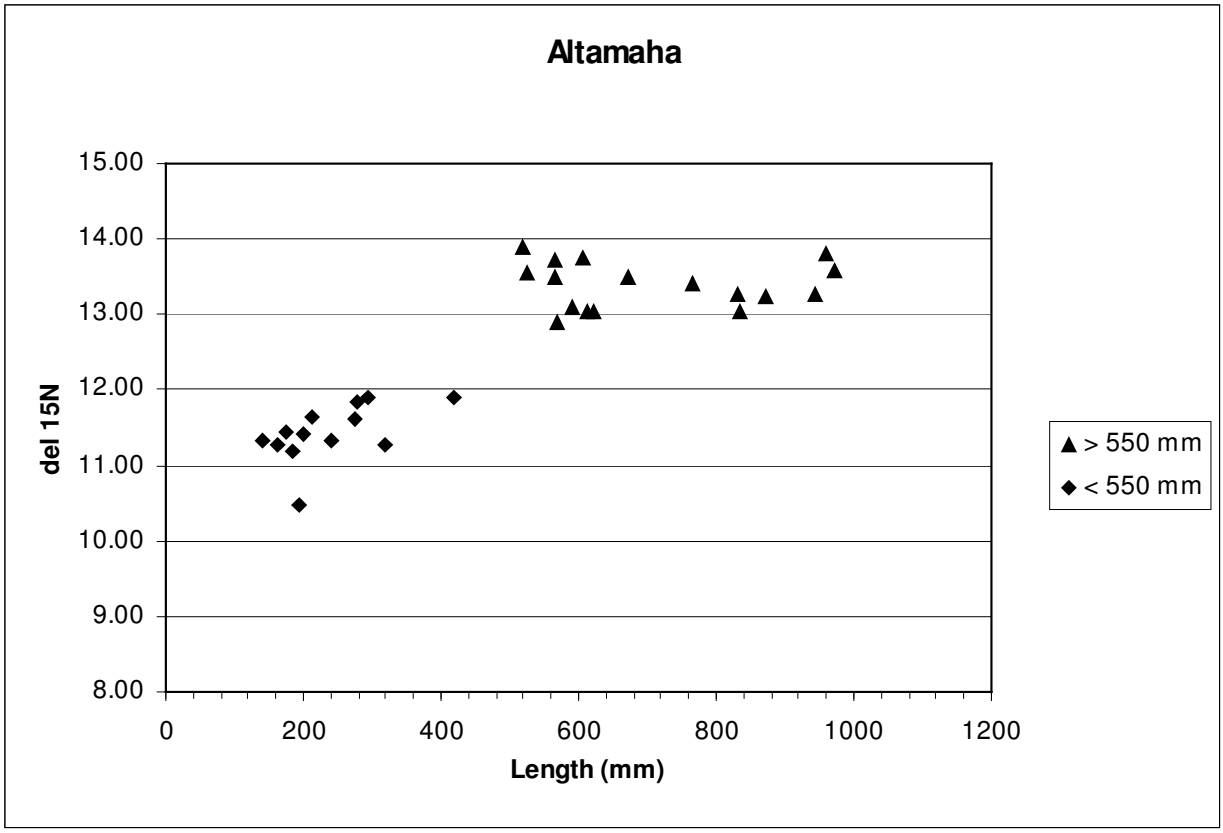

Satilla
-26.50
-26.00
-25.50
-25.00
-24.50
-24.00
-23.50
-23.00
-22.50
-22.00
-21.50
del 13C
0
200
400
600
800
1000
1200
Length (mm)
> 550 mm
< 550 mm

Figure 2.4. $\delta^{15}N$ values vs. length. Altamaha and Satilla Rivers.


Altamaha
15.00
14.00
13.00
12.00
11.00
10.00
9.00
8.00
del 15N
0
200
400
600
800
1000
1200
Length (mm)
> 550 mm
< 550 mm

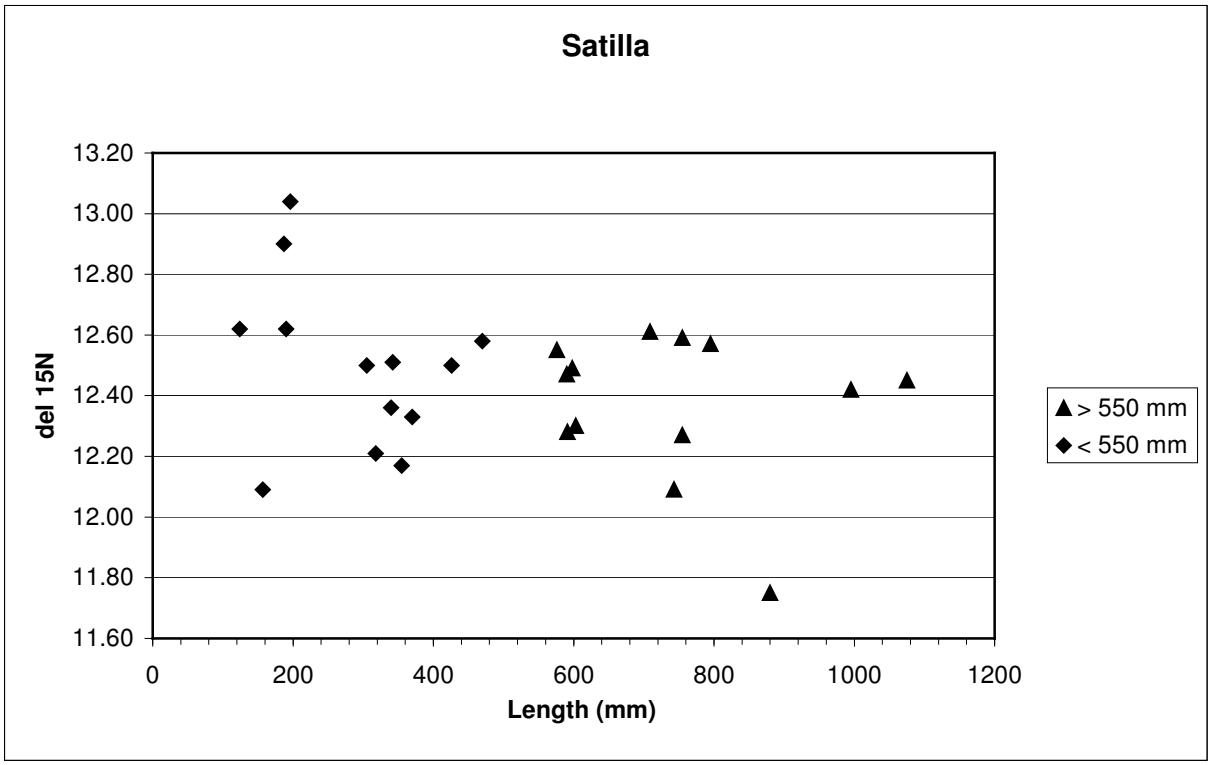
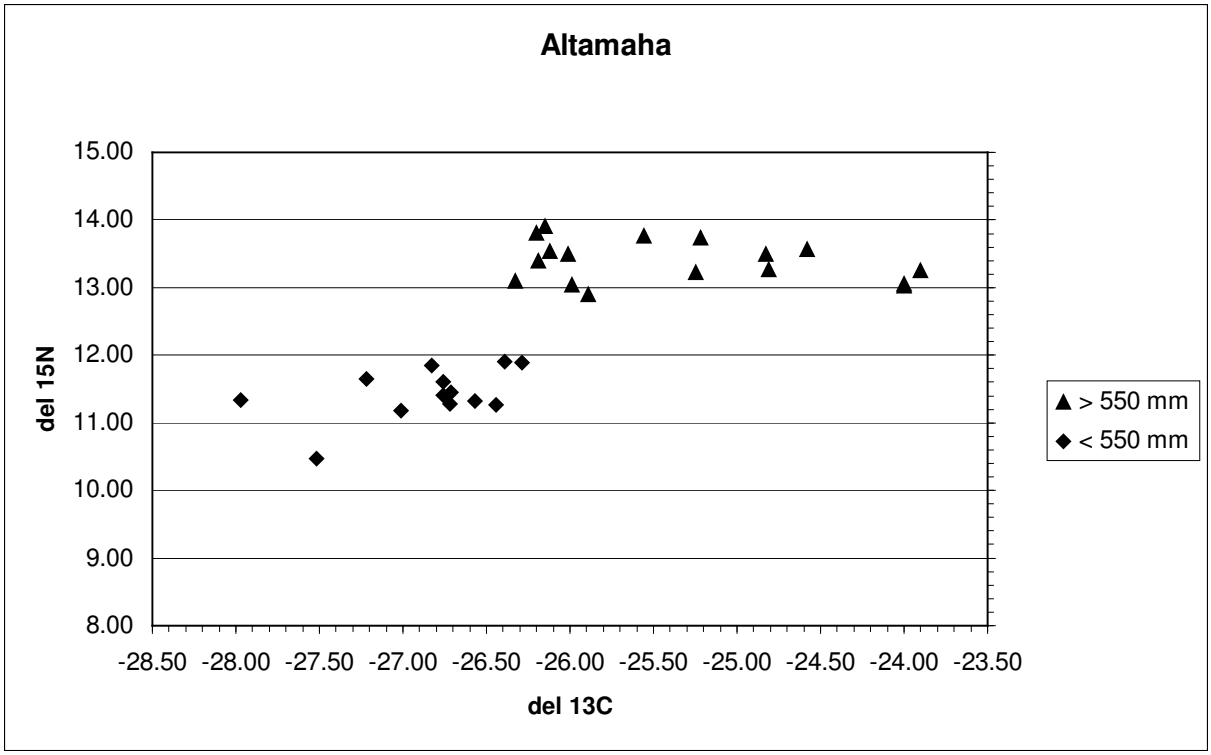
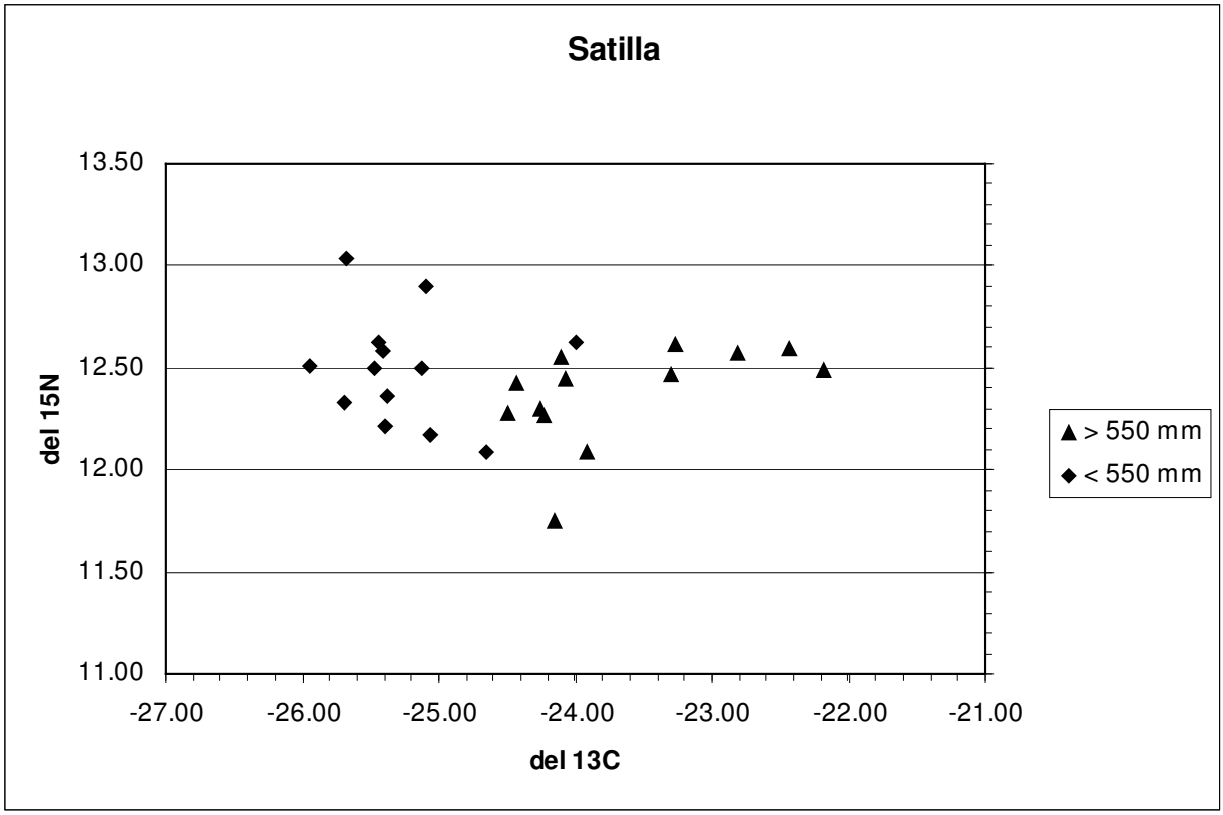

Satilla
13.20
13.00
12.80
12.60
12.40
12.20
12.00
11.80
11.60
del 15N
0
200
400
600
800
1000
1200
Length (mm)
> 550 mm
< 550 mm

Figure 2.5. $\delta^{13}C$ vs. $\delta^{15}N$. Altamaha and Satilla Rivers.


Altamaha
15.00
14.00
13.00
12.00
11.00
10.00
9.00
8.00
del 15N
-28.50 -28.00 -27.50 -27.00 -26.50 -26.00 -25.50 -25.00 -24.50 -24.00 -23.50
del 13C
> 550 mm
< 550 mm


Satilla
13.50
13.00
12.50
12.00
11.50
11.00
del 15N
-27.00 -26.00 -25.00 -24.00 -23.00 -22.00 -21.00
del 13C
> 550 mm
< 550 mm

CHAPTER 3

ANALYSIS OF A COASTAL MARSH CREEK FOOD WEB TROPHIC STRUCTURE

Food web research qualitatively describes food web patterns and interactions across vastly different ecosystem communities (Pimm 1982, Pimm et al. 1991, Polis 1994). Understanding a given food webs trophic structure is crucial for any ecosystem analysis, because ecological processes are described by analyzing community food webs (see Paine 1966, Cohen and Newman 1985, Power 1990). Food webs provide a basis for community and systems analysis by providing a logical structure linking species together by organic matter flows. Scientists can better understand organic matter flows through a given system, trophic classification and interactions, crucial keystone species dynamics, biodiversity shifts, and population control mechanisms from a food web framework. Various quantitative methods can identify connectivity between different organisms (or components) found within a food web (i.e., cycles, linkages, energy flow, etc.). Linkages may be simple elemental flow rates (Baird and Ulanowicz 1989) or feeding links (Martinez 1991, Pimm et al. 1991). An interaction food web analysis is based on linkages of space and/or resource control and requires experimental protocols and systems to replicate and quantify links (Hall et al. 1990, Paine 1992).

Although provoking useful discussion in ecology, food web theory has had little impact on conservation policy or management. This is because until recently no techniques allowed a simple, comprehensive examination of a given community. Traditionally, studies of food web structure have relied on gut content analysis, usually binary feeding data, to delineate trophic structure and

food web pathways (Odum 1968, Tagatz 1968, Odum and Heald 1972, Schoenly and Cohen 1991). This incomplete approach does not examine overall feeding patterns and is not always helpful for determining trophic rank or linkages. Classification of nutrient sources of estuarine and marsh biotic compartments (benthic and pelagic) poses a major problem for researchers. Gut content analysis is difficult to execute, is confounded by material that has been partially digested, and only provides a "snapshot" of food components consumed by an individual (Hughes and Shen 1983). Also, gut content analyses only provide an instantaneous image of material ingested. They do not provide a measure of food sources or allow examination of food source integration over time.

Stable isotope analysis has developed over the last 20 years to become an effective tool for documenting flows within communities and determining trophic level for consumers and producers (Peterson and Fry 1987, Fry 1988, 1991, Duggins et al. 1989, Hamilton et al. 1992, Kling et al. 1992). Stable isotope analysis provides process and tracer information essential for studying food web structure of large communities within reasonable time and financial constraints (Peterson and Fry 1987). Carbon and nitrogen stable isotope ratios have provided important insights useful for understanding trophic status and nutrient flows. Differential assimilation and bio-magnification of nitrogen isotope ratios are useful for separating consumers, predators and prey while ratios of carbon ($\delta\,^{13}C$) can be analyzed to separate food web components by organic matter flows within the food web (DeNiro and Epstein 1981, Rounick and Winterbourn 1986, Peterson and Howarth 1987, Parker 1989, Schell and

Ziemann 1989, Sullivan and Moncrief 1990, Gearing et al. 1991, Hamilton 1992, Kennicutt et al. 1992). The stable isotope signature for a given element in a food web compartment is an integration of the chemical or isotope composition of assimilated sources of energy and nutrients. Furthermore, these ratios change ontogenetically or as metabolic processes change with time or age and are useful for examining trophic shifts throughout the life history of a given organism.

Carbon isotopes can be used to ascertain primary energy source (eg. C3 photosynthesis vs. C4 photosynthesis) and nitrogen isotopes are used to discriminate trophic state or level. It is important to recognize that while an isotopic ratio for a specific plant or animal is thought to represent or reflect the diet or nutrient source there are many spatio-temporal specific factors that can and do cause shifts in average ratio. Isotopic turnover rates, anthropogenic impacts (site specific pollution), shifts in vegetation patterns, hydrography, soil characteristics, and ontogenetic shifts need to be considered when comparing different sampling periods among many possible sources of data being used for comparisons.

Salt marsh ecosystems are considered to be some of the most productive communities in the world (Teal 1962, Reimold et al. 1973, Wiegert and Pomeroy 1981, Wiegert and Freeman 1990), and, are complicated by food webs comprised of pelagic and benthic communities. Salt marsh ecosystem production supports food webs in rivers, creeks, and estuaries as well as adjacent terrestrial systems (Kraeuter and Wolf 1973). Taxa within these systems exhibit a diverse range of feeding strategies, complicating our view of

salt marsh trophic relationships. Salt marsh food webs are dominated by bacterial production with secondary utilization facilitated by benthic-pelagic coupling agents such as diatoms, harpacticoid copepods, polychaetes, amphipods and grazers/shredders in the form of crab and shrimp species as well as many fish species (Wiegert and Pomeroy 1981, Wiegert and Freeman 1990). Obscuring our understanding of flows of organic matter in these systems are a number of factors including bacterially mediated detrital pathways as well as primary production by C3 and C4 plants as well as benthic and planktonic diatoms and phytoplankton (Mann 1972, Odum et al. 1973, Pomeroy and Wiegert 1981).

I examined a food web of a saltwater marsh creek on Sapelo Island. I used stable isotope analysis of carbon and nitrogen and evaluate the efficacy of stable isotopes and enrichment to identification of trophic structure and organic matter flows (food sources) within the salt-marsh food web. The salt marsh – estuary ecosystems of Sapelo Island, Georgia have been extensively studied since the 1950's. The studies encompassed research of autecological and synecological processes defining this complex marsh-estuary ecosystem. While these marshes are important nursery grounds for many species of invertebrates and fish, the trophic level of many taxa is still unclear (see Haines and Montague 1979, Pomeroy and Wiegert 1981). Previous isotopic research of the trophic structure, community organization, and organic matter utilization within the salt marsh food web has been limited to the examination of natural abundance levels of carbon isotope ratios in various community compartments (Fry and Sherr

1984, Haines 1976a, Haines 1976b, Haines 1977, Haines and Montague 1979, Hughes and Sherr 1983, Knieb and Stiven 1980, Sherr 1982) and one study by Peterson and Howarth, 1987 which utilizes sulfur, carbon, and nitrogen isotope ratios to examine organic matter flow in Sapelo Island salt-marsh systems. A major problem with their interpretation of trophic structure and state is that they use spatially disparate sampling points for a given species but treat these samples as static populations without reflection or analysis on differences and bias introduced by different sampling sites.

Research using natural abundance of stable carbon and nitrogen isotope ratios of organisms from a small Sapelo Island, Georgia salt marsh creek, I attempted to describe food web interactions from both a connectance viewpoint as well as a detailed trophic organization viewpoint. This system, while extensively studied, is inadequately described. As an initial study, I amended the creek over a 40 day period with an enriched nitrogen salt (ammonium chloride) to examine problematic compartments at the base of the food web with the goal that the enriched isotopic signatures would provide data useful to clarify trophic positions and, possibly, benthic-pelagic coupling. This experiment was partially successful and provides useful insights for future studies of the salt marsh ecosystem and food web. In this paper, I examine and describe a salt marsh creek food web to: 1) determine the trophic base of the food web, 2) discriminate species thought to be critical benthic-pelagic coupling agents through the use of a nitrogen enrichment, and 3) contrast past studies of Sapelo Island food webs

results that may have confounded relationships with a food web sampled from one location.

METHODS

Study Site

This study was conducted during the late summer 2000 in a small tidal saltwater marsh creek on the south end of Sapelo Island. The creek is a smaller branch (total volume is approx. 2500 m^3) off South End Creek located in a diked area and runs beside a small road in a southeast to northwest direction This creek drains a relatively small area of mixed marsh and is impacted by runoff from the road during storm conditions. The water column is well mixed. The first 100 meters of the creek, from the main branch of South End Creek, has a bottom substrate composed of oyster reefs (live and archaic) separated by soft mud. The remaining creek bottom is soft mud. Mean tidal range is 2 – 3 meters (Pomeroy and Wiegert 1981). *Spartina alterniflora* is the dominant plant species in this small marsh system. There is some bank slumping at 150 meters from the mouth of this creek. Samples of representative trophic compartments (Table 3.1), prior to and throughout the duration of a nitrogen enrichment experiment, were collected in August 2000.

Sampling Methods

Samples taken every other day until the last 10 days when I switched to daily sampling. POM and *S. alterniflora,* were collected at approximately 45 minutes before low tide at the earliest daylight time on an every other day cycle.

All of the remaining taxa were sampled on a 2 to 4 day cycle. There are gaps in sampling for many taxa because of the variability in movement or abundance during a given tidal cycle. Salinity and temperature data were collected daily using a handheld YSI meter. Water samples collected at 2-4 day intervals for NH_4 analysis were stored on ice and transported to the University of Georgia analytical laboratory for processing.

The primary sampling site was located at the right angle bend in the creek. This 10-meter area is characterized by a large, active oyster reef, remains of an old oyster reef, and an area of soft mud. *Spartina alterniflora* dominated the high banks of this region. This area is located 30 meters from the marriote bottle used for dripping the labeled nitrogen. This 30 meter buffer distance was an attempt to insure that the label would be well mixed before reaching the sampling site. All samples, whole animals or muscle tissue, were placed in labeled, ashed scintillation vials and were dried for 24 hours at 60 degrees C in a vacuum oven. They were then placed in a desiccator with a small vial of 10 M HCL for 4 hours. I did not acid wash specimens because Bunn et al. 1995 reported an increase in variation among $\delta\ ^{15}N$ and $\delta\ ^{13}C$ samples, which may confound the analysis of food web trophic structure.

POM was sampled at the primary location and at a site 200 meters from the primary sampling site. POM samples were collected by filling a rinsed 10-liter carboy from each site and filtering onto an ashed Whatman GF/F filter under vacuum until the filter clogged. Filters were rinsed, dried, acidified, and stored for analysis. POM samples were duplicated and combined for each sample. After

drying, POM samples were scraped off of the GF/F with a razor and ground for analysis.

Two producers (*Spartina alterniflora* and benthic diatoms (*Nitzschia* and *Navicula*), POM, detritus, and 14 animal taxa, which span a range of functional groups, were used for this study (Table 3.1). These taxa were the most abundant found in the study site and are representative of the major compartments in this salt marsh creek system. Furthermore, previous studies examined many of the taxa I chose to examine which facilitates the examination of changes over time and/or across areas.

For macrofauna and fish, I combined 3 or more specimens for each sample point. Benthic organisms, *Crassostrea virginica*, *Geukensia demissa*, *Sesarma cinereum*, and *Eurytium limosum* were collected by hand and placed in separate containers in a cooler for later processing. A D-net and cast-net were used to collect Fundulus heteroclitus, Penaeus setiferus, *Mugil cephalus*, *Micropogonias undulates*, *Callinectes sapidus*, *Palaemonetes pugio*, and *Cynoscion nebulosus*. These were stored in whirl-pak bags and placed in a cooler for processing in the lab. To collect *Neanthes succinea*, I filled nylon mesh bags with detritus wrack and anchored these to the creek bed with heavy gauge wire (Steve Pennings, pers. Comm.). Multiple bags were placed in the creek prior to the start of the enrichment. One bag was collected per sampling period, stored in a large 1-gallon baggie for processing in the lab. The contents were filtered and elutriated using 100-micron stainless steel sieves. *Neanthes succinea* were picked using forceps, rinsed, and placed in scintillation vials.

Benthic diatoms were collected at low tide from blooms on mud banks by placing 2 sheets of 64-micron nylon mesh with clean beach sand sandwiched between them over the bloom area (see Couch 1989). The top sheet was rinsed using deionized water into a bottle and this was later filtered onto a GF/F filter. *Spartina alterniflora* was collected by hand, rinsed to remove residual mud, and then dried in brown paper bags. Three *S. alterniflora* stems were cut and combined for each sample. *Orchestia grillus* was collected by cutting *S. alterniflora* at the base of the sediment. The stems were cut into manageable pieces and placed in 1-gallon Baggies, which were then filled with hot water (Ronald Kneib, pers. Comm.). This water was then filtered using a 100-micron sieve. *O. grillus* was then picked using forceps, rinsed, and placed in scintillation vials. Benthic copepods were collected using a diaphragm pump with a series of stainless steel sieves with 63 and 100-micron openings. These samples were resuspended in a watch glass and the copepods were picked using fine forceps and placed in a scintillation vial. Copepods were pooled together, irrespective of their size to assure minimum mass for analysis. All fish taxa sampled were restricted to young size classes so that their muscle tissue would probably reflect recent food sources. All fish and macrofauna were dissected and muscle tissue was collected for analysis. Detritus was picked off the surface of the mud until a scintillation vial was half filled.

All samples were ground in a Spex ball mill or by hand. Samples were analyzed on a Finnigan Delta C mass spectrometer connected to a Carlo-Erba NA1500 carbon-nitrogen analyzer via Finnigan's Conflo II interface in the

University of Georgia Institute of Ecology Analytical Laboratory. The Carlo-Erba CHN analyzer oxidizes the samples at 1050C, reducing the nitrogen oxides to N_2 and removes O_2 at 650C with copper as a catalyst, and removes H_2O with magnesium perchorate. CO_2 and N_2 were chromatographically separated and helium is used to carry the gases to the mass spectrometer. Raw data were corrected for shifts within and bias of instrumental effects. Because the samples were relatively large with the exception of Orchestia, copepod, and diatom samples, machine error is relatively low. The data are recorded as $\delta^{15}N$ and $\delta^{13}C$ values relative to isotopic ratios of NIST standard bovine serum samples. The δ values are calculated by:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Enriched samples will be "heavier" than values from unenriched samples.

<u>NH_4 Enrichment</u>

I labeled the incoming tidewater, on each flood tide, by continuously dripping 15N-enriched NH_4CL using a Marriote bottle calibrated for different flow rates from 8 August to 29 September, 2000. The creek/marsh volume was assumed to always be 2500 m^3 (calculated by taking length and width measurements at spring tide and adding 35% for overflow onto adjacent marsh (Jackson Blanton, pers. comm.). To regulate the drip rate, I used a series of stainless steel hose clamps marked for different flow rates. These clamps were calibrated and marked by etching with a steel nib and stripping the screw with a file. A 100-liter carboy was used to construct my Marriote bottle. I built a

wooden platform in the marsh to support the marriote bottle and drip stem above the creek. The flow rate of ^{15}N being added to the incoming tidal flow was set approximately 100% relative to background levels of NH_4. The concentration of nitrogen added to the incoming tide was approximately 0.038 mg/l. $^{15}NH_4Cl$ was chosen because it is a labile form of nitrogen for bacterial and planktonic uptake.

RESULTS

Daily temperature and salinity data were recorded daily. Temperatures averaged 30.49C with a low of 26.90C and a high of 33.59C (Figure 3.2). Salinity ranged from 26.5 to 33.1 ppt. (Figure 3.1). Ammonium levels ranged from 37.2 mg/m3 to 39.29 mg/m3 (Figure 2). The ammonium levels are similar to those previously reported (Haines et al. 1977, Haines 1979, Pomeroy and Wiegert 1981). During the study a low-pressure system formed over coastal Georgia resulting in large rainfall amounts and cooler, overcast days. The temperature and salinity data from 9/3/2000 to 9/8/2000 reflect this weather event (Figure 3.2).

The major primary producers in this salt marsh creek were benthic diatoms and were visible as large golden brown to green blooms on the surface of mud flats at low tide. Benthic diatom blooms on the creek banks can reach densities of 6400 cells/mm^2 (Wiegert and Pomeroy 1981). *Nitzchia* and *Navicula* species as well as some other unidentified pennate and centric diatoms characterized these blooms (see Wiegert and Pomeroy 1981). It is possible that the unidentified diatoms were various life stages of the identified species,

Nitzchia and *Navicula*. The creek banks and marsh area were dominated by *Spartina alterniflora*. The periphery of the marsh nearest the road supported fragmented populations of *Juncus roemarianius, Borrichia frutescens, Myrica cerifera,* and *Salicornia virginica*.

Sediments were characterized by deep mud composed of silt, sand and organic matter. Gelatinous mud is found in patches throughout the creek bottom. The creek sediments support high populations of *C. virginica* and *G. demissa* as well as a diverse population of infauna including *N. succinea* and harpacticoid copepods (Wiegert and Pomeroy 1981).

Zooplankton species were dominated by calanoid copepods including *Eurytemora affinis* and *Acartia tonsa*.

The creek and surrounding waters support a wide variety of fish. Many different species were collected during preliminary sampling, including *Fundulus majalis, Fundulus heteroclitus, Dasyatis Americana, Brevoortia smithi, Anchoa mitchilli, Opsanus tau, Poecilia latipinna, Archosargus probatocephalus, Leiostomus xanthurus, and Trinectes maculatus*.

Primary producers sampled were *Spartina alterniflora, Juncus roemerianus,* and benthic diatoms (pooled sample with *Nitzchia* and *Navicula* comprising the majority of the samples). *S. alterniflora* $\delta\,^{13}C$ and $\delta\,^{15}N$ values ranged from 5.44 to 7.85 with an initial value of 7.17 and –13.32 to –12.21 with an initial value of -12.22, respectively. J. roemerianus had $\delta\,^{15}N$ values ranging from 4.77 to 5.17 with an initial value of 4.98 and $\delta\,^{13}C$ values ranging from –26.45 to –25.81 with an initial value of –26.21. Benthic diatoms values for $\delta\,^{13}C$

and $\delta^{15}N$ ranged from 4.03 to 16.78 with an initial value of 5.1 and from –19.21 to 18.20 with an initial value of –18.2. POM samples taken from the primary site had d13N values ranging from 4.36 to 480.27 with an initial value of 4.36 and $\delta^{13}C$ values ranging from –22.11 to –19.39 with an initial value of –21.25. POM samples from the secondary sample site had $\delta^{15}N$ values ranging from 4.06 to 9.77 with an initial value of 5.95 and $\delta^{13}C$ values ranging from –23.01 to –20.77 with an initial value of –21.97. Detritus had $\delta^{15}N$ values ranging from 2.98 to 9.12 with an initial value of 6.23 and $\delta^{13}C$ values ranging from –20.83 to -15.89 with an initial value of –16.2.

Suspension feeders sampled were *Crassostrea virginica* and *Geukensia demissa*. *C. virginica* had $\delta^{15}N$ values ranging from 7.72 to 10.92 with an initial value of 6.79 and $\delta^{13}C$ values ranging from –19.08 to –17.52 with an initial value of -17.78. *G. demissa* had $\delta^{15}N$ values ranging from 7.27 to 8.94 with an initial value of 7.39 and $\delta^{13}C$ values ranging from –18.98 to –17.97. Both suspension feeders were labeled with ^{15}N, *C. virginica* was moderately labeled *and G. demissa* was weakly labeled (Figure 3.11).

Deposit-suspension feeders sampled were *Palaemonetes pugio*, *Mugil cephalus*, and *Litopenaeus setiferus*. *P. pugio* had $\delta^{15}N$ values ranging from 6.56 to 10.21 with an initial value of 6.01 and $\delta^{13}C$ values ranging from –18.92 to –17.18 with an initial value of –17.29. *M. cephalus* had $\delta^{15}N$ values ranging from 8.49 to 10.12 with an initial value of 8.53 and $\delta^{13}C$ values ranging from –17.23 to –14.6 with an initial value of –16.48. *L. setiferus* had $\delta^{15}N$ values

ranging from 7.99 to 9.66 with an initial value of 8.73 and $\delta^{13}C$ values ranging from –15.89 to –14.48 with an initial value of –15.48. *P. pugio* was moderately labeled by a factor of 2‰ (Figure 3.12). It is unclear why *P. pugio* did not exhibit better labeling.

Sesarma cinereum, a deposit feeder, had $\delta^{15}N$ values ranging from 6.74 to 7.66 with an initial value of 6.91 and $\delta^{13}C$ values ranging from –19.72 to –18.87 with an initial value of –19.26.

Omnivores sampled were *Fundulus heteroclitus*, *Callinectes sapidus*, *Eurytium limosum*, *Neanthes succinea* and benthic copepods. *F. heteroclitus* had $\delta^{15}N$ values ranging from 9.92 to 14.34 with an initial value of 10.88 and $\delta^{13}C$ values ranging from –18.59 to -15.44 with an initial value of –17.39. *C. sapidus* had $\delta^{15}N$ values ranging from 9.89 to 10.67 with an initial value of 10.24 and $\delta^{13}C$ values ranging from –14.8 to –13.99 with an initial value of –14.12. *E. limosum* had $\delta^{15}N$ values ranging from 7.99 to 8.94 with an initial value of 8.94 and $\delta^{13}C$ values ranging from –18.90 to –18.00 with an initial value of –18.62. *N. succinea* had $\delta^{15}N$ values ranging from 8.45 to 18.12 with an initial value of 8.83 and $\delta^{13}C$ values ranging from –17.2 to -15.02 with an initial value of –15.87. Benthic copepods had $\delta^{15}N$ values ranging from 5.0 to 9.12 with an initial value of 6.12 and $\delta^{13}C$ values ranging from –17.05 to -16.01 with an initial value of –17.05. Of the omnivores sampled, only *N. succinea* exhibited strong labeling with over half of the samples clearly above the pre-treatment base level (Figure 3.8). *F. heteroclitus* and benthic copepods were weakly labeled with 30‰ of samples above pre-treatment base levels (Figure 3.8).

Predators sampled were *Micropogonias undulatus* and *Cynoscion nebulosus*. *M. undulatus* had $\delta^{15}N$ values ranging from 12.27 to 13.91 with an initial value of 13.91 and $\delta^{13}C$ values ranging from –17.82 to –17.07 with an initial value of –17.45. *C. nebulosus* had $\delta^{15}N$ values ranging from 14.21 to 15.03 with an initial value of 14.21 and $\delta^{13}C$ values ranging from –18.21 to –16.23 with an initial value of –16.92. Neither of the top predators sampled were labeled with enriched ^{15}N (Figure 3.9). This may be attributed to turnover rates or variable movements in/out of the creek system.

Orchestia (Urlochestia) grillus is problematic to classify to feeding type. It is considered a scavenger, detritivore, and predator (see Pollock 1997). *O. grillus* had $\delta^{15}N$ values ranging from 6.98 to 17.23 with an initial value of 6.9 and $\delta^{13}C$ values ranging from –18.9 to –17.41 with an initial value of –18.51. Examining Figure 3.8, it is obvious that *O. grillus* is highly labeled with enriched ^{15}N with over 60% of the samples well above the baseline pre-treatment level.

Only a few taxa or compartments exhibited strong labeling with enriched nitrogen (Table 3.3). POM, *N. succinea*, and *O. grillus* had post-enrichment values much higher than pre-enriched values (Figures 3.10 and 3.13). Moderately enriched taxa were *C. virginica* and benthic diatoms (Figures 3.10 and 3.11). Detritus, *G. demissa*, *P. pugio*, *F. heteroclitus*, and benthic copepods displayed were weakly enriched (Figures 3.11, 3.12, and 3.13).

Results from other Sapelo Island Food Web and Stable Isotope Research

This summary of previous stable isotope research on Sapelo Island Salt marshes includes the goal of each study and data for taxa or compartments that were sampled during the current study.

Haines (1976) examined stable carbon isotope values in the salt marsh near Sapelo Island, Ga. The goal of the study was to examine the utility of stable carbon isotope analysis to trace carbon flow. See Table 3.4 for a summary of isotope data from this study. *S. alterniflora* and benthic diatoms stable carbon isotope values were highly variable and were similar to soil $\delta^{13}C$ values. This study concluded that the invertebrates sampled reflected the $\delta^{13}C$ values of the primary producers but that temporal integration of source material combined with interacting physical and biological made it impossible to see direct relationships between consumers and producers.

Haines and Montague (1979) examined the usefulness of stable isotopes to determine food sources for selected invertebrate taxa. As in previous studies, only stable carbon isotopes were examined.

DISCUSSION

It is very likely that POM and benthic diatoms are the principal benthic pelagic coupling agents within these tidal creek systems, serving as vectors of nutrients to both the benthos and water column. Detritus plays an obvious role,

as does fresh *S. alterniflora* and other marsh plants. POM is the first compartment examined to demonstrate a clear enrichment phenomenon. Benthic diatoms are not far behind POM in this respect. The role of bacteria was not determined other than to the extent that it is a known component of POM and is responsible for much of the detritus isotopic signature (Haines 1976a, Wiegert and Pomeroy 1981, Newell and Fallon 1982, Newell 1984). The enrichment of POM is probably mediated by diatom, planktonic and benthic, and bacteria remineralization. Clearly, a more rigorous enrichment experiment which uses new methods to isolate and document bacterial biomass and isotopic ratios (see Molina 2001) is critical to formulate mixing models capable of accurately assessing isotopic signals with regard to trophic status and percentage of production for a given producer compartment.

The examination of $\delta^{13}C$ ratios for the different compartments or taxa suggests that there is high variability in the carbon isotope ratios. For example, detritus had relatively high variances compared to some of the other food web base compartments (Table 3.3). This variability may be a function of mixing processes during tidal flow (ebb and flood) with water from the main channel as well as associated smaller creeks off of the main channel.

Stable isotope ratios or signatures of prey and nutrient sources change from source to consumer in a predictable pattern because of selective fractionation and uptake (Peterson and Fry 1987, Lajtha and Michener 1994, and vander Zanden et al. 1997). Carbon isotope ratios are typically enriched by 1-2‰ from one trophic level to the next, which facilitates comparison of taxa with

respect to food or nutrient source (Michener and Schell 1994). Nitrogen isotopes exhibit a trophic shift of 3-5‰ from source or prey to consumer and is frequently used to indicate trophic position for taxa with a given food web (Yoshioka et al. 1994, Bootsma et al. 1996, vander Zanden and Rasmussen 1996, vander Zanden et al. 1997). Because the rate of change of isotopic ratios in response to a change in source ratios is dependent on metabolic processes, an enrichment study may provide useful information for one functional group or taxa while providing none for others. Kendall (1998) examined spatial differences and found that sampling sites that are relatively close together may still exhibit vastly different isotopic ratios for a given food web compartment.

In this study, pre-treatment samples for the "base" of the food web varied in $\delta^{15}N$ from 4.36 (POM) to 7.17 (*S. alterniflora*). For 1st order consumers, $\delta^{15}N$ varied from 6.12 (benthic copepods) to 10.88 (*F. heteroclitus*) with a mean of 8.17 and variance of 2.54. These values make delineating trophic position for many taxa problematic because there is no consistent 3-5‰ change from one trophic level to the next. Post-treatment data is clearly different with distinct shifts between the functional feeding groups. Producers, POM, and detritus, after the enrichment, have values ranging from 4.59 (detritus) to 14.1 (POM). Post-treatment 1st order consumer data ranged from 7.31 (*S. cinereum*) to 18.12 (*N. succinea*) with a mean value of 10.53 and variance of 14.14. The resulting "separation" of the taxa shows clear separation between many of the taxa or compartments that were close in value in the pre-treatment data. Table 3.3 compares the data from the pre- and post-enrichment sampling.

This study demonstrates clear differences in ^{15}N labeling between taxa and compartments, showing differential use of production from benthic and pelagic producers (benthic diatoms, detritus, and POM). POM enrichment is likely a function of phytoplankton, benthic diatoms entrained within the water column, and bacterial assimilation and production. To better differentiate between organic matter (or nutrient) sources, the role of bacteria within this ecosystem should be examined. Current technology, including the examination of bacteria specific phospholipid fatty acids, will, in time, provide data critical to realistic construction of food webs and the production that drives them. But, as evidenced by the data in this study, nitrogen enrichment in a tidal system is possible and provides data important for the analysis and understanding of problematic linkages in marsh-creek food webs.

It seems unlikely that invertebrates, including *N. succinea*, *C. virginica*, *P. pugio*, *O. grillus*, and benthic copepods, become labeled without ingesting bacterial carbon at a primary or secondary level. While we know much about feeding habits there is still much to learn. For instance, *O. grillus* and *N. succinea* pose particular problems because of a lack of consensus on preferred feeding habits and the possibility that they may not fit within any standard defined feeding mode. The clear labeling of *O. grillus* and *N. succinea* indicates that they are assimilating large quantities of bacteria, indirectly or not. *O. grillus* is thought to prefer *S. alterniflora* detritus as a food source (Kneib 1982, Fell et al. 1998) but has isotopic ratios that reflects utilization of benthic diatoms and POM (Table 3.2). This suggests that algae and general grazing might play a more important

nutritional role that previously thought for this species. *N. succinea*, a deposit feeder (Cammen 1980a, 1980b) has isotopic ratios that reflect an indiscriminate deposit feeding mode (Table 3.2).

Progressing "up" the food chain, there is more variability in the ^{15}N ratio. This is probably a result of the fact that as you move "up" the food chain, and are farther removed from producers, prey species may have vastly different feeding modes, and exhibit highly variable isotopic signals. Secondary and tertiary consumers would need to consistently eat enriched prey species such as benthic copepods or other grazers of the bacterial and diatom compartments. Further confounding the interpretation of isotopic signals in "higher" trophic levels is the inherent variability of many predators and omnivores diets. The two predators that I sampled for this study, *M. undulates* and *C. nebulosus*, were not enriched and had relatively constant isotopic ratios across the sampling period (Figures 3.9 and 3.14). This constancy in the isotopic ratios is probably a function of their highly temporally variable diet, slower metabolism and isotopic turnover, in contrast to the invertebrates sampled, and buffering ability of the relatively larger mass of muscle tissue.

The results of this study shows that stable isotope enrichment in tidal creek systems is not only possible but may provide critical information about flows of organic matter and nutrients. Combining this approach with life history data, biogeochemical budgets, and population data will allow examination of relationships heretofore confounded by lack of data or simply unknown interrelated ecosystem processes. The data from this study show that there may

be differential utilization and relationships of bacterial (via POM and indiscriminate benthic grazing) and planktonic compartments that were and are unknown.

In summary, the primary plant species (*S. alterniflora* and *J. romerianius*) and benthic diatoms were characterized by distinct nitrogen and carbon isotope compositions. Detritus and POM samples had very similar carbon isotope ratios and nitrogen isotope ratios separated by 1‰. The deposit feeder and deposit suspension feeders had carbon isotopic ratios that reflect a detritus or benthic diatom signal but the pre- and post-enrichment nitrogen isotopic ratios were confounding in that they reflect detritus, benthic diatoms, and S. alterniflora nitrogen isotope ratios. It is possible that the short duration of the enrichment experiment or inconsistent labeling of base compartments may be part of the problem. The suspension feeders had stable carbon and nitrogen isotope ratios similar to POM. Omnivores had $\delta^{15}N$ ratios that were highly variable and enriched compared to the other functional feeding groups. It is interesting to note that *N. succinea* and *O. grillus* are well studied but data is lacking concerning their role within the marsh creek food web. I speculate that they, as well as related infauna, are more important for the transfer of bacterial and PP carbon to secondary and tertiary trophic levels than previously thought.

The southend creek of Sapelo Island offers a potentially perfect place for the utilization of enrichment studies to better understand OM dynamics and trophic – food web relationships in tidal creek systems that have little to no freshwater input. The creek is moderate in size and drains/floods a restricted

area of marsh contained with a diked region. Future research in this area, founded on historical data, could provide important information about benthic-pelagic coupling agents. This study utilized the work of ecological research at Sapelo Island dating back to the 1950's (see Wiegert and Pomeroy 1981, Wiegert and Freeman 1990, and Chalmers 1997 for reviews).

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Table 3.1. Taxa used in this study, including scientific names and feeding classification. Functional feeding groups follow Pollock 1998 and Peterson and Howarth 1987.

Producers and misc. OM sources	Detritus
	Benthic Diatoms
	POM
	Spartina alterniflora
	Juncus romerianius
Suspension Feeders	*Crassostrea virginica*
	Geukensia demissa
Deposit Suspension Feeders	*Mugil cephalus*
	Penaeus setiferus
	Palaemonetes pugio
Deposit Feeders	*Sesarma cinereum*
Omnivores	*Benthic copepods*
	Callinectes sapidus
	Eurytium limosum
	Fundulus heteroclitus
	Neanthes succinea
Unclassified	*Orchestia grillus*
Predators	*Micropogonias undulates*
	Cynoscion nebulosus

Table 3.2. Stable isotope ratio statistics for all taxa and compartments.

Taxa/Compartment	Mean		Variance		Range	
	N	C	N	C	N	C
Detritus	5.18	-18.23	2.95	2.43	2.98-9.12	-20.83 - -15.89
POM–1	7.51	-20.86	7.43	0.50	4.36 – 14.25	-22.11 - -19.9
POM-2	6.15	-21.52	1.71	0.19	4.06 – 9.77	-23.01 - --20.77
Benthic Diatoms	6.92	-18.57	18.23	0.09	4.03 – 16.78	-19.21 - -18.2
S. alterniflora	6.97	-12.71	0.29	0.16	5.44 – 7.85	-13.32 - -12.21
J. romerianius	4.94	-26.15	0.021	0.04	4.77 – 5.17	-26.45 - -25.81
C. virginica	7.76	-18.14	0.94	0.139	7.72 – 10.92	-19.08 - -17.52
G. demissa	7.73	-15.56	0.16	.097	7.27 – 8.94	-17.97 - -18.98
M. cephalus	8.79	-12.24	0.26	0.95	8.49 – 10.12	-17.23 - -14.6
P. setiferus	8.86	-15.05	0.18	0.14	7.99 – 9.66	-15.89 - -14.48
P. pugio	7.82	-17.64	1.08	0.21	6.56 – 10.21	-18.92 - -17.18
S. cinereum	7.0	-19.29	.07	0.065	6.74 – 7.66	-19.72 - -18.87
Benthic Copepods	6.23	-16.52	1.42	0.13	5.0 – 9.12	-17.05 - -16.01
C. sapidus	10.26	-14.38	0.077	0.087	9.89 – 10.67	-14.80 - -13.99
E. limosum	8.64	-18.54	0.096	0.065	7.99 – 8.94	-18.90 - -18.00
F. heteroclitus	11.41	-18.37	1.18	0.72	9.92 – 13.24	-18.55 - -15.44
N. succinea	10.61	-15.99	11.9	0.43	8.45 – 18.12	-17.20 - -15.02
O. grillus	10.93	-18.21	20.52	0.18	6.87 – 17.23	-18.9 - -17.41
M. undulates	13.08	-17.42	0.49	.077	12.27 – 13.91	-17.82 - -17.07
C. nebulosus	14.68	-16.87	0.12	.64	14.21 – 15.03	-18.21 - -16.23

Table 3.3. Pre- and post enrichment $\delta^{15}N$ ratios. Predator = PR, Omnivore = OM, Deposit Feeder = DF, Deposit Suspension Feeder = DSF, Suspension Feeder = SF, Producer = PD

Compartment	**Feeding Group**	**Baseline**	**Enriched Data**	**Difference**
POM		4.36	14.1	+9.74
J. romerianius	PD	4.98	5.12	+0.14
Benthic Diatoms	PD	5.1	9.21	+4.11
Detritus		6.23	4.59	-1.64
S. alterniflora	PD	7.17	7.81	+0.64
Benthic copepods	OM	6.12	9.12	+3.0
C. virginica	SF	6.79	10.92	+4.13
O. grillus	**	6.9	17.23	10.33
S. cinereum	DF	6.91	7.31	+0.4
P. pugio	DSF	6.01	8.78	+1.87
G. demissa	SF	7.39	8.94	+1.55
M. cephalus	DSF	8.53	8.49	-0.04
P. setiferus	DSF	8.73	9.45	+0.72
N. succinea	OM	8.83	18.12	+9.29
E. limosum	OM	8.94	8.83	-0.11
C. sapidus	OM	10.24	10.67	+0.43
F. heteroclitus	OM	10.88	13.94	+3.06
M. undulates	PR	13.91	12.27	-1.64
C. nebulosus	PR	14.21	15.03	+0.82

Table 3.4. Summary of stable isotope data from Sapelo Island, Georgia. $\delta^{13}C$ data are followed by $\delta^{15}N$ data when available. Current column has both pre- and post-enrichment $\delta^{15}N$ data.

	Haines (1976)	Haines/Montague (1977)	Peterson/Howarth (1987)	Current
Group				
S. alterniflora	-13.6	-12.1	-12.8 and 7.2	-12.71 7.17 | 7.81
Diatoms	-16.2	-16.2		-18.57 5.1 | 9.21
POM	-23.2	(-18)–(-24.7)		-20.86 4.36 | 14.1
J. romerianus	-23.0	-22.8	-25.1 and 5.1	-26.15 4.98 | 5.12
C. virginica	-21.0		-19.1 and 8.7	-18.14 6.79 | 10.92
P. pugio	-13.6		-17.1 and 8.8	-17.64 6.91 | 8.78
S. cinereum	-19.1	-13.5		-19.29 6.91 | 7.31
E. limosum		-13.4		-18.54 8.94 | 8.83
G. demissa			-19.5 and 7.4	-15.56 7.39 | 8.94
L. setiferus			-17.1 and 9.6	-15.05 8.77 | 9.45
M. cephalus			-15.6 and 10.8	-12.24 8.53 | 8.49
F. heteroclitus			-15.6 and 11.5	-18.37 10.88 | 13.94
C. sapidus			-16.7 and 10.5	-14.38 10.24 | 10.67

Figure 3.1. Characteristics of water, Road Creek, Sapelo Island, Georgia, 2000. Temperature and salinity at primary sampling site.

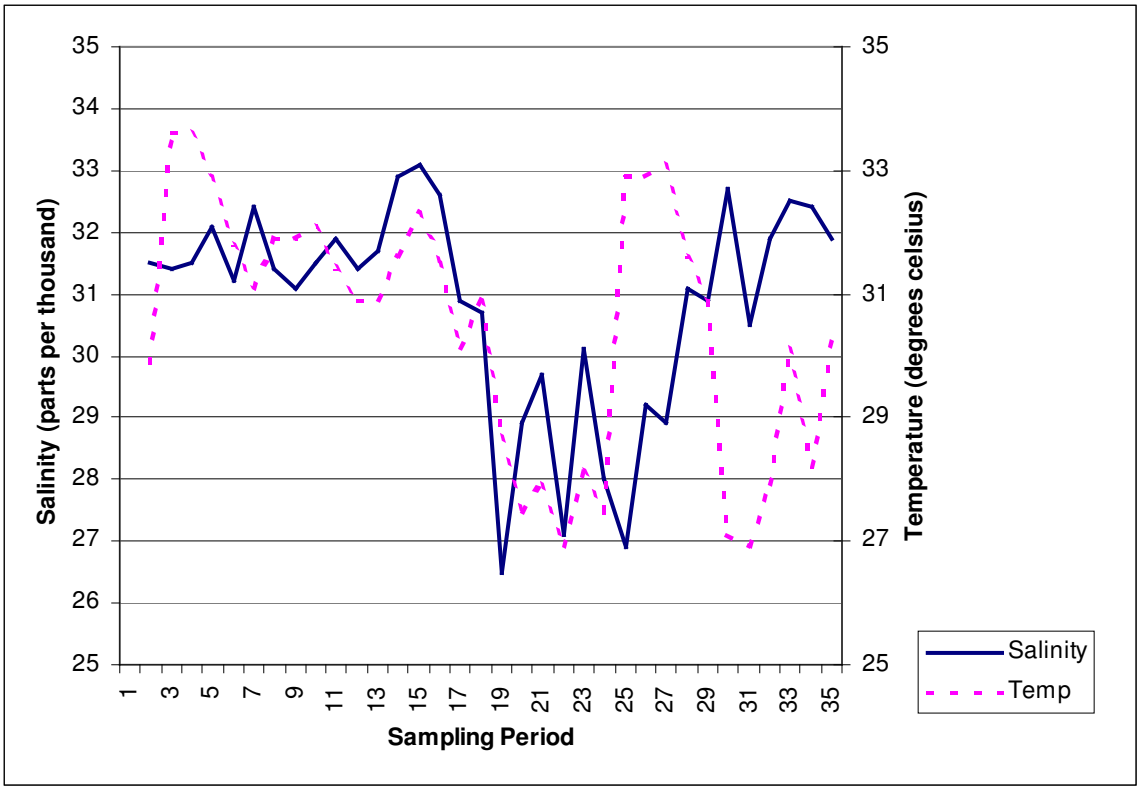
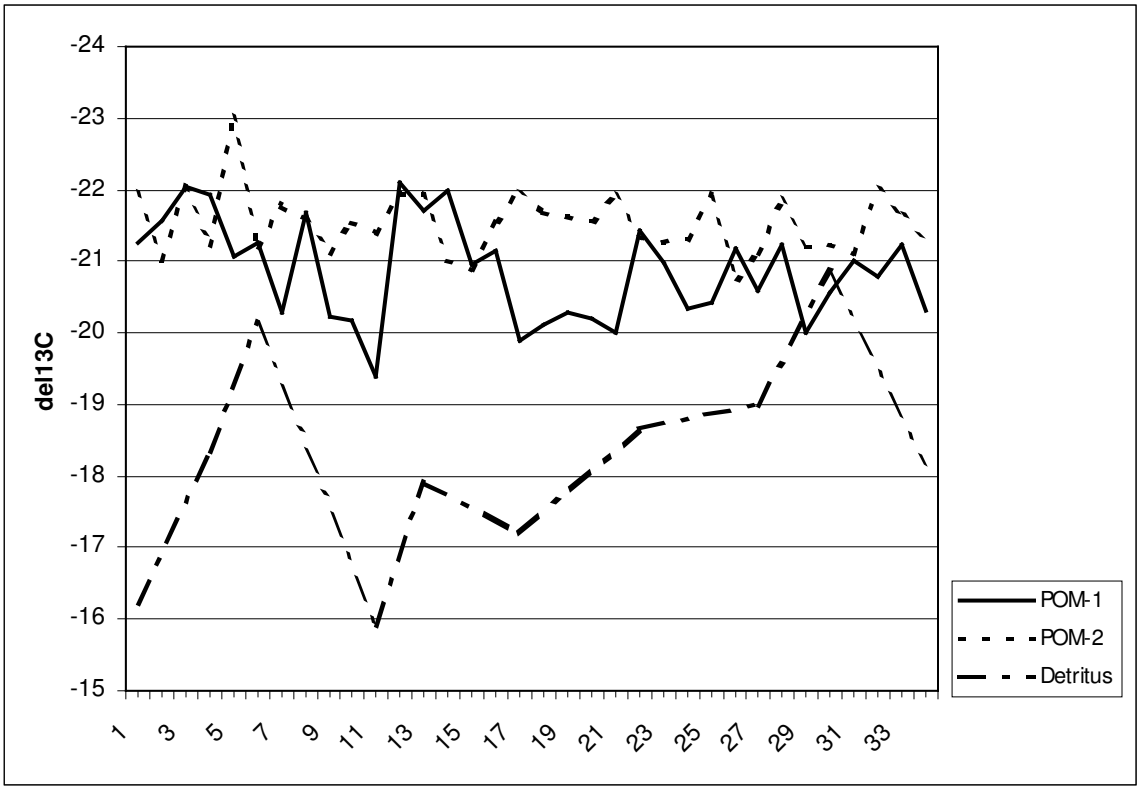

Salinity (parts per thousand)
Temperature (degrees celsius)
Sampling Period
Salinity
Temp
35
34
33
32
31
30
29
28
27
26
25
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35

Figure 3.2. Ammonium concentration for Road Creek, Sapelo Island, Georgia, 2000. Temperature and salinity at primary sampling site.


39.5
39
38.5
38
37.5
37
NH4 (mg/m3)
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
Sample Period

Figure 3.4. Isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) for POM samples and detritus of Road creek, Sapelo Island, Georgia, 2000.


-24
-23
-22
-21
-20
-19
-18
-17
-16
-15
del13C
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
POM-1
POM-2
Detritus

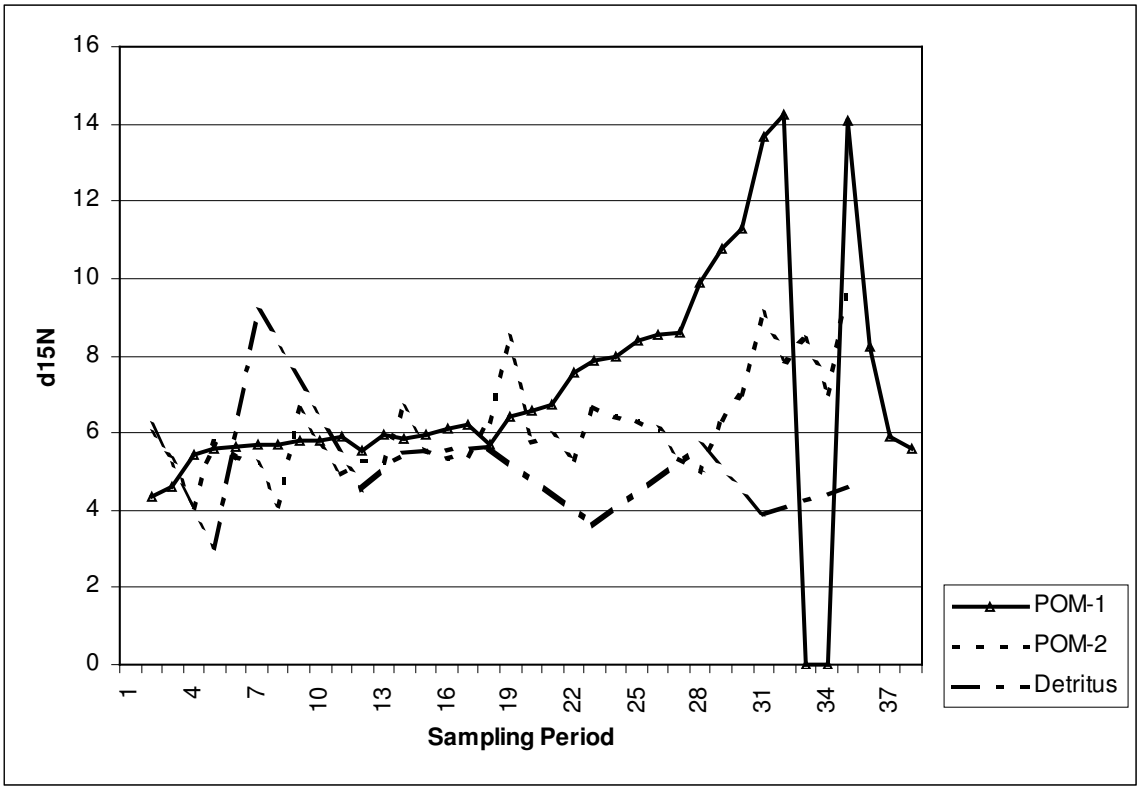
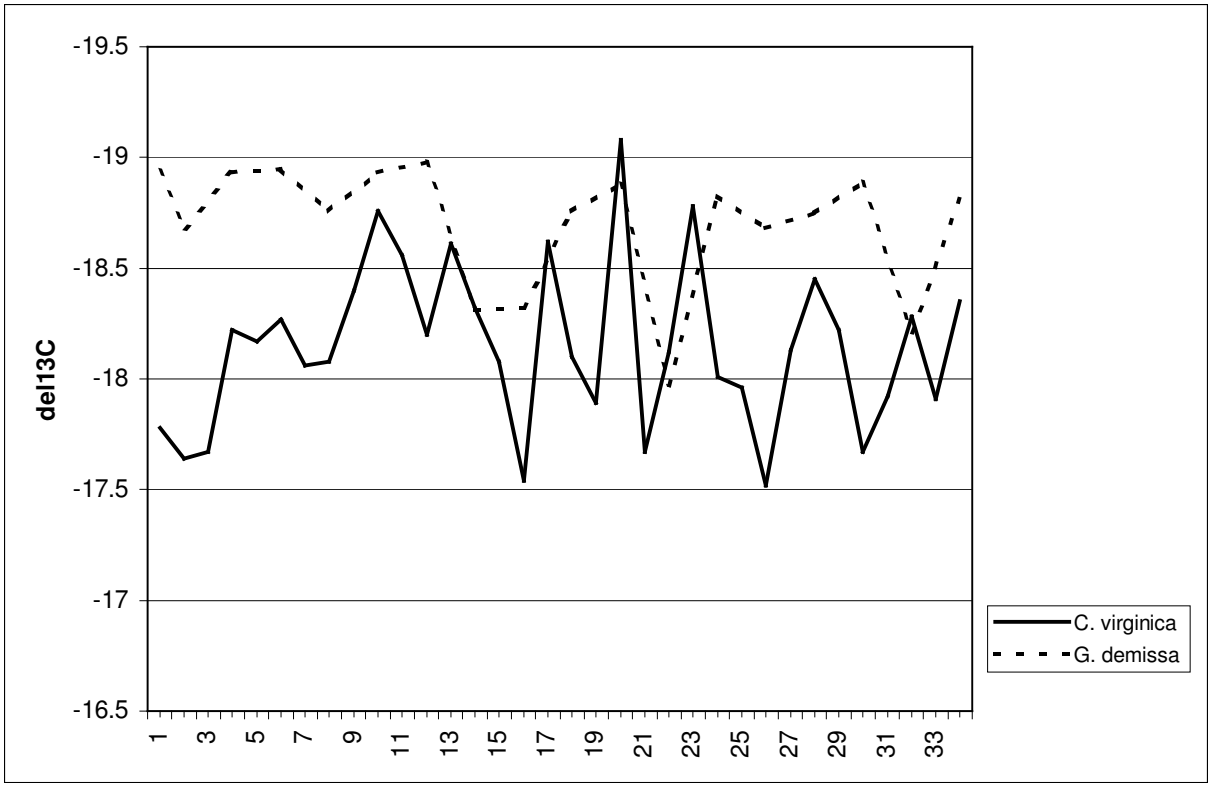

16
14
12
10
8
6
4
2
0
d15N
1
4
7
10
13
16
19
22
25
28
31
34
37
Sampling Period
POM-1
POM-2
Detritus

Figure 3.5. Isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) for suspension feeders of Road creek, Sapelo Island, Georgia, 2000.


-19.5
-19
-18.5
-18
-17.5
-17
-16.5
del13C
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
C. virginica
G. demissa

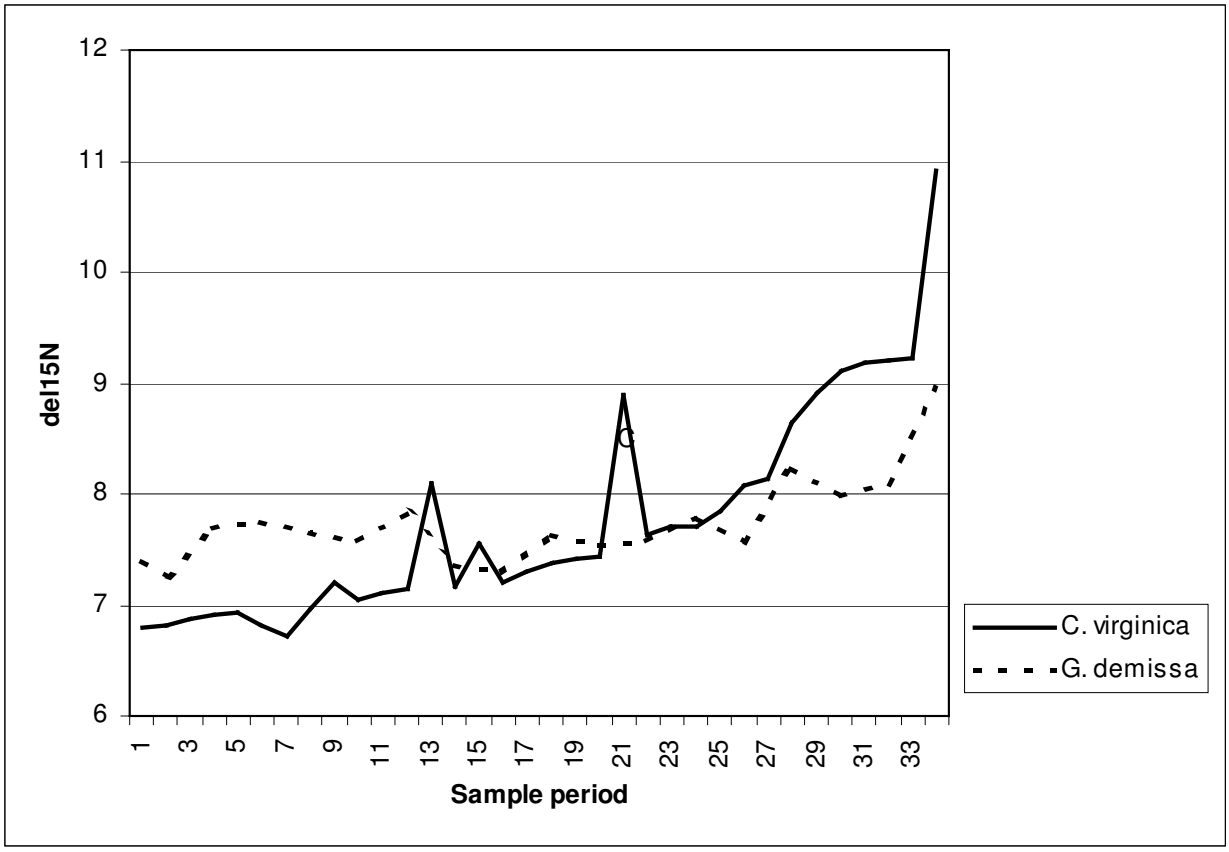
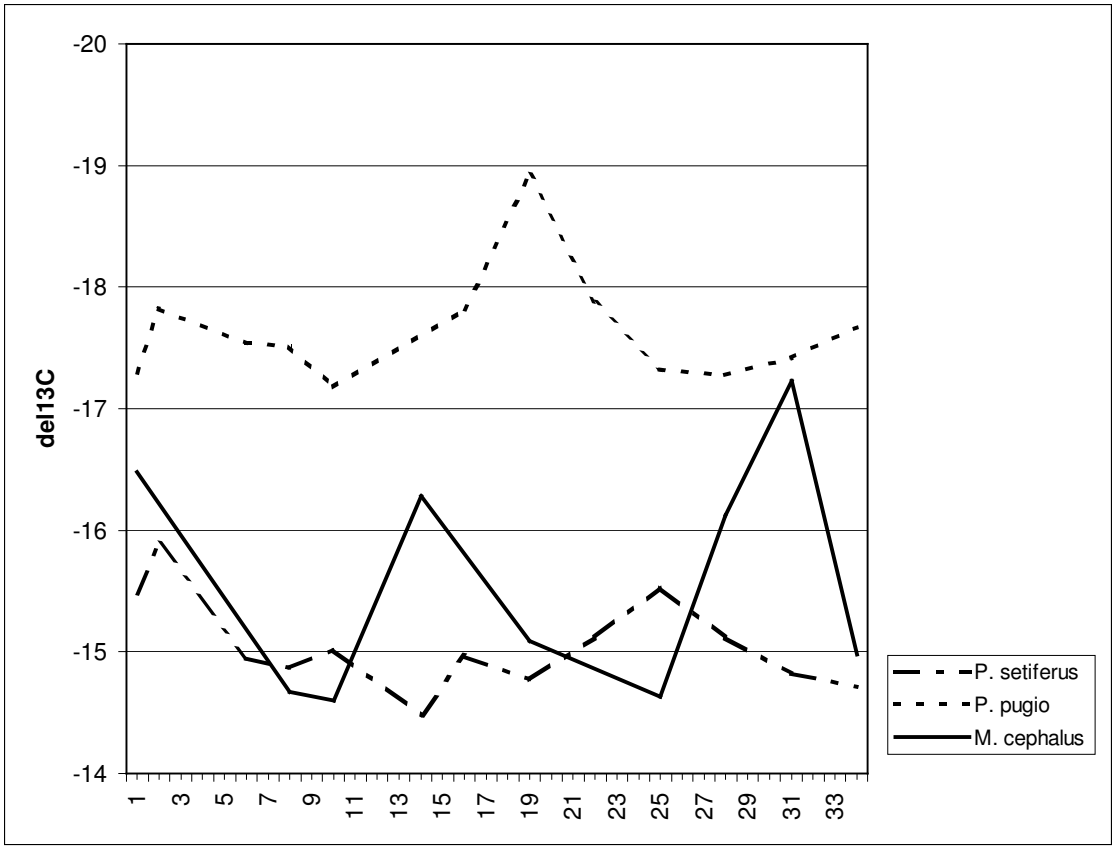

12
11
10
9
8
7
6
del15N
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
Sample period
C. virginica
G. demissa

Figure 3.6. Isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) for deposit suspension feeders of Road creek, Sapelo Island, Georgia, 2000.


-20
-19
-18
-17
-16
-15
-14
del13C
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
P. setiferus
P. pugio
M. cephalus

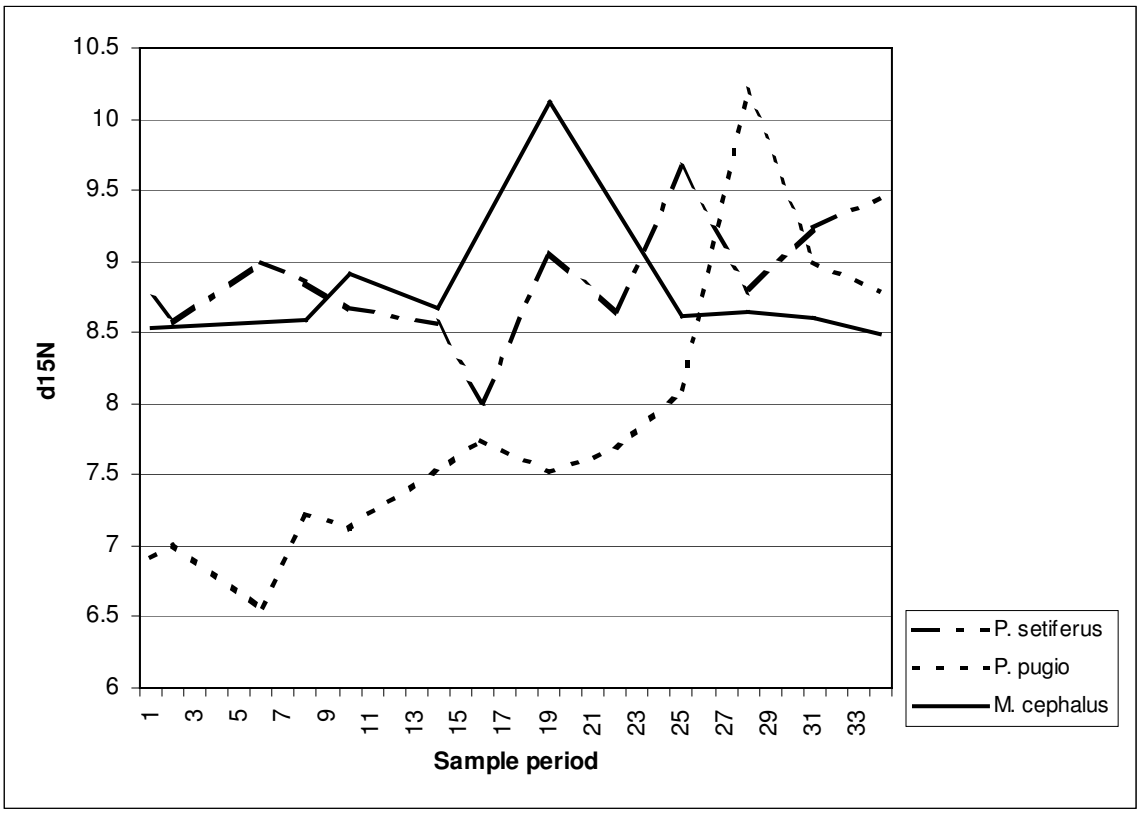
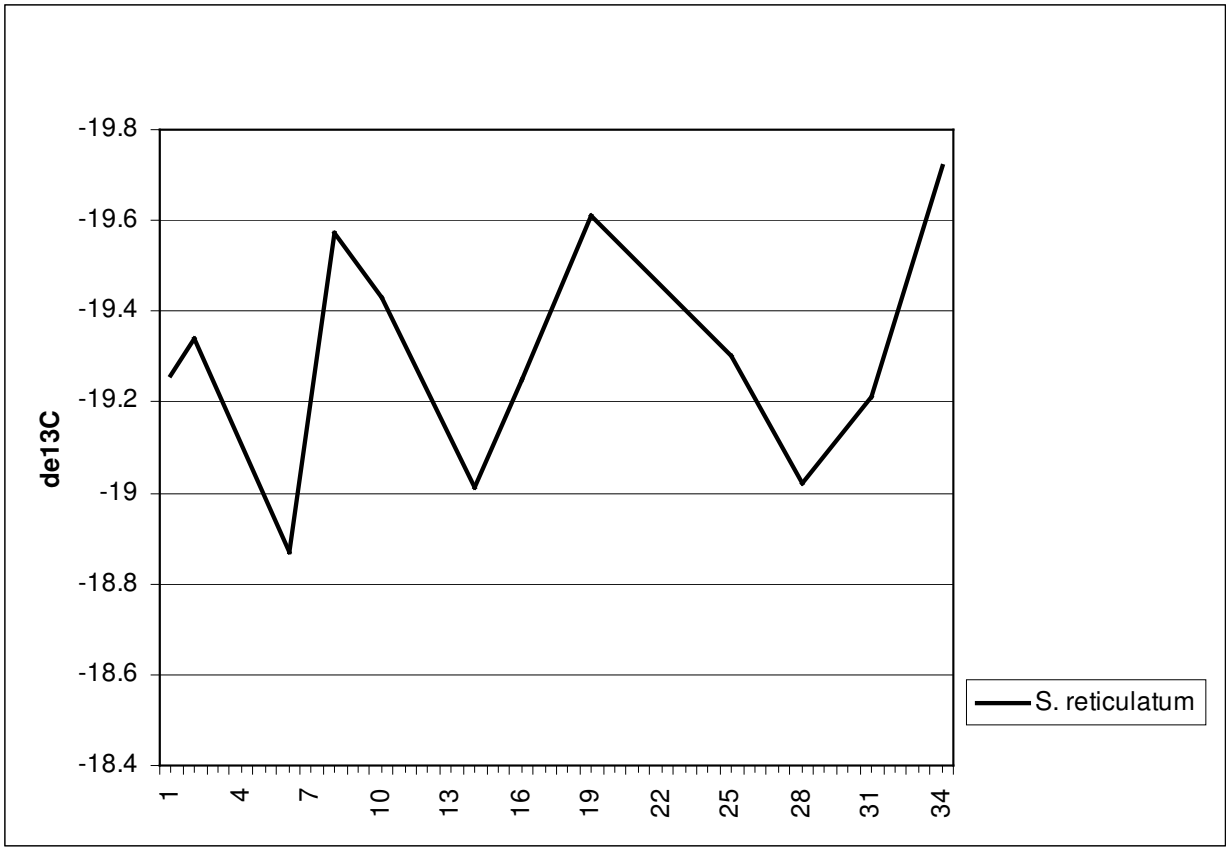

10.5
10
9.5
9
8.5
8
7.5
7
6.5
6
d15N
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
Sample period
P. setiferus
P. pugio
M. cephalus

Figure 3.7. Isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) for deposit feeders of Road creek, Sapelo Island, Georgia, 2000.


-19.8
-19.6
-19.4
-19.2
-19
-18.8
-18.6
-18.4
de13C
1
4
7
10
13
16
19
22
25
28
31
34
S. reticulatum

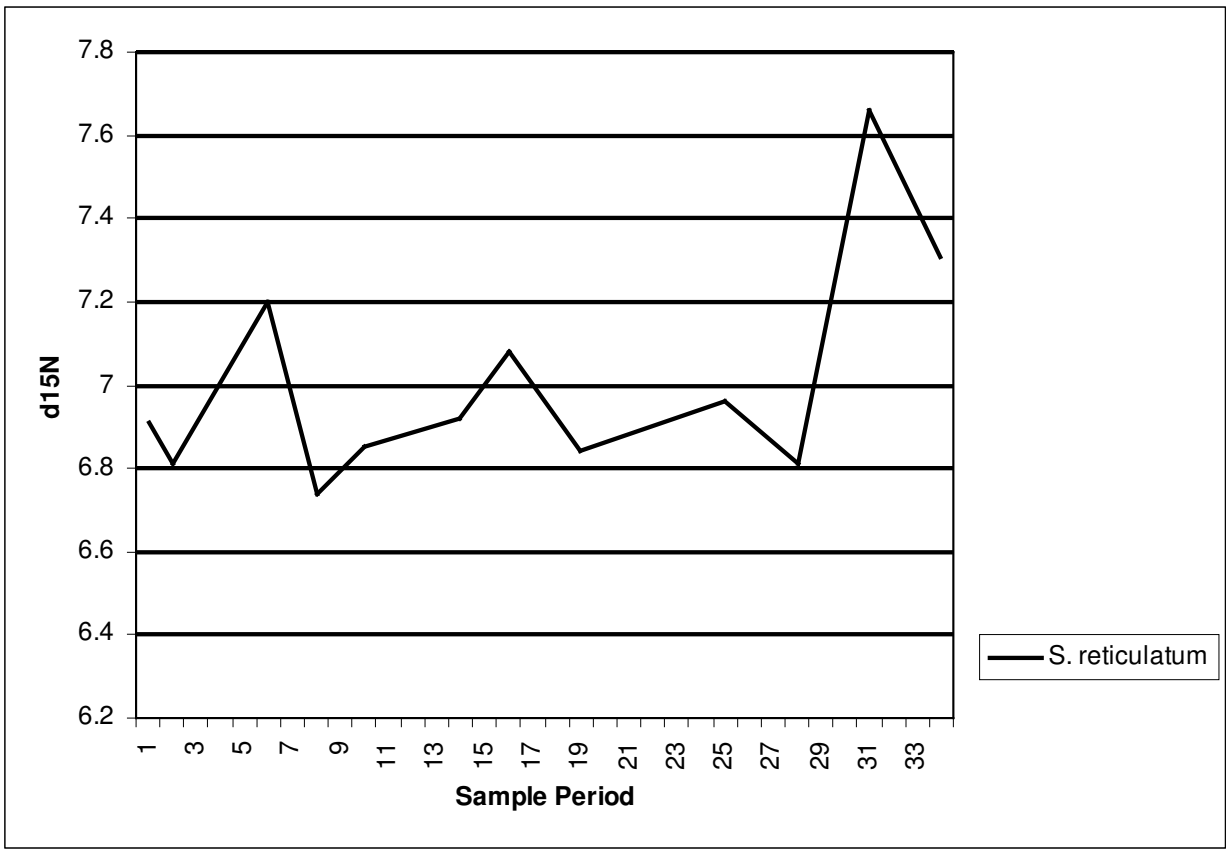
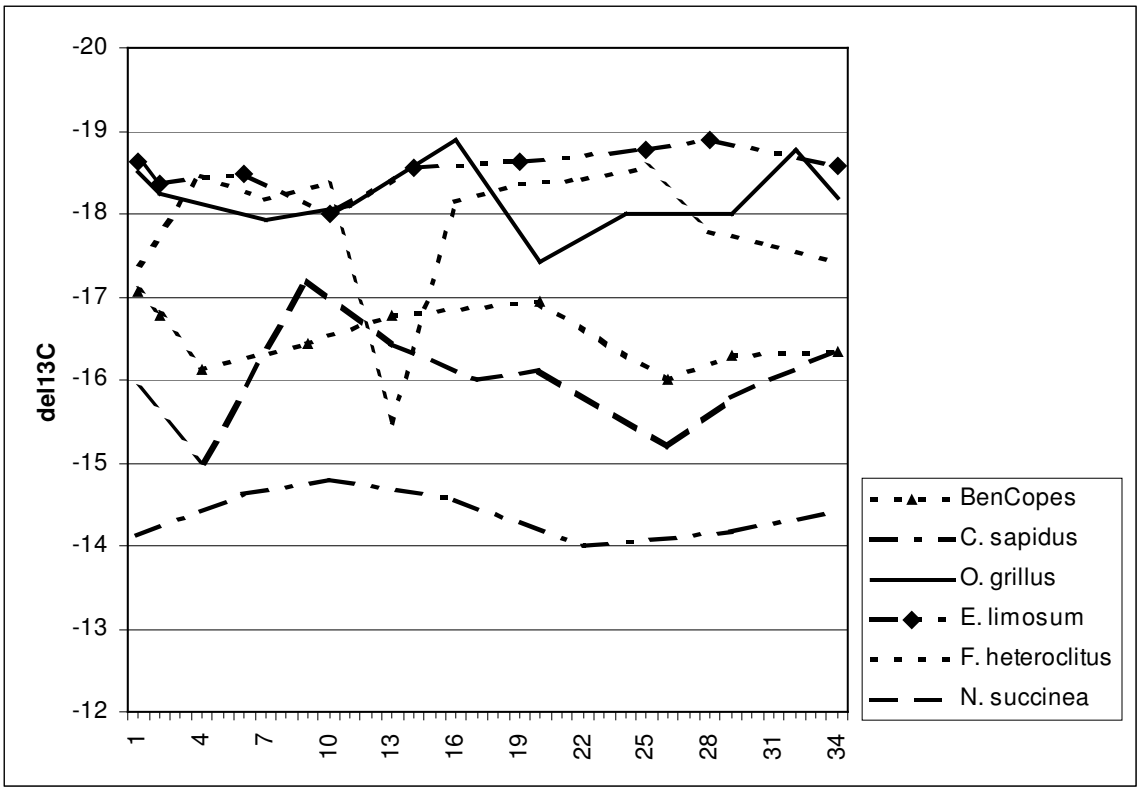

7.8
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
d15N
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
Sample Period
S. reticulatum

Figure 3.8. Isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) for omnivores of Road creek, Sapelo Island, Georgia, 2000.


-20
-19
-18
-17
-16
-15
-14
-13
-12
del13C
1
4
7
10
13
16
19
22
25
28
31
34
BenCopes
C. sapidus
O. grillus
E. limosum
F. heteroclitus
N. succinea

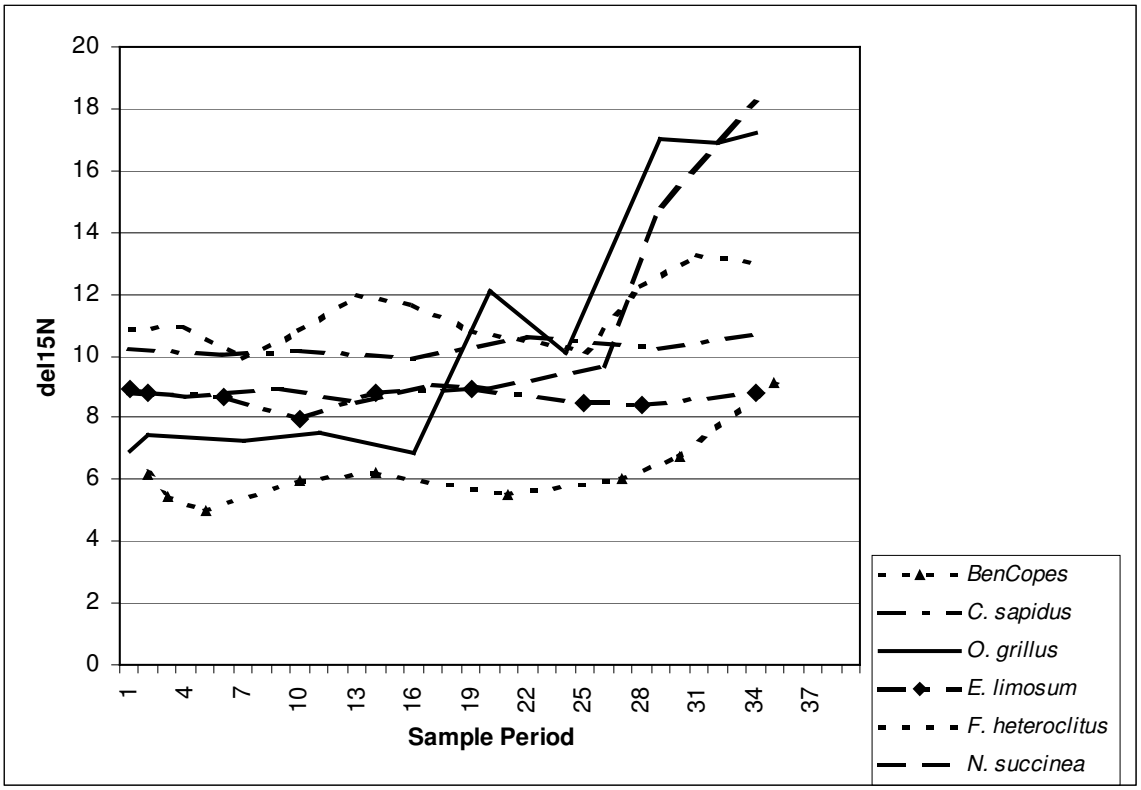
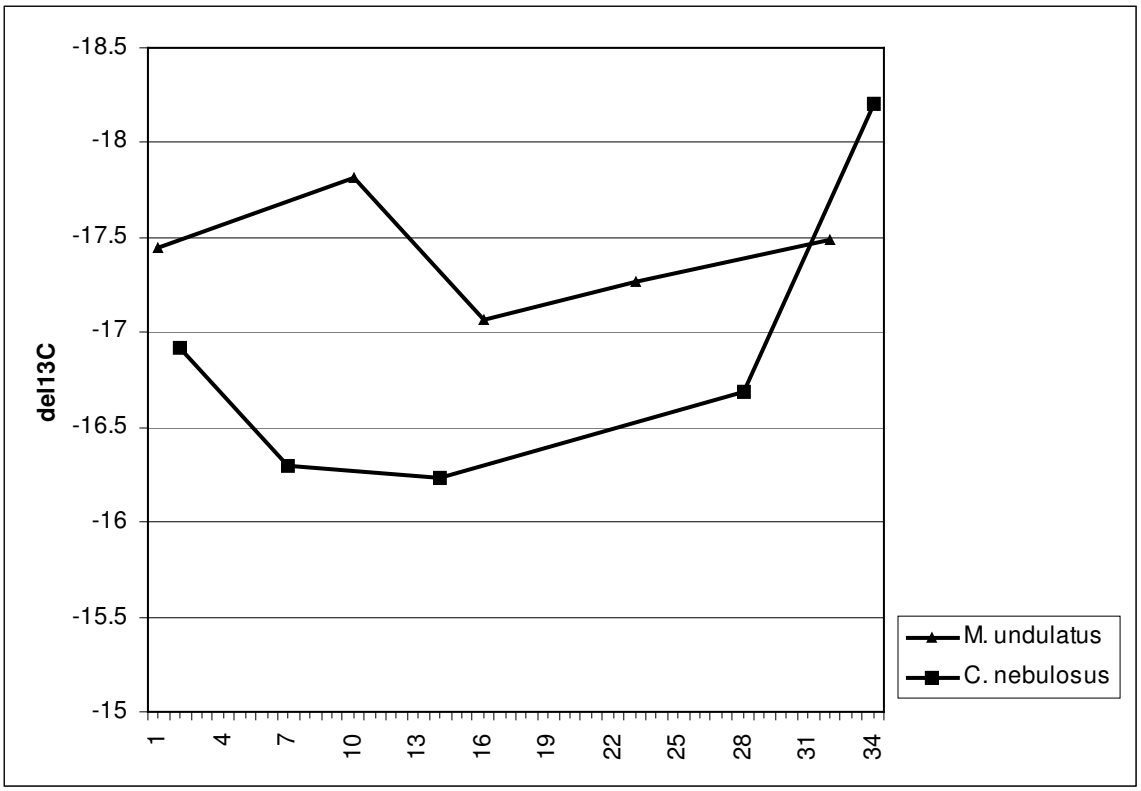

20
18
16
14
12
10
8
6
4
2
0
del15N
1
4
7
10
13
16
19
22
25
28
31
34
37
Sample Period
BenCopes
C. sapidus
O. grillus
E. limosum
F. heteroclitus
N. succinea

Figure 3.9. Isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) for predators of Road creek, Sapelo Island, Georgia, 2000.


-18.5
-18
-17.5
-17
-16.5
-16
-15.5
-15
del13C
1
4
7
10
13
16
19
22
25
28
31
34
M. undulatus
C. nebulosus

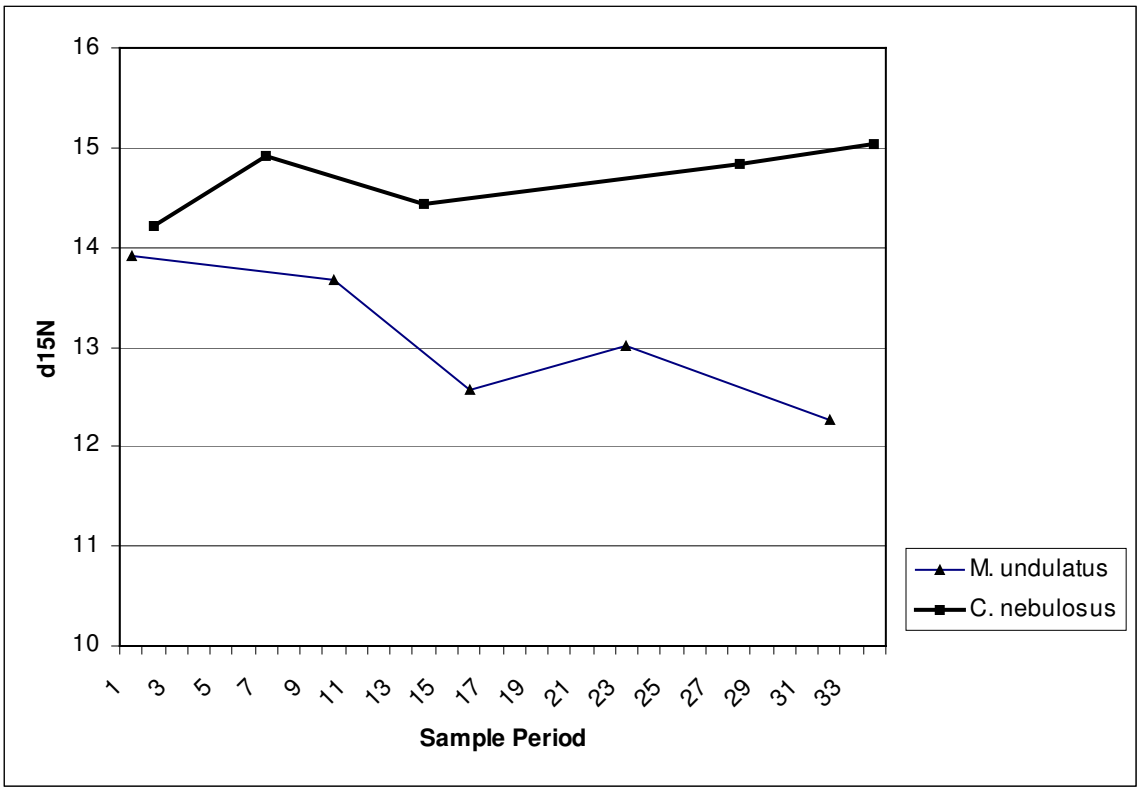

16
15
14
13
12
11
10
d15N
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
Sample Period
M. undulatus
C. nebulosus

Figure 3.10. Isotopic ratios endpoints ($\delta\ ^{15}N$) for producers, POM, and detritus of Road creek, Sapelo Island, Georgia, 2000.

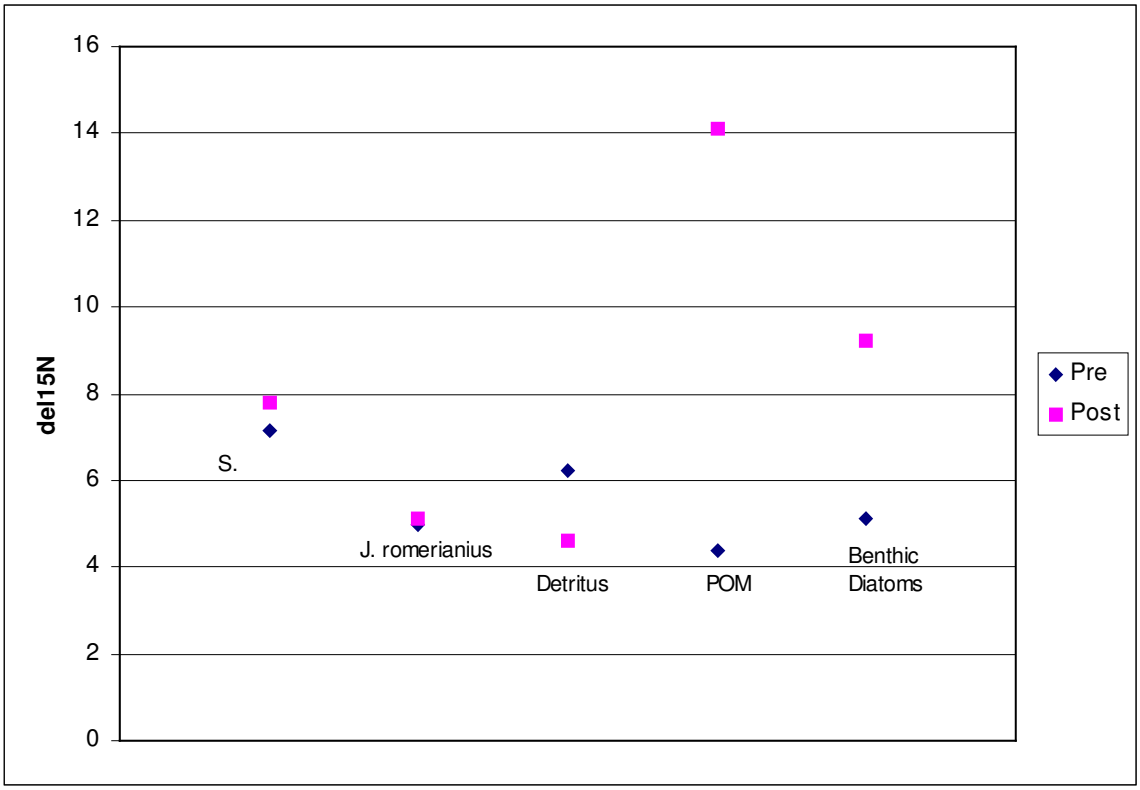

16
14
12
10
8
6
4
2
0
del15N
S.
J. romerianius
Detritus
POM
Benthic
Diatoms
Pre
Post

Figure 3.11. Isotopic ratio endpoints ($\delta\ ^{15}N$) for suspension feeders of Road creek, Sapelo Island, Georgia, 2000.

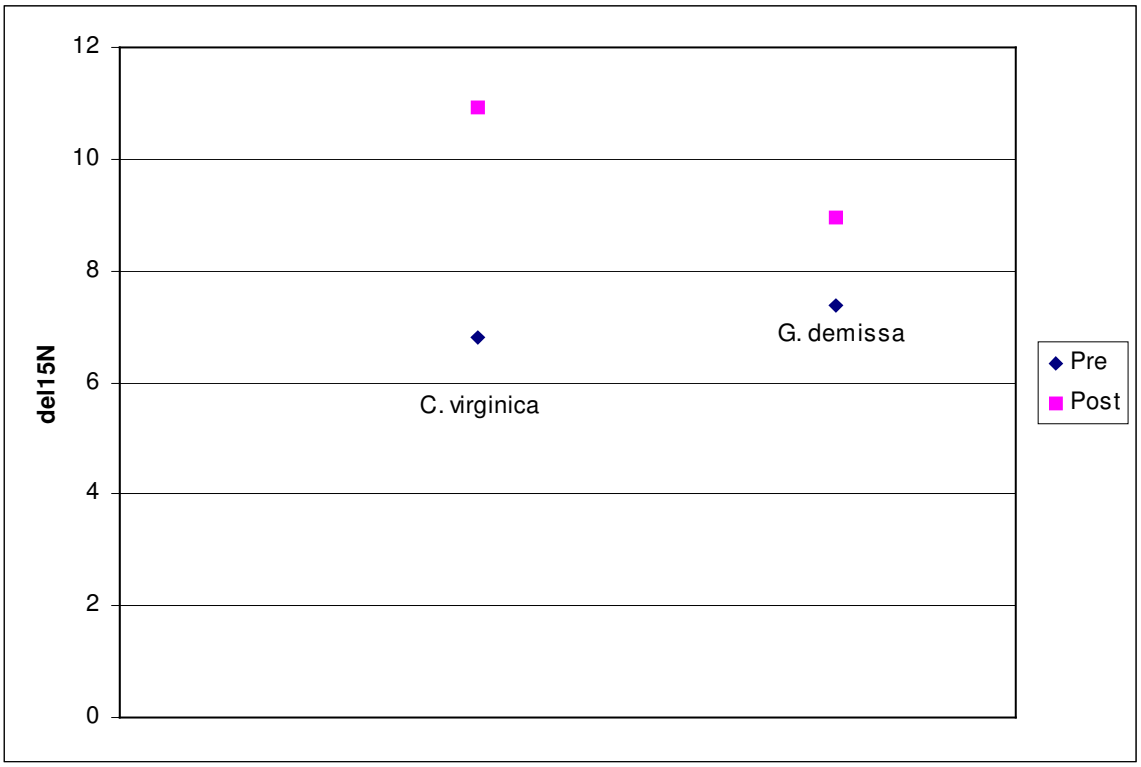

12
10
8
6
4
2
0
del15N
C. virginica
G. demissa
Pre
Post

Figure 3.12. Isotopic ratio endpoints ($\delta\ ^{15}N$) for deposit suspension feeders of Road creek, Sapelo Island, Georgia, 2000.

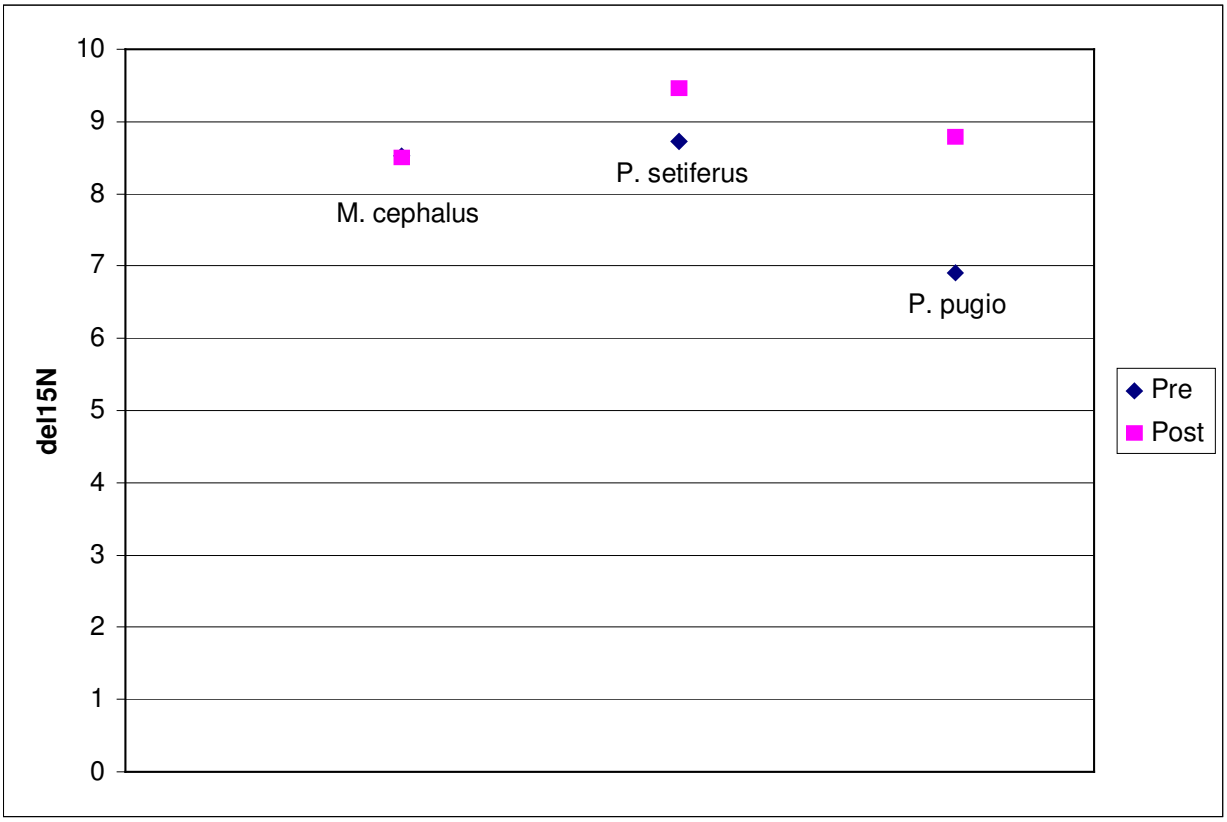

10
9
8
7
6
5
4
3
2
1
0
del15N
M. cephalus
P. setiferus
P. pugio
Pre
Post

Figure 3.13. Isotopic ratio endpoints ($\delta\ ^{15}N$) for deposit feeders of Road creek, Sapelo Island, Georgia, 2000.

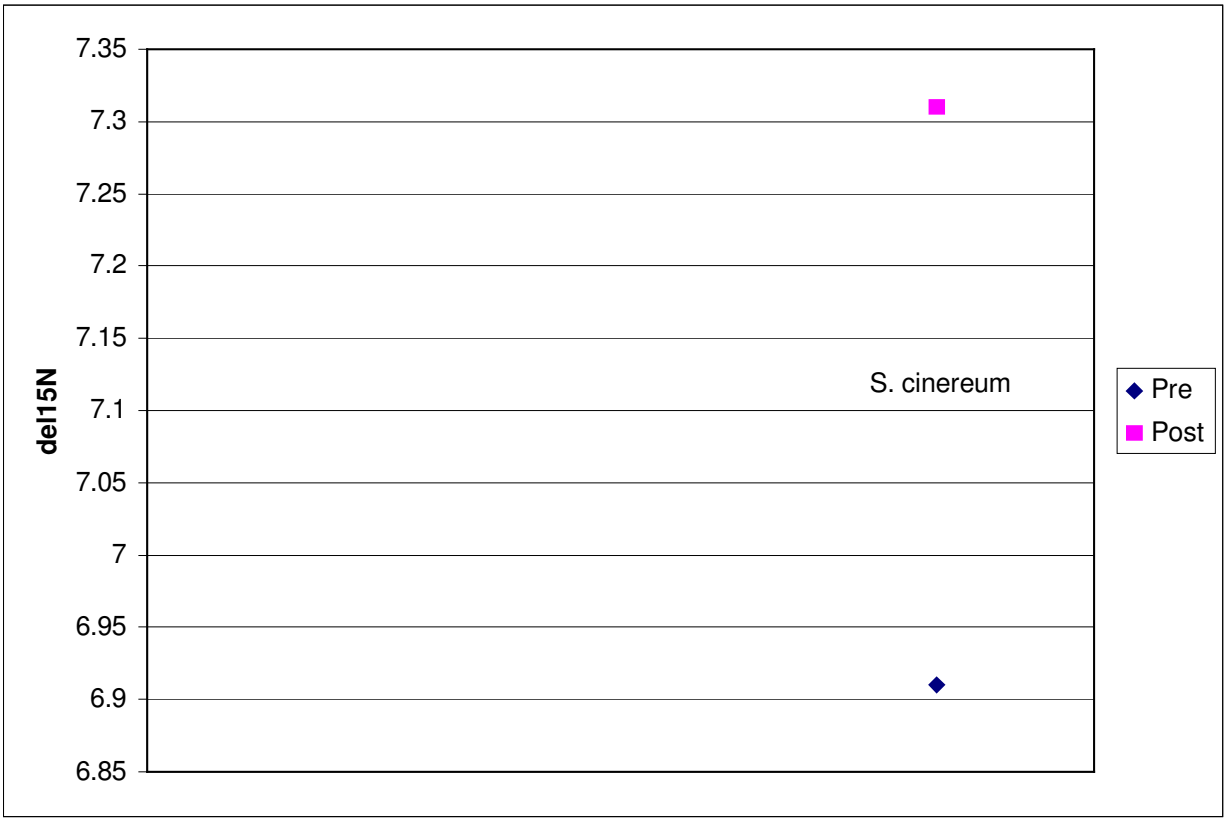

7.35
7.3
7.25
7.2
7.15
7.1
7.05
7
6.95
6.9
6.85
del15N
S. cinereum
Pre
Post

Figure 3.14. Isotopic ratio endpoints ($\delta\ ^{15}N$) for omnivores of Road creek, Sapelo Island, Georgia, 2000.

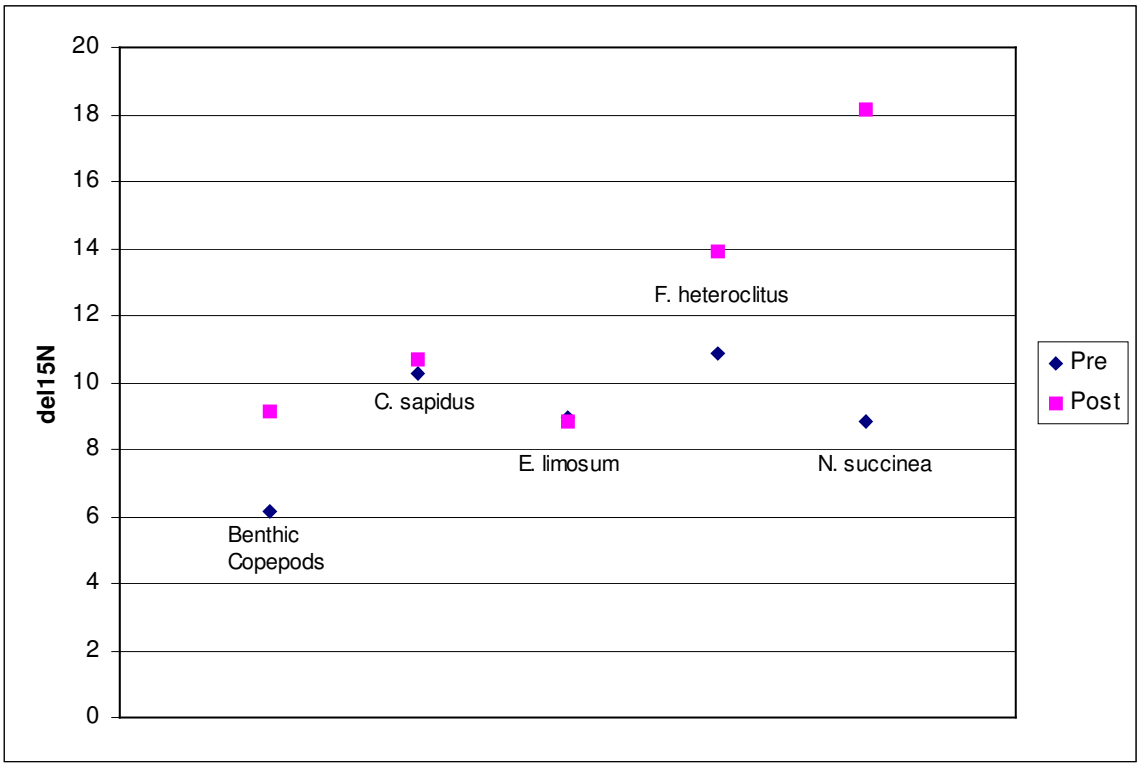

20
18
16
14
12
10
8
6
4
2
0
del15N
F. heteroclitus
C. sapidus
E. limosum
N. succinea
Benthic
Copepods
Pre
Post

Figure 3.15. Isotopic ratio endpoints ($\delta\ ^{15}N$) for predators of Road creek, Sapelo Island, Georgia, 2000.

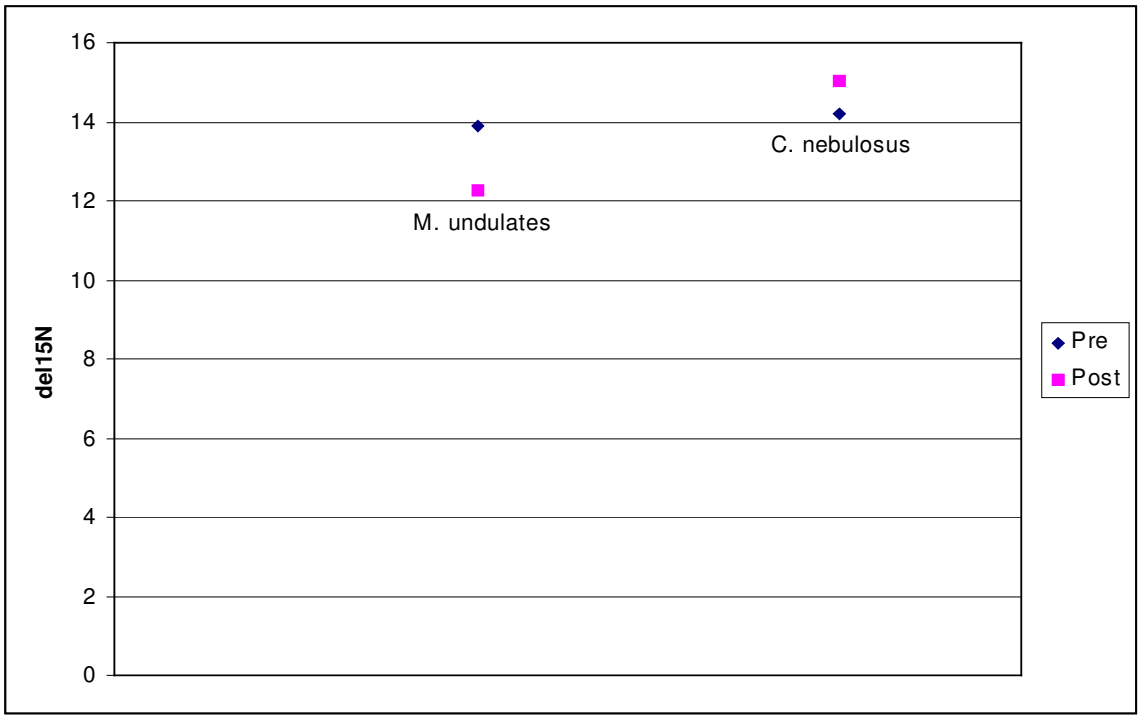

16
14
12
10
8
6
4
2
0
del15N
M. undulates
C. nebulosus
Pre
Post

Figure 3.16. Summary $\delta^{13}C$ data for all compartments.

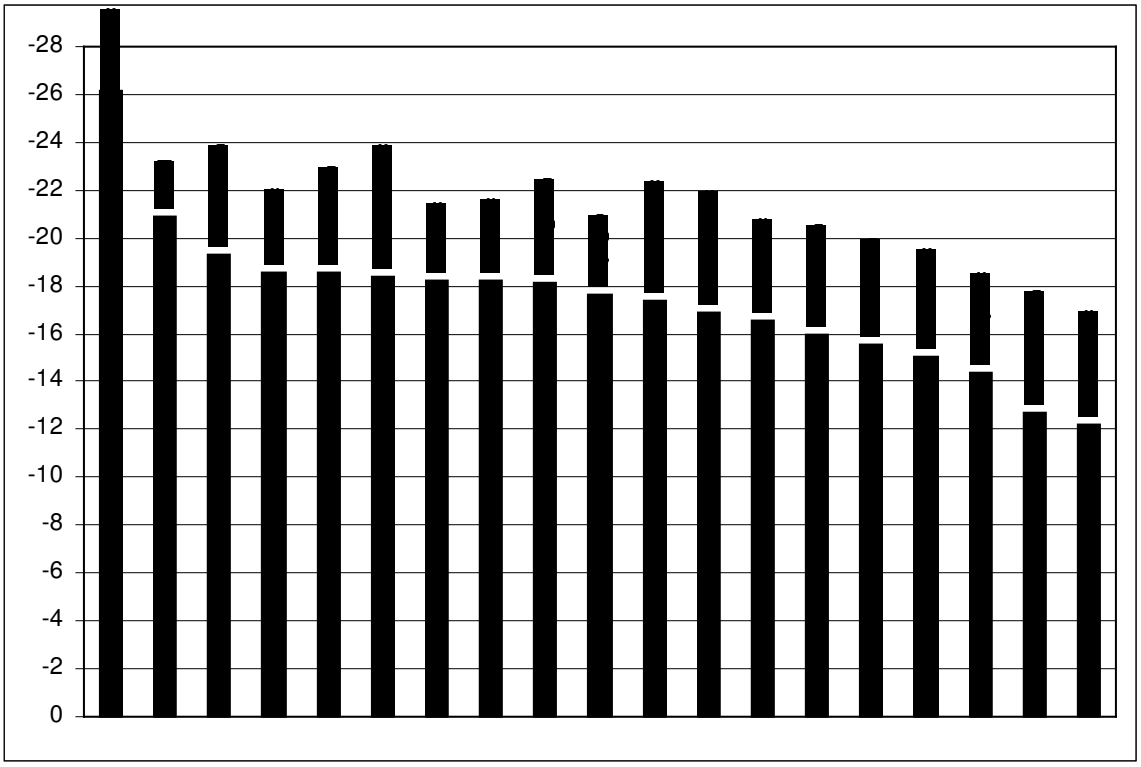
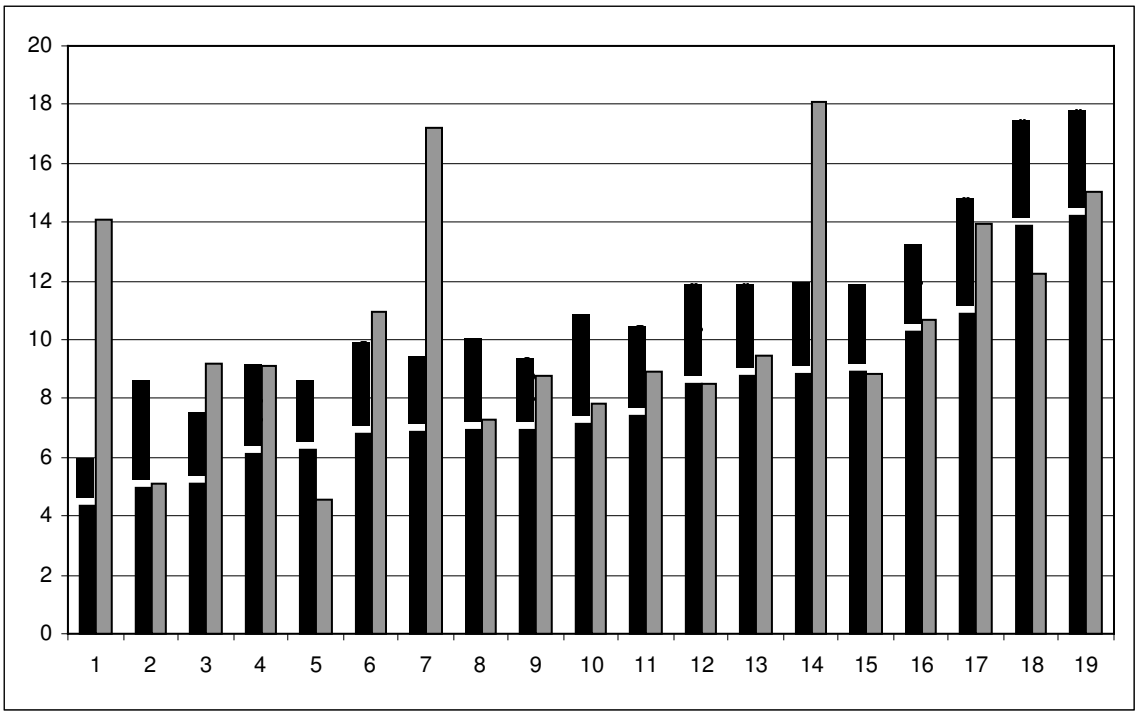

-28
-26
-24
-22
-20
-18
-16
-14
-12
-10
-8
-6
-4
-2
0

Figure 3.17. Summary data for pre- and post-enrichment $\delta^{15}N$


20
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19