An investigation of nutrient transfer in a restored eelgrass, *Zostera marina*, meadow.

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Abstract

The contribution of seagrass primary production to nutrient flow in temperate marine systems is widely debated. The purpose of this work was to ascertain whether eelgrass (*Zostera marina*) comprises a measurable fraction of the diet of consumers in recently restored eelgrass meadows in South Bay, Virginia. Dominant primary producers, consumers, and higher trophic level fish were collected from eelgrass meadows and adjacent barren sediment habitats dominated by ephemeral floating macroalgae. Two methods were employed to identify the nutritional contributions from local primary producers. First, the stable isotope values of primary producers were compared with that of consumers within both site types. Stable isotope analysis gave little indication that consumers were consuming live eelgrass directly. However, it was found that significant differences exist between the isotope values of fish from the eelgrass meadows compared with the unvegetated sites, suggesting that the base primary production sources differ between the two sites. In particular, there was a strong deviation in consumer isotope values between the two sites in the late summer following a major algal biomass crash. The second method employed involved monitoring the transfer of primary producer essential fatty acids to primary and higher level consumers. Two invertebrate species and one fish from eelgrass meadows contained small amounts of seagrass-specific fatty acid

biomarkers. Relatively high levels of bacterial fatty acid markers were noted in all consumers. As bacteria are major arbiters of nutrient recycling in marine systems it is likely that bacteria mediate the transfer of seagrass primary production to higher consumers via decomposition of detrital material. The decomposing matter then enters the local food web through detritivores.

This study has identified direct and indirect food-web related effects related to restoration of eelgrass to South Bay. The structural presence and seasonal persistence of eelgrass in South Bay has indirectly influenced overall primary production utilization by consumers. While the direct dietary importance of live eelgrass matter appears to be very limited, there are indications that a few species assimilate eelgrass-derived dietary fatty acids. Assimilation of additional eelgrass matter is also likely via ingestion of detritus and associated microbiota.

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I. Chapter 1: Introduction.

1.1. History of eelgrass in the Chesapeake Bay region

The Chesapeake Bay is the largest estuary in the United States, stretching from Virginia to northern Maryland and is home to over 3600 species of plants and animals. The extensive littoral zone and wide salinity range supports many different types of submerged aquatic vegetation (SAV). Freshwater from large rivers such as the Susquehanna mixes with tidal inputs of seawater from the mouth of the estuary, resulting in waters that range from fresh to brackish to saline. Few species are able to tolerate this full spectrum of salinities and are range-limited within the Bay. Fresh and brackish water species such as *Potamogeton perfoliatus* (redhead grass), are restricted to the upper and middle portions of the estuary. In contrast, the more salt-tolerant species such as *Zostera marina* L. (eelgrass) are more prevalent to the lower reaches of the Bay and the coastal embayments where salinities are closest to that of seawater (Orth and Moore, 1984).

Submerged aquatic vegetation in the Chesapeake Bay region has been recognized as a valuable resource for many decades, which has resulted in widespread efforts to understand and protect these plants. The National Marine Fisheries Service designated seagrass beds as an "Essential Fish Habitat," resulting in efforts to restore seagrass to locations where it had been historically

recorded. The restoration efforts in the southern Delmarva coastal bays are considered successful, having survived and thrived for nearly a decade. The remaining seagrass meadows in the Chesapeake Bay and Delmarva coastal bays are still under threat from anthropogenic effects, mainly algal blooms caused by increased nutrient runoff from local agricultural activities and physical damage due to fisheries practices such as clam dredging (Orth *et al.*, 2006a).

Seagrass beds sustain complex floral and faunal communities that fill several roles critical to ecosystem function. The extensive root and rhizome systems that anchor the plants to the seafloor form an interconnected net that binds the soft sediments and prevents erosion (De Leeuw *et al.*, 1995). The roots and rhizomes also take up the majority of the assimilated nutrients from the sediments (Clarke & Kirkman, 1989, Mann, 2000). The seagrass leaves, meanwhile, dampen wave speed, resulting in deposition of sediment and organic matter which becomes available to local organisms (Kikuchi, 1980; Hemminga and Duarte, 2000). By decreasing wave action along coastlines, seagrass meadows also decrease beach erosion.

These systems maintain high productivity rates and export large quantities of energy and nutrients to coastal food webs, providing the base structure for a diverse assortment of organisms (Fry and Parker, 1979; Kikuchi, 1980; Mann, 1982). The shoots serve as an anchor for epiphytic flora and fauna while the dense canopy provides protection for both indigenous and larval or

juvenile fishes (Harris *et al.*, 2004). Extensive root and rhizome structures anchor beds to the soft sediments and provide the majority of nutrients necessary for growth (Clarke and Kirkman, 1989; Mann, 2000).

Eelgrass was abundant in the Delmarva coastal bays and Chesapeake Bay in the early 1900s. The rapid decline of eelgrass populations in the western Atlantic that started in the 1930's caused lasting changes to these fragile ecosystems (Short *et al*., 1988). In 1933 a combination of a major storm in August and a disease resulted in the disappearance of eelgrass from the coastal bays and substantial reduction in eelgrass abundance in the Chesapeake Bay (Vergeer *et al*., 1995; Orth *et al*., 2006a, 2010). While recovery of eelgrass was reported in the Chesapeake Bay and the northern coastal bays of the Delmarva Peninsula, eelgrass did not recover in the southern Virginia coastal bays. The discovery of a small natural patch in South Bay in the late 1990s was followed initially by small-scale experiments with eelgrass seeds and adult plants. Further large-scale, seed-based restoration in 2001 and 2002 resulted in the establishment of a substantial eelgrass population in South Bay by 2004.

The widespread disappearance of eelgrass meadows dramatically altered the Chesapeake Bay region. Most notably, numerous species dependent on eelgrass during one or more life-stages suffered population losses throughout the Bay area (Orth *et al*., 2006a, 2006b; Fonseca and Uhrin, 2009). For instance, the bay scallop (*Argopecten irradians*) population in the Chesapeake Bay has not

returned to commercially harvestable levels since the eelgrass decline in the 1930's (Fonseca and Uhrin, 2009). In addition, shorelines were subjected to increased sediment erosion following the loss of the sediment-stabilizing effects provided by the seagrass leaves and root-rhizome systems (Rasmussen, 1973). In many locations floating macroalgae eventually became the dominant macrophyte in subtidal areas previously populated by eelgrass meadows.

1.2. Virginia Coast Reserve

South Bay is a shallow coastal lagoon on the eastern shore of Virginia, which lies within the Virginia Coast Reserve Long-Term Ecological Research (VCR-LTER) site. The VCR LTER program is a large-scale, multi-disciplinary project that encompasses long-term monitoring and experiments as well as short-term process-level studies and modeling. Since the inception of the program in 1987, VCR scientists have investigated ecosystem patterns, processes and interactions at multiple spatial and temporal scales.

The VCR itself is a dynamic coastal system comprising numerous barrier islands, shallow lagoons, tidal marshes, mudflats, and tidal creeks. The 14000 ha reserve was established in 1970 by the Nature Conservancy. The barrier islands are currently uninhabited, though the islands once supported a prosperous fishing and farming community. In addition to long-term environmental changes, the entire system is frequently subjected to short-term disturbances, mainly storms.

A main part of the synthesis effort of the VCR LTER program is to understand the trophic consequences of a state change in order to gain the ability to predict the consequences of future state changes. Thus, the return and expansion of eelgrass as a 'foundation species' has become a fundamental component of the current VCR program. The ecosystem-level effects of a rapid state change back to an eelgrass-vegetated state will be monitored from the outset.

Few large-scale studies have been undertaken which have continued to monitor the development of a restored eelgrass system following successful recolonization. Thus very little is known about the impact of restoration on fish and invertebrate communities, especially in regards to the nutritional status of local fish and invertebrate communities. To that end, the primary objective of this study is to determine whether eelgrass tissues comprise a measurable fraction of consumer diets in South Bay.

1.3. Questions and hypotheses

Although the scientific community in general acknowledges that seagrass meadows provide numerous ecosystem services, media outlets and published reports generally focus on coral reefs, mangroves, marshes, and tropical forests which are less widely distributed worldwide (Orth *et al.*, 2010). Tidal salt marshes have received much attention in temperate regions; many scientific studies of recent decades have examined the contribution of salt marsh plants

such as *Spartina alterniflora* as major carbon sources to temperate estuaries (Boesch and Turner, 1984; Peterson and Howarth, 1987; Kwak and Zedler, 1997; Weinstein and Kreeger, 2000). Early works posited large-scale transport of salt marsh primary production, generally as detritus, to nearby estuarine waters, a pattern that has been referred to as the 'Outwelling Hypothesis' (Taylor and Allanson, 1995). Further studies found that some marshes showed net importation of carbon, while others showed no net exchange (Weinstein and Kreeger, 2000). Therefore, it has become clear that identifying and when possible quantifying the various sources of organic matter is essential to understanding nutrient dynamics in estuarine systems (Kwak and Zedler, 1997; Childers *et al.*, 2000).

Eelgrass has the potential to affect the nutritional status of consumers by direct as well as indirect means. Consumers may rely on live and/or detrital fragments of eelgrass as a direct dietary source. On the other hand, eelgrass may indirectly affect consumer diets via recruitment of diverse species into the meadows. In general, previous studies have found that eelgrass meadows support numerically greater and more species-rich consumer populations than nearby sites lacking attached macrophytes (see Beck *et al.*, 2001 and Heck *et al.*, 2003 for reviews). Secondary and higher level consumers benefit from the greater number and variety of prey items available.

I sought to address the long-standing subject of energy transfer in eelgrass systems by tracing the transfer of eelgrass primary production into the food web of a temperate coastal ecosystem. The information gathered will be used to assess the nutritional importance of eelgrass productivity to the local ecosystem in South Bay, Virginia. The two overarching questions that will be addressed seek to determine whether *Zostera marina* provides a measurable influence on the nutritional status of organisms in the restored meadows. The two questions are as follows:

1. Does the presence of eelgrass habitat affect the dietary sources utilized by consumers in restored *Zostera marina* meadows?

2. Are South Bay consumers directly ingesting and assimilating live and/or detrital eelgrass tissues?

The first question focused on eelgrass as a structural habitat and was addressed from two angles. First, the stable isotope values of consumers captured in the restored eelgrass meadows were compared with individuals of the same species captured in adjacent eelgrass-free sites in order to assess potential differences in nutritional sources. I hypothesized that the diets of consumers captured in restored sites differed from those of consumers of the same species from the eelgrass-free sites. Second, the dietary implications of the continuous structural presence of eelgrass meadows were evaluated. Floating, ephemeral macroalgae experienced a mid to late summer die-off throughout

South Bay during the 2005 season, so that little or no live macroalgal biomass remained within the system by early fall. I hypothesized that the persistence of eelgrass provided stability to consumer nutritional sources relative to eelgrass-free sites that were dominated by ephemeral primary production sources. Regarding the second question, above, I hypothesized that eelgrass primary production comprised a measurable dietary component of consumer diets.

A second, naturally-populated eelgrass meadow believed to be more than ten years old was discovered adjacent to Fisherman's Island at the mouth of the Chesapeake Bay. It is one of few known natural meadows along the lower seaside coast of the Delmarva Peninsula and is the closest in terms of distance to South Bay. The eelgrass meadow at Fisherman's Island is likely subject to high nutrient loading from the eutrophic waters of the Chesapeake Bay, while the restored meadows lie in a relatively oligotrophic lagoon. Consumers from the natural site were compared with those from the restored meadows in South Bay to determine whether consumers utilized different nutrient sources at the two sites and whether evidence of high nutrient loading from the Bay could be detected in consumers at the Fisherman's Island site.

Two approaches were employed to explore the study questions. The first analyzed nutritional sources of carbon, nitrogen, and sulfur in consumer tissues via stable isotope analysis. This method was used to create a general characterization of the food web in South Bay restored eelgrass beds. The bulk

isotope values obtained helped to identify those species that were most likely to obtain a measure of nutrition from the eelgrass itself. The second approach investigated the sources of specific compounds in consumer diets, namely fatty acids. Fatty acids are ubiquitous dietary compounds that are required by organisms for normal, healthy development (Bell *et al.*, 1986; Sargent *et al.*, 1999) and thus are potentially useful indicators of nutritional sources.

1.4. Potential pathways of eelgrass utilization by consumers

There is a high degree of interconnectedness in the food webs of coastal marine systems. Two major pathways of nutrient transfer and utilization, one based on live material and the other on dead and decomposing material, combine and allow for the recycling of nutrients within the system. A third pathway, not addressed here, is via the excretion of dissolved organic compounds by eelgrass, which are then assimilated by microorganisms.

The flow of nutrients and energy within an ecosystem is often shown visually through the linkages of a food web. A food web is a network of energy and matter flow that links plants with the herbivores and omnivores that consume them, and so on to upper level predators. The food web depicted in Figure 1.1 is a hypothetical food web of a coastal marine ecosystem. Autrotrophs and detrital material comprise the base materials utilized by primary and higher level consumers. In the South Bay system autotrophs include phytoplankton, benthic and epiphytic microalgae, macroalgae, and eelgrass. Detritus consists of

all of the non-living organic matter, from fragments of plant matter to fecal pellets and dead animal remains, combined with the microbial community that colonizes and decomposes the organic matter. Invertebrate grazers, zooplankton, detritus grazers, and a few fish and birds that eat macrophytes comprise the primary consumers responsible for transferring new and recycled autotrophic energy into the heterotrophic food web. Secondary and higher-level consumers include invertebrate predators, omnivorous and carnivorous fish, and birds.

For simplicity, a few minor aspects of this food web have been omitted or simplified. For example, detrital excretory material is not shown to add to the dissolved nutrient pool. Also, no pathways show that some species of fish, zooplankton and invertebrates feed upon other species in the same category.

Eelgrass ecosystems are generally very productive, with large quantities of plant and animal biomass created and contained within the system (Deegan *et al*., 2002; Mateo *et al*., 2006; Jaschinski and Sommer, 2008). While it is relatively rare that animals eat live eelgrass, it is not unheard of, and there is a precedent on the Atlantic coast of the United States. Pinfish (*Lagodon rhomboides*), which are common in South Bay, are known to be omnivorous and have been captured with fragments of eelgrass blades in their stomachs (Montgomery and Targett, 1992). Members of the isopod genus *Idotea* are also known eelgrass grazers (Williams and Ruckelshaus, 1993; Thom *et al*., 1995). Still, though eelgrass meadows contribute a large proportion of the primary productivity of coastal

ecosystems only a very small proportion of live production is believed to enter the food web, while the majority becomes detritus (Kikuchi, 1980; Vähätalo and Søndergaard, 2002). Nutrient recycling by microbial decomposers becomes a very important process in such a system for making previously fixed nutrients available to plants. Without decomposers to free up nutrients bound in dead organic matter, plant productivity rates would likely be far lower.

Many researchers assert that, at least in most temperate systems, the majority of macrophyte carbon enters marine food webs through the detrital pathway (Fry and Parker, 1979; Nichols *et al*., 1985; Thresher *et al.*, 1992; de Leeuw *et al*., 1995). Thus, the majority of the scientific literature regarding the dietary importance of eelgrass tissue emphasizes microbially-based decomposition and utilization of detritus (Stevenson, 1988; de Sylva, 1975). These studies indicate that the role that eelgrass primary production plays as a detrital organic matter contributor is far more important than the minor input made of live material to local food webs (De Leeuw *et al.*, 1995; Klumpp *et al.*, 1989; Wiedemeyer and Schwamborn, 1996; Vähätalo and Søndergaard, 2002).

1.5. Stable isotopes in ecosystem studies

Stable isotopic analysis is an invaluable tool for delineating food web structure and distinguishing consumer diets in aquatic systems. Assessing the relationship between the natural stable isotopic composition of marine plants and animals to examine food webs is relatively simple and straightforward

(Haines and Montague, 1979). Natural abundances of stable isotopes in the tissues of consumers reflect that of assimilated food (DeNiro and Epstein, 1978; 1981). The relationship varies somewhat between species, though in general, consumer tissues have a greater percentage, or enrichment, of the heavier isotopes of carbon and nitrogen owing to fractionation during metabolism (Hoefs, 1997; Focken and Becker, 1998). The trophic enrichment in isotopic composition can provide information on nutritional sources, food web structure, and the trophic levels of different species within an ecosystem (Vizzini and Mazzola, 2004). Sulfur isotopic values of organisms, on the other hand, generally approximate the values of the sulfur sources (Hoefs, 1997, Peterson, 1999). Consequently, patterns in marine plant-herbivore interactions have been identified and interpreted using bulk stable isotopic analyses (e.g. Haines and Montague, 1979; Vizzini and Mazzola, 2004).

Stable isotope values of bulk tissues of coexisting primary producers in South Bay vary considerably. There are two main reasons for the broad ranges. The first is that different species obtain nutrients from different source pools with different isotopic compositions. For instance, marine and saltmarsh plants that root in reduced sediments have been shown to incorporate δ^{34}S-depleted porewater sulfides (Peterson *et al*., 1986; Fredericksen *et al*., 2006) or the similarly δ^{34}S-depleted oxidized sulfur species formed from sulfides near the root surface into their tissues in addition to seawater-derived sulfate through the leaves (Fry

et al., 1982). Sedimentary sulfides tend to hover near -10 per mil (‰) (Michener and Schell, 1994); therefore, the sulfur isotope values of these plants are largely depleted compared to free-floating algae. Floating macroalgae are limited mainly to the seawater sulfate (+20 to +21 ‰) present in the water column (Fry *et al*., 1982; Kharlamenko *et al*., 2001; Connolly *et al.*, 2004). The second source of variation is the extent of fractionation that occurs during the uptake and metabolism of nutrients. The magnitude of the fractionation varies, based on the ratio of incorporation versus loss of the heavier isotope during synthesis and catabolism and is dependent on several factors including, but not limited to, nutrient limitation, metabolic pathway, and temperature.

Plants tissues generally have a lower $^{13}C/^{12}C$ ratio than that of their carbon source owing to discrimination against ^{13}C (fractionation) uptake during photosynthetic and metabolic processes. Different photosynthetic pathways result in different fractionation. CO_2 in the atmosphere is the main carbon source available to terrestrial plants and is fairly well-mixed, resulting in a small range in source $\delta^{13}C$ values. Thus, the carbon isotope values of a terrestrial plant can generally be used to identify which photosynthetic pathway it utilizes.

Eelgrass is a C_3 plant, though carbon isotope values found in the literature for eelgrass and other seagrass species are similar to those of terrestrial C_4 plants (Haines and Montague, 1979). C_4 plants have carbon isotopic values ranging from -9 to -17 ‰ (Ballentine *et al.*, 1998), which is enriched by 10 to 15 ‰ relative

to values of C_3 plants (Meier-Augenstein, 2002). Carbon isotope values for bulk tissues of Zostera marina, though, typically range from -8 to -11 ‰. The main reason for the discrepancy is that the concentration and diffusion rate of CO_2 in the marine environment are relatively low compared to those found in terrestrial systems. Not only are CO_2 concentrations far lower in water than in air, but CO_2 solubility is 10-15% lower in salt water than in fresh water due to dissolved salts in the water column. More importantly, CO_2 diffuses on the order of 10,000 times more slowly in water than in air (Nichols *et al.*, 1985). Since CO_2 diffusion is a rate-limiting step, there is less isotopic discrimination, which results in the relatively enriched values (Hemminga and Duarte, 2000). In addition, eelgrass is able to utilize marine bicarbonate in addition to dissolved CO_2. Marine bicarbonate is enriched by approximately 8.5 ‰ relative to atmospheric CO_2, which further increases the $\delta^{13}C$ of live eelgrass tissues.

1.6. Fatty acids

Carboxylic acids, more commonly referred to as fatty acids, comprise one group of critical compounds for animals. Carbon chain lengths can extend to around eighty, though the most common biologically active fatty acids range from two (acetic acid) to twenty four (lignoceric acid) carbon atoms. The majority of fatty acids originating from plants and animals are straight-chained and even numbered, while branched and odd-chain fatty acids are indicative of bacterial sources (Christie, 2003). Animal fatty acids commonly vary from fourteen to

twenty two carbons per chain terminating at one end with a carboxyl group, and have one or more double bonds in specific positions relative to the carboxyl group (mono- or polyunsaturated, respectively). Short-chain fatty acids are synthesized *de novo* by plants from an acetate precursor. The initial products are saturated but can be partially desaturated to form monoenoic acids (Hepher, 1988). A number of fatty acids, which cannot be synthesized *de novo* by animals yet are crucial for healthy growth, must be obtained entirely through the diet. These required fatty acids are designated essential fatty acids (EFA) when the organism in question does not possess the necessary enzymes needed to synthesize these fatty acids. The suite of EFA differs between species depending on metabolic requirements.

Most of the fish that have been studied require food sources containing ω3 and ω6 polyunsaturated fatty acids (PUFA) (NRC, 1993). Each of these fatty acids may serve as a precursor for eicosanoids, which are necessary for certain metabolic functions. Fatty acids are also critical components of the phospholipids of cell membranes. If these membranes do not remain in a fluid state, the membrane permeability decreases considerably (Kimelberg and Papahadjopoulos, 1972, in Hepher, 1988). Membrane fluidity is important for two reasons. First, studies have found that the incorporation of these fatty acids into the phospholipids allow cell membranes to remain fluid at far lower temperatures (Hepher, 1988; NRC, 1993) because PUFA, especially of the

ω3 type, have lower melting points. Thus PUFA are particularly important for cold-water marine species. Second, phospholipids may play an important role in the osmoregulatory system of marine fish by activating a membrane-bound enzyme responsible for regulation of K^+ and Na^+ permeability (Hepher, 1988).

Fatty acid profiles from eelgrass tissues can be found in the literature. The major FA components of *Z. marina* from the Sea of Japan were as follows, in order of increasing percentage: 16:3ω3 [hexadecatrienoic acid], 18:2ω6 [linoleic acid], 16:0 [palmitic acid], and 18:3ω3 [α-linolenic acid], comprising nearly 90% of total FAs (Khotimchenko, 1993; Sanina *et al.,* 2004). All other fatty acids with carbon chain lengths ranging from 14 to 24 were each less than 2 percent of the total fatty acid content. *Z. marina* contained small amounts of saturated FAs with relatively long chain lengths ranging from 20:0 to 25:0 (Khotimchenko, 1993). In summary, eelgrass is both rich in C_{18} polyunsaturated fatty acids and contains long-chain saturated fatty acids, which is a combination typical only of seagrass in marine systems (Khotimchenko, 1993).

The suite of fatty acids as well as the relative concentrations of these fatty acids has been shown to differ between *Z. marina* and other macrophyte primary producers commonly found in South Bay, particularly marine macroalgae. The fatty acid composition of eelgrass and other primary producers vary somewhat between different locations and among individuals. However, it can be expected

that the overall fatty acid chemistry and carbon isotopic values of macrophytes differ enough to be resolved as separate sources.

The second portion of this study focuses on fatty acids for several reasons. First, these compounds are carbon-rich. Therefore, fatty acid compositions can be determined from extracts of relatively small sample sizes (Boschker and Middleburg, 2002). Second, they are ubiquitous biological compounds that are relatively easy for organisms to metabolize when assimilated through the diet. Thus to a heterotrophic organism, fatty acids may contribute nutrients required for growth and reproduction and energy supplies, as well as serving as a necessary element of cell membranes (Alfaro *et al.*, 2006). Third, the methods used to extract and concentrate fatty acids for analysis are relatively simple and straight-forward. The fatty acid preparation methods are modified from Barnes *et al*. (1979, in Ballentine, 1997).

Although preliminary bulk isotope analyses of tissues suggested little input from live *Z. marina* to consumer diets, the use of fatty acid analysis would help to clarify whether eelgrass organic matter was assimilated when the results from bulk isotope analysis proved unclear. First, it is possible that the eelgrass isotopic signal was masked by more substantial dietary sources in the bulk isotope analysis. Fatty acids give a time-integrated representation of animal diets, which is similar to the results of stable isotope analysis. Stable isotopes, however, integrate all sources of each individual element, which somewhat

limits how specific the conclusions based on such analyses can be. Several fatty acids are specific to certain taxa and so can be used to trace energy transfers through food webs (Napolitano, 1999). Additionally, many fatty acids are metabolically stable following assimilation and thus have the potential to indicate specific sources. To that end, I sought to identify and trace the transfer of individual dietary fatty acids by comparing the fatty acid composition of macrophytes with those of consumers and by searching for the presence of biomarker fatty acids. Second, decomposition is a complicating factor in the interpretation of food webs using stable isotopes. The stable isotope composition of marsh plants and eelgrass can change during decomposition due to either differential decay rates of individual components of the original source material or due to microbial metabolism (Peterson *et al.*, 1980; Benner *et al.*, 1987; Peterson, 1999). Therefore, the eelgrass isotopic signal in South Bay consumers may be masked by a bacterial signal. Fatty acid analysis will indicate bacterial markers in consumers and thus whether microbially-reworked eelgrass matter is utilized by consumers.

1.7. Chapter descriptions

The uptake of eelgrass primary production by local consumers is addressed in the following chapters. Chapter II elaborates on the methods utilized for this study. Chapters III and IV give an investigation of the influence of eelgrass presence on the nutritional status of fish and invertebrates using

carbon, nitrogen, and sulfur isotope values of bulk tissue samples. Chapter III details comparisons of consumers in the restored eelgrass meadows with those in adjacent eelgrass-free sites to assess whether eelgrass structural presence affects dietary sources. Additional comparisons were made with consumers from a nearby naturally-populated eelgrass meadow. Chapter IV explores the seasonal component of consumer dietary change through the growing season. The continued presence of eelgrass in the late summer, especially after the sharp drop observed in macroalgal biomass, is scrutinized as a variable affecting changes in consumer isotope values. The fatty acid chemistry of South Bay flora and fauna is examined in Chapter V to determine whether eelgrass primary production, whether as live material or detritus, is assimilated by local consumers. Finally, a summary and synthesis of the previous chapters is presented in Chapter VI.

1.8. References

Alfaro, A.C., Thomas, F., Sergent, L., and Duxbury, M. 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuarine Coastal and Shelf Science 70: 271-286.

Ballentine, D.C. 1997. Tracing combustion-derived atmospheric pollutants using compound specific isotope analysis. Ph.D. Dissertation, Department of Chemistry, University of Virginia. 172 pages.

Ballentine, D.C., Macko, S.A., and Turekian, V.C. 1998. Variability of stable carbon isotopic compositions in individual fatty acids from combustion of C4 and C3 plants: implications for biomass burning. Chemical Geology 152: 151-161.

Beck, M.W., Heck, K.L. Jr., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., and Weinstein, M.P. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. Bioscience 51 (8): 633-641.

Bell, M.V., Henderson, R.J., and Sargent, J.R. 1986. Minireview: The role of polyunsaturated fatty acids in fish. Comparative Biochemistry and Physiology 83B (4): 711-719.

Benner, R., Fogel, M.L., Sprague, E.K., Hodson, R.E. 1987. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. Nature 329 (6141): 708-710.

Boesch, D.F., and Turner, R.E. 1984. Dependence of fishery species on salt marshes: the role of food and refuge. Estuaries 7: 460-468.

Boschker, H.T.S., and Middleburg, J.J. 2002. Minireview: Stable isotopes and biomarkers in microbial ecology. FEMS Microbiology Ecology 40: 85-95.

Childers, D.L., Day, J.W. Jr., McKellar, H.N. Jr. 2000. Twenty more years of marsh and estuarine flux studies: Revisiting Nixon (1980). Pages 391-423 in: Weinstein, M.P., and Kreeger, D.A. (eds.), *Concepts and controversies in tidal marsh ecology.* New York: Kluwer Academic Publishers. 875 pages.

Christie, W. 2003. Lipid analysis: isolation, separation, and structural analysis of lipids. Bridgewater, England: The Oily Press. 416 pages.

Clarke, S.M., and Kirkman, H. 1989. Seagrass Dynamics. Pages 304-345 in: A.W.D. Larkum, A.J. McComb and S.A. Shepherd (eds), *Biology of Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region.* Amsterdam: Elsevier Science Publishing. 841 pages.

Connolly, R.M., Guest, M.A., Melville, A.J., and Oakes, J.M. 2004. Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia 138: 161-167.

de Leeuw, J.W., Rijpstra, W.I.C., and Nienhuis, P.H. 1995. Free and bound fatty acids and hydroxy fatty acids in the living and decomposing eelgrass *Zostera marina*. Organic Geochemistry 23 (8): 721-728.

DeNiro, M.J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42 (5): 495-506.

DeNiro, M.J., and Epstein, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45: 341-351.

de Sylva, D.P. 1975. Nektonic food webs in estuaries. Pages 420-443 in *Estuarine Research.* Myrtle Beach: 2nd International Estuarine Research Conference.

Deegan, L.A., Wright, A., Ayvazian, S.G., Finn, J.T., Golden, H., Rand Merson, R., and Harrison, J. 2002. Nitrogen loading alters seagrass ecosystem structure and support of higher trophic levels. Aquatic Conservation: Marine and Freshwater Ecosystems 12: 193-212.

Focken, U., and Becker, K. 1998. Metabolic fractionation of stable carbon isotopes: implications for different proximate compositions for studies of the aquatic food webs using $\delta^{13}C$ data. Oecologia 115: 337-343.

Fonseca, M., and Uhrin, A.V. 2009. The status of eelgrass, *Zostera marina*, as bay scallop habitat: consequences for the fishery in the western Atlantic. Marine Fisheries Review 71 (3): 20-33.

Frederiksen, M.S., Holmer, M., Borum, J., and Kennedy, H. 2006. Temporal and spatial variation of sulfide invasion in eelgrass (*Zostera marina*) as reflected by its sulfur isotopic composition. Limnology and Oceanography 51 (5): 2308-2318.

Fry, B., and Parker, P.L. 1979. Animal diet in Texas seagrass meadows: $\delta^{13}C$ evidence for the importance of benthic plants. Estuarine and Coastal Marine Science 8: 499-509.

Fry, B., Scalan, R.S., Winters, K., and Parker, P.L. 1982. Sulphur uptake by salt grasses, mangroves, and seagrasses in anaerobic sediments. Geochimica et Cosmochimica Acta 46: 1121-1124.

Haines, E.B., and Montague, C.L. 1979. Food sources of estuarine invertebrates analyzed using $^{13}C/^{12}C$ ratios. Ecology 60 (1): 48-56.

Harris, L.A., Buckley, B., Nixon, S.W., and Allen, B.T. 2004. Experimental studies of predation by bluefish *Pomatomus saltatrix* in varying densities of seagrass and macroalgae. Marine Ecology Progress Series 281: 233-239.

Heck, K.L. Jr., Hays, G., and Orth, R.J. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series 253: 123-136.

Hemminga, M.A., and Duarte, C.M. 2000. *Seagrass Ecology*. Cambridge: Cambridge University Press. 298 pages.

Hepher, B. 1988. *Nutrition of Pond Fishes*. Cambridge: Cambridge University Press. 388 pages.

Hoefs, J. 1997. *Stable Isotope Geochemistry*. Berlin: Springer-Verlag. 201 pages.

Jaschinski, S., and Sommer, U. 2008. Top-down and bottom-up control in an eelgrass-epiphyte system. Oikos 117: 754-762.

Kharlamenko, V.I., Kiyashko, S.I., Imbs, A.B., and Vyshkvartzev, D.I. 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analysis. Marine Ecology Progress Series 220: 103-117.

Khotimchenko, S.V. 1993. Fatty acids and polar lipids of seagrasses from the Sea of Japan. Phytochemistry 33 (2): 369-372.

Kikuchi, T. 1980. Faunal relationships in temperate seagrass beds. Pages 153-172 in: R.C. Phillips and C.P. McRoy (eds.), *Handbook of Seagrass Biology: An Ecosystem Perspective*. New York: Garland STPM Press. 353 pages.

Kimelberg, H.K., and Papahadjopoulos, D. 1972. Phospholipid requirements for (Na++K+)-ATPase activity: head group specificity and fatty acid fluidity. Biochimica et Biophysica Acta 282 (1): 277-292.

Klumpp, D.W., Howards, R.K., and Pollard, D.A. 1989. Trophodynamics and nutritional ecology of seagrass communities. Pages 394-457 in: A.W.D. Larkum, A.J. McComb, and S.A. Shepherd (eds.), *Biology of Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region.* Amsterdam: Elsevier Science Publishing. 841 pages.

Kwak, T.J., and Zedler, J.B. 1997. Food web analysis of southern California coastal wetlands using multiple stable isotopes. Oecologia 110 (2): 262-277.

Mann, K.H. 1982. Seagrass, marsh grass and mangrove systems. Pages 18-52 in: Anderson, D.J., Greig-Smith, P. and Pitelka, F.A. (eds.), *Studies in Ecology vol. 8: Ecology of Coastal Waters, a Systems Approach*. Berkeley: University of California Press. 332 pages.

Mann, K.H. 2000. *Ecology of Coastal Waters: with implications for management.* 2nd ed. Malden: Blackwell Science. 406 pages.

Mateo, M.A., Cebrián, J. Dunton, K., and Mutchler, T. 2006. Carbon flux in seagrass ecosystems. Pages 159-192 in: A.W.D larkum, R.J. Orth, and C.M. Duarte (eds.), *Seagrasses: Biology, Ecology and Conservation*. Dordrecht (Netherlands): Springer. 691 pages.

Meier-Augenstein, W. 2002. Review: Stable isotope analysis of fatty acids by gas chromatography-isotope ratio mass spectrometry. Analytica Chimica Acta 465: 63-79.

Michener, R.H., and Schell, D.M. 1994. Stable isotope ratios as tracers in marine aquatic food webs. Pages 138-157 in: Lajthe, K. and Michener, R.M. (eds.), *Methods in Ecology: Stable Isotopes in Ecology and Environmental Science.* Oxford (UK): Blackwell Scientific Publications. 316 pages.

Montgomery, J.L.M., and Targett, T.E. 1992. The nutritional role of seagrass in the diet of the omnivorous pinfish *Lagodon rhomboides*. Journal of Experimental Marine Biology and Ecology158 (1): 37-57.

Napolitano, G.E.1999. Fatty acids as trophic and chemical markers in freshwater ecosystems. Pages 21-44 in: Arts, M.T. and Wainman, B.C. (eds.), *Lipids in Freshwater Ecosystems*. New York, NY: Springer-Verlag. 319 pages.

Nichols, P.D., Klumpp, D.W., and Johns, R.B. 1985. A study of food chains in seagrass communities III. Stable carbon isotope ratios. Australian Journal of Marine Freshwater Research 36: 683-690.

National Research Council (U.S.) Committee on Animal Nutrition. 1993. *Nutrient Requirements of Fish.* Washington D.C.: National Academy Press. 114 pages.

Orth, R.J., and Moore, K.A. 1984. Distribution and abundance of submerged aquatic vegetation in Chesapeake Bay: An historical perspective. Estuaries 7 (4B): 531-540.

Orth, R.J., Luckenbach, M.L., Marion, S.R., Moore, K.A., and Wilcox, D.J. 2006a. Seagrass recovery in the Delmarva Coastal Bays, USA. Aquatic Botany 84: 26-36.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Jr., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., and Williams, S.L. 2006b. A global crisis for seagrass ecosystems. Bioscience 56 (12): 987-996.

Orth, R.J., Marion, S.R., Moore, K.A., and Wilcox, D.J. 2010. Eelgrass (*Zostera marina* L.) in the Chesapeake Bay Region of Mid-Atlantic Coast of the USA: Challenges in Conservation and Restoration. Estuaries and Coasts 33 (1): 139-150.

Peterson, B.J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. Acta Oecologia 20 (4): 479-487.

Peterson, B.J., and Howarth, R.W. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnology and Oceanography 32 (6): 1195-1213.

Peterson, B.J., Howarth, R.W., and Garritt, R.H. 1986. Sulfur and carbon isotope as tracers of salt-marsh organic matter flow. Ecology 67 (4): 865-874.

Peterson, B.J., Howarth, R.W., Lipschultz, F., and Ashendorf, D. 1980. Salt marsh detritus: and alternative interpretation of stable carbon isotope ratios and the fate of *Spartina alterniflora*. Oikos 34: 173-177.

Rasmussen, E. 1973. Systematics and ecology of the Isefjord marine fauna (Denmark): with a survey of the eelgrass (*Zostera*) vegetation and it's communities. Ophelia 11 (1/2): 1–495.

Sanina, N.M., Goncharova, S.N., and Kostetsky, E.Y. 2004. Fatty acid composition of individual polar lipid classes from marine macrophytes. Phytochemistry 65: 721-730.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., and Estevez, A. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177: 191-199.

Short, F.T., Ibelings, B.W., and den Hartog, C. 1988. Comparison of a current

eelgrass disease to the wasting disease in the 1930s. Aquatic Botany 30: 295–304.

Stevenson, J.C. 1988. Comparative ecology of submersed grass beds in freshwater, estuarine, and marine environments. Limnology and Oceanography 33 (4, part 2): 867-893.

Taylor, D.I., and Allanson, B.R. 1995. Organic carbon fluxes between a high marsh and estuary, and the inapplicability of the Outwelling Hypothesis. Marine Ecology Progress Series 120: 263-270.

Thom, R., Miller, B., and Kennedy, M. 1995. Temporal patterns of grazers and vegetation in a temperate seagrass system. Aquatic Botany 50 (2): 201-205.

Thresher, R.E., Nichols, P.D., Gunn, J.S., Bruce, B.D., and Furlani, D.M., 1992. Seagrass detritus as the basis of a coastal planktonic food chain. Limnology and Oceanography Notes 37 (8): 1754-1758.

Vähätalo, A.V., and Søndergaard, M. 2002. Carbon transfer from detrital leaves of eelgrass (*Zostera marina*) to bacteria. Aquatic Botany 73: 265-273.

Vergeer, L.H.T, Aarts, T.L., and de Groot, J.D. 1995. The 'wasting disease' and the effects of abiotic factors (light intensity, temperature, salinity) and infection with *Labyrinthula zosterae* on the phenolic content of *Zostera marina* shoots. Aquatic Botany 52: 35-44.

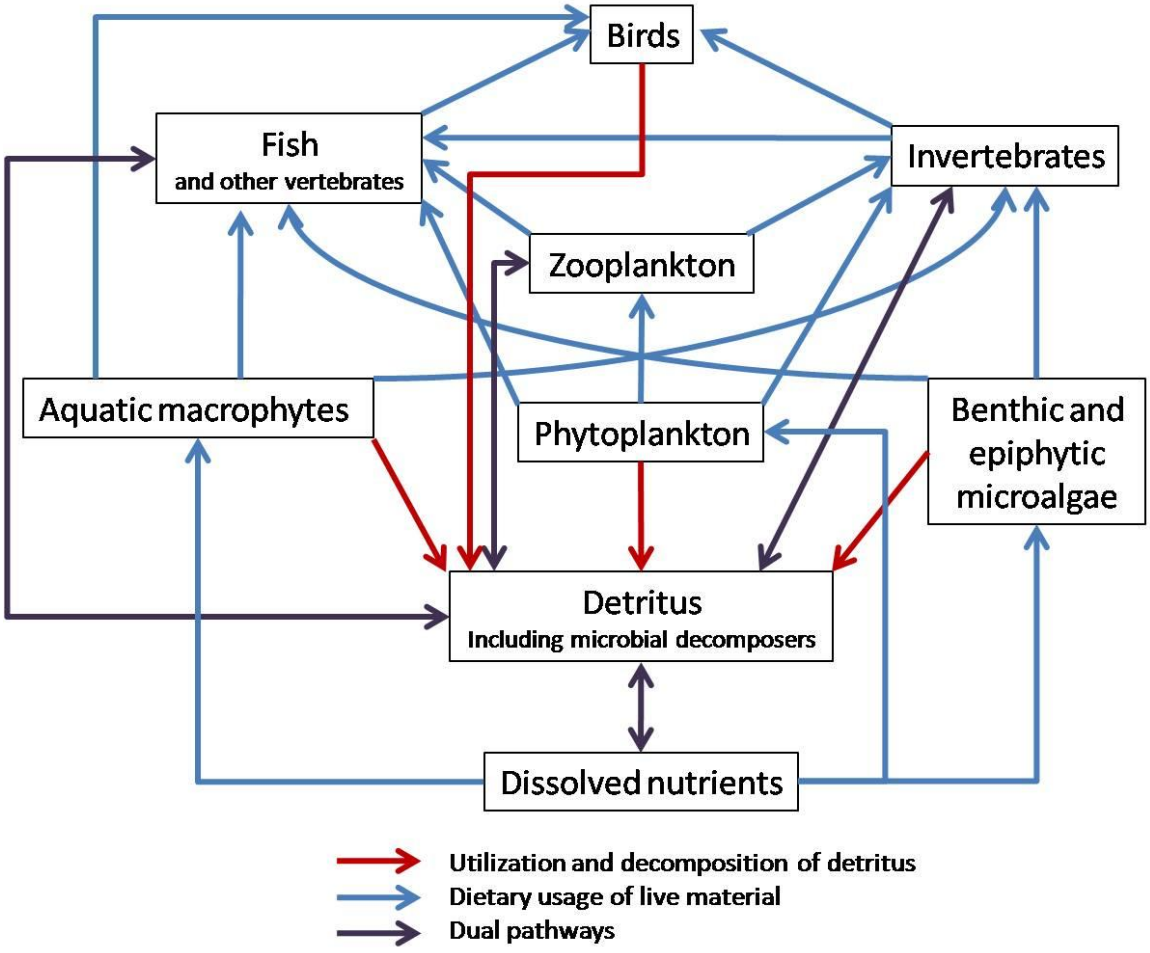
Vizzini, S., and Mazzola, A. 2004. The trophic structure of the pipefish community (Pisces: *Syngnathidae*) from a Western Mediterranean seagrass meadow based on stable isotope analysis. Estuaries 27 (2): 325-333.

Weinstein, M.P., and Kreeger, D.A. 2000. *Concepts and controversies in tidal marsh ecology.* New York: Kluwer Academic Publishers. 875 pages.

Wiedemeyer, W.L., and Schwamborn, R. 1996. Detritus derived from eelgrass and macroalgae as potential carbon source for *Mytilus edulis* in Kiel Fjord, Germany: a preliminary carbon isotopic study. Helgoland Marine Research 50 (3): 409-413.

Williams, S., and Ruckelshaus, M.H. 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. Ecology 74 (3): 904-918.

Figure 1.1. Simplified coastal marine food web emphasizing dietary and detrital pathways of nutrient transfer.



II. Chapter 2: General analytical methods

2.1. Introduction

Approximately 1700 samples of fish, invertebrates, primary producers, and particulate matter were collected for the current study. The same analytical procedures were utilized for the investigations in several of the following chapters. Therefore, the general analytical methods are explained in detail in this chapter, while condensed versions of the methods used are included within each following chapter so that each may be considered as an individual paper. Specific statistical methods used are described within the appropriate chapter.

2.2.1. Field methods: study sites

The southern bays adjacent to the eastern shore of Virginia were essentially devoid of eelgrass cover from 1933 until 1998. The discovery of several small, natural eelgrass beds led to the initiation of an eelgrass restoration program in South Bay, Virginia, by scientists at the Virginia Institute of Marine Science (Orth et al., 2006a). South Bay is a shallow coastal bay on the southern Delmarva Peninsula, approximately 6 miles off the Virginia coast (Figure 2.1).

Small-scale planting efforts using adult plants as well as seeds were conducted with the help of volunteers in 1998 through 2000 (Orth et al., 2003). Following the success of the original test plots, the Virginia Marine Resources Commission (VMRC) set aside a 400 acre subtidal area within South Bay for

eelgrass restoration (Canuel and Orth, 2002). Twenty-four one-acre plots were restored via reseeding in 2001; twelve plots received 100,000 seeds per plot, while the remaining twelve received 200,000 seeds per plot. In 2002, twenty-four plots additional plots were restored, with twelve restored at 50,000 seeds per plot and the final twelve restored at 100,000 seeds per plot (Figure 2.2) (Orth *et al.*, 2006).

A naturally-populated eelgrass meadow was recently discovered adjacent to Fisherman's Island, Virginia. The meadow was believed to have developed in the early 1990's and was selected to compare with the South Bay sites. Eelgrass was estimated to cover a subtidal area of just over 6800 m^2 at the site in 2004 (Orth *et al*., 2005) and was at a comparable depth and subject to a similar tidal range to South Bay seagrass plots. The meadow was too small to allow for multiple internal seining locations and was therefore treated as a single site throughout this study.

2.2.2. Field methods: sampling methods

Fish, invertebrates, and macrophytes were collected monthly from June through September of 2004 and 2005 in South Bay. Specific sampling dates were chosen such that low tide occurred in the early afternoon. A 150-foot seine was used to sweep an area averaging 750 square meters per site within the restored eelgrass beds (see Figure 2.2) and adjacent near-shore eelgrass-free habitats characterized by the visual absence of eelgrass cover. The vast majority of fish collected were juveniles, with few exceptions. In 2004, each of the six potential

combinations of year and density for eelgrass plots (Year: 2001 or 2002; Seed density: 50,000, 100,000, or 200,000 seeds/acre) was sampled randomly three times throughout the four-month period, as were eelgrass-free habitats (Table 2.1). The scheme was selected to investigate whether fish dietary preferences varied in the eelgrass meadows relative to the density of seeds disbursed during restoration and/or the age of the restored eelgrass plots. Different sets of restored eelgrass plots and eelgrass-free sites were sampled during each trip in 2004, without repetition.

At the start of the 2005 season, three restored eelgrass plots were chosen prior to the first sampling trip. One eelgrass plot was selected at random from plots respresenting each of three different combinations of year and seed density (restored eelgrass plots 36, 110, and 148; see Figure 2.2) and three nearby eelgrass-free plots were selected upon arrival in South Bay (Table 2.2); GPS coordinates were taken to mark the locations of eelgrass-free sites. Eelgrass-free sites were identified by the visual absence of eelgrass cover. Sample collections for the following months continued at these six pre-determined sites to investigate short-term seasonal variation in consumer isotope values. Individual specimens were separated by species upon return to VIMS prior to transport to the University of Virginia for isotopic and fatty acid analysis. Samples were kept on ice during transport.

The sampling regime for the natural eelgrass meadow at Fisherman's Island was similar to that of the restored meadows of South Bay, though only one eelgrass sampling site was possible due to the relatively small size of the meadow. Fish, invertebrates, and macrophytes were collected monthly from July through September 2006 using a 30-foot seine within the seagrass bed. Net contents of three to four sweeps of approximately 950 square feet were combined for each sampling period. As was the case with the South Bay sites, few adults were collected. An eelgrass-free site was again chosen based on a visual lack of live eelgrass and was sampled under the same parameters. Samples were kept on ice during transport to UVA, at which time individuals were separated by species in preparation for analysis.

Water samples were collected for isotopic analysis of seston at each site during each sampling period for bulk isotopic analysis (C, N, and S). Seston is defined here as the material remaining on a glass-fiber filter following filtration of water samples. Two water samples each of two liters were collected per site then stored on ice for 6 to 18 hours until filtration upon return to UVA.

2.3.1. Laboratory methods: stable isotope analyses

Water samples for seston were filtered through pre-combusted Whatman® 47 mm GF/C glass-fiber filters. Dilute HCL was added to samples for carbon isotopic analysis to remove dissolved carbonates. Particulates were

carefully scraped off of filters and transferred to tin cups prior to combustion and isotopic analysis.

Primary producers, fish, and invertebrates were freeze-dried and homogenized upon arrival at UVA. Primary producers were scraped clean of epiphytes using a scalpel, and then rinsed with tap water followed by deionized water prior to freeze drying. Samples of white muscle tissue of macroconsumers were selected for stable isotope analysis because even after lipid extraction, white muscle tissue exhibits the smallest bulk isotopic variation (Pinnegar and Pulonin, 1999). Samples that were too small to discriminate between tissue types were homogenized with care taken to avoid skin, bone or shell, and internal organs. Samples were weighed out to between 0.8 and 1.5 mg for carbon and nitrogen analysis, and 3.0 and 4.0 mg for sulfur in tin cups. Samples were combusted in a Carlo Erba elemental analyzer coupled to a GV Micromass Optima continuous flow isotope ratio mass spectrometer for isotopic analysis. Carbon, nitrogen and sulfur isotope values of common constituents of the marine environment are depicted in Figure 2.3a-c.

Isotopic measurements are expressed as δ, where:

$\delta^{N}E$ (‰) = (R_{sam} / R_{std} – 1) * 1000.

In the above equation, E refers to the element analyzed, N is the atomic mass of the heavy isotope, and R is the ratio of the abundance of the heavy isotope to the light isotope (i.e. $^{13}C/^{12}C$). Isotopic values are recorded in per mil

(‰). Carbon, nitrogen and sulfur isotope values are reported relative to the Pee Dee Belemnite, atmospheric nitrogen, and the Canyon Diablo Troilite standards, respectively, which are each defined as 0 ‰.

2.3.2. Laboratory methods: fatty acids

Total fish abundance in eelgrass plots and algae sites was greatest during July sample periods for both sampling seasons. The majority of fish selected for fatty acid analysis were collected during July such that the greatest possible range of species was represented. The exception was a group of spot (*Leiostomus xanthurus*) captured in September, 2005, which exhibited significantly different bulk stable isotope values compared to individuals collected during previous months. These individuals will be compared with spot from July to determine whether the fish fatty acid profiles can shed light on the origin(s) of the observed bulk isotope differences. The major primary producers present in South Bay were also subject to fatty acid analysis.

Samples were lipid extracted and derivatized to fatty acid methyl esters (FAMEs) using the method of Ballentine (1996, modified from Barnes *et al.*, 1979). See the flow chart in Figure 2.4 for a representation of the fatty acid preparation methods. Glassware used for fatty acid sample preparation was washed thoroughly then ashed in a 550-degree Celsius muffle furnace for a minimum of 4 hours prior to use with the exception of the Soxhlet extraction tube and water-cooled condenser. All solvents (chromatography grade) were pre-distilled in

order to ensure purity. Glassware was cleaned and rinsed several times with the first-cut removed from distillation of dichloromethane (DCM). Extreme care was used not to touch the extraction thimble so as to prevent sample contamination by lipids from fingerprints.

Homogenized tissue samples were lipid-extracted in DCM for approximately 16 hours in a glass Soxhlet extraction tower to allow for continuous extractions of lipids. Enough solvent was added so that the siphon of the extractor tube was covered plus excess so that the flask did not go dry; approximately 150 mL. The lipid extract was concentrated using a rotary evaporator. Triglycerides were saponified to individual fatty acid salts by heating under reflux with 10 mL of 1 N KOH-methanol (31.9 grams 87.9 % assay KOH/500 ml distilled methanol). Three hexane rinses of 10, 5, and 5 ml in a separatory funnel separated non-saponifiable materials (straight-chain lipids such as alkanes) from the remaining fatty acids in methanol. The bottom layer of saponifiable fatty acids was collected and acid neutralized (1N HCl) drop by drop until neutral or slightly acidic. Neutral FA's were then extracted with three portions of 10, 5 and 5 ml hexane. Total FA's were concentrated by rotoevaporation (if necessary) and vacuum desiccation to dryness then refrigerated until derivatization.

Relatively non-volatile molecules such as fatty acids require derivatization to the corresponding methyl esters prior to analysis. The procedure used to

derivatize FAs to FAMES was as follows. Approximately 2 mL of 15% Boron trifluoride in excess methanol (BF_3 CH_2OH) were added to vials containing the dried fraction, which were then sealed and heated to 60°C for 8 minutes. Samples were cooled to room temperature then transferred to a separatory funnel using two 5 mL hexane washes. The solution was then washed with two 5 mL portions of saturated KCl solution. The bottom fraction containing KCL was removed and discarded while the top hexane fraction was transferred to a new vial. Water was removed from the hexane fraction by addition Na_2SO_4 (anhydrous) to excess. Samples were transferred by pipette to a clean, ashed vial sealed with a Teflon-lined cap. Vials were stored, refrigerated, until analysis (from Ballentine, 1997).

The FAMES were analyzed using a Hewlett Packard 5890 Series II gas chromatograph fitted with a flame ionization detector (FID). A 60 meter J&W DB-5 column was used for analysis with helium as the carrier gas. The general temperature program used for the separation of fatty acids for gas chromatographic analysis was as follows: hold at 50°C for 1 minute, ramp at 15°C/minute to 120°C, ramp at 2°C/minute to 210°C, hold for 5 minutes, ramp at 2°C/minute to 250°C, hold for 15 minutes. The injector temperature was 200°C. A Hewlett Packard 3390A integrator was used to record the elution of fatty acid peaks through the FID.

FAMES were identified by comparison of peak retention times with commercial SUPELCO C16 – C18 FA in water standard mix (#17973; all

components at concentration of .4% w/v), SUPELCO Bacterial Acid FAMES mix in methyl caproate (#47080; 10mg/ml total concentration) as well as in-house standard mixes. Three to five injections were performed for each sample, depending on the reproducibility of the analysis.

2.3.3. Fatty acid nomenclature

The fatty acid shorthand nomenclature used here is of the 'ω' form. For this shorthand, given the fatty acid 18:2ω3, the '18' refers to the carbon chain length, '2' is the total number of double bonds in the chain, and the 'ω3' refers to the position of first double bond nearest the carboxyl group. To clarify, a designation of 'ω3' for a given fatty acid is equivalent to a designation of 'n-3' which is also commonly used. The polyunsaturated fatty acids observed in this study are methylene interrupted – i.e. separated by a CH_2 group – as is typical for the more common fatty acids with multiple double bonds (Napolitano, 1999).

2.4. Statistical methods

SAS statistical software v.9.1.3 (SAS Institute Inc., Cary, NC) was used for all statistical analyses performed in the following chapters. Student's t-test, one-way analysis of variance, and multivariate analysis of variance were all used. Tukey's Studentized Range (HSD) test was used when needed to test for significant differences at $p < 0.05$. Statistical tests used will be identified and described for each individual chapter.

2.5. References

Ballentine, D.C., Macko, S.A., Turekian, V.C., Gilhooly, W.P., and Martincigh, B. 1996. Compound specific isotope analysis of fatty acids and polycyclic aromatic hydrocarbons in aerosols: implications for biomass burning. Organic Geochemistry 25 (1-2): 97-104.

Ballentine, D.C. 1997. Tracing combustion-derived atmospheric pollutants using compound specific isotope analysis. PhD. Dissertation, Department of Chemistry, University of Virginia. 172 pages.

Barnes, P.J., Brassell, S.C., Comet, P., Eglinton, G., McEvoy, J., Maxwell, J.R., Wardoper, A.M.K, and Volkman, J.K. 1979. Preliminary lipid analysis of core sections 18, 24 and 30 from hole 402A. Pages 965-976 in: Montadert, L. and Roberts, D.G. (eds.), *Initial reports of the Deep Sea Drilling Project, vol. 48.* Washington D.C.: United States Government Printing Office.

Canuel, E.A., and Orth, R.J. 2002. Use of stable isotopes for developing a food web model in a recovering seagrass system in Virginia coastal bays. Final Report submitted to Virginia Coastal Resources Management Program.

Kumar, S., Ramesh, R., Bhosle, N.B., Sardesai, S., and Sheshshayee, M.S. 2005. Natural isotopic composition of nitrogen in suspended particulate matter in the Bay of Bengal. Biogeosciences 1: 63-70.

Michener, R.H., and Schell, D.M. 1994. Stable isotope ratios as tracers in marine aquatic food webs. Pages 138-157 in: Lajthe, K. and Michener, R.M. (eds.), *Methods in Ecology: Stable Isotopes in Ecology and Environmental Science.* Oxford (UK): Blackwell Scientific Publications. 316 pages.

Nadelhoffer, K.J., and Fry, B. 1994. Nitrogen isotope studies in forest ecosystems. Pages 22-44 in: Lajtha, K. and Michener, R.M. (eds.), *Methods in Ecology: Stable Isotopes in Ecology and Environmental Science.* Oxford (UK): Blackwell Scientific Publications. 316 pages.

Napolitano, G.E. 1999. Fatty acids as trophic and chemical markers in freshwater ecosystems. Pages 21-44 in: Arts, M.T. and Wainman, B.C. (eds.), *Lipids in Freshwater Ecosystems.* New York: Springer-Verlag Inc. 319 pages.

Orth, R.J., Wilcox, D.J., Nagey, L.S., Owens, A.L., Whiting, J.R., and Kenne, A.K. 2005. 2004 Distribution of submerged aquatic vegetation in the Chesapeake Bay and Coastal Bays. VIMS Special Scientific Report Number 146. Final report to U.S. EPA, Chesapeake Bay Program, Annapolis, MD. Grant No.CB973013-01-0, http://www.vims.edu/bio/sav/sav04

Orth., R.J., Luckenbach, M.L., Marion, S.R., Moore, K.A., and Wilcox, D.J. 2006. Seagrass recovery in the Delmarva Coastal Bays, USA. Aquatic Botany 84 (1): 26-36.

Mann, K.H. 2000. *Ecology of Coastal Waters: with implications for management.* 2nd ed. Malden, Massachusetts: Blackwell Science. 406 pages.

Peterson, B.J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. Acta Oecologia 20 (4): 479-487.

Peterson, B.J., Howarth, R.W., and Garritt, R.H. 1986. Sulfur and carbon isotopes as tracers of salt-marsh organic matter flow. Ecology 67: 865-874.

Pinnegar, J.K., and Pulonin, N.V.C. 1999. Differential fractionation of the $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. Functional Ecology 13 (2): 225-231.

Wigand, C., McKinney, R.A., Cole, M.L., Thursby, G.B., and Cummings, J. 2004. Varying stable nitrogen isotope ratios of different coastal marsh plants and their relationships with wastewater nitrogen and land use in New England, USA. Environmental Monitoring and Assessment 131 (1-3): 71-81.

Table 2.1. Site characteristics and sampling dates, summer 2004.

Plot/Site	Year Seeded	Seed Density (acre^{-1})	Sampling Date
3	2002	50,000	06/09/2004
29	2001	200,000	
91	2001	100,000	
Algae 1	n/a	n/a	
Algae 2	n/a	n/a	
Algae 3	n/a	n/a	
137	2002	100,000	07/13/2004
145	2002	50,000	
148	2001	100,000	
Algae 4	n/a	n/a	
Algae 5	n/a	n/a	
Algae 6	n/a	n/a	
30	2001	200,000	08/10/2004
39	2002	100,000	
49	2002	100,000	
Algae 7	n/a	n/a	
Algae 8	n/a	n/a	
Algae 9	n/a	n/a	
21	2001	100,000	09/8/2004
50	2002	50,000	
120	2001	200,000	
Algae 10	n/a	n/a	
Algae 11	n/a	n/a	
Algae 12	n/a	n/a	

Table 2.2. Site characteristics and sampling dates, summer 2005.

Plot/Site	Year Seeded	Seed Density (acre^{-1})
36	2001	200,000
110	2002	50,000
148	2001	100,000
Algae 20	n/a	n/a
Algae 21	n/a	n/a
Algae 22	n/a	n/a

Figure 2.1. Map of the Southern portion of the Delmarva Peninsula, courtesy of the VCR-LTER. Boxed location indicates South Bay study site and circled location indicates Fisherman's Island study site.

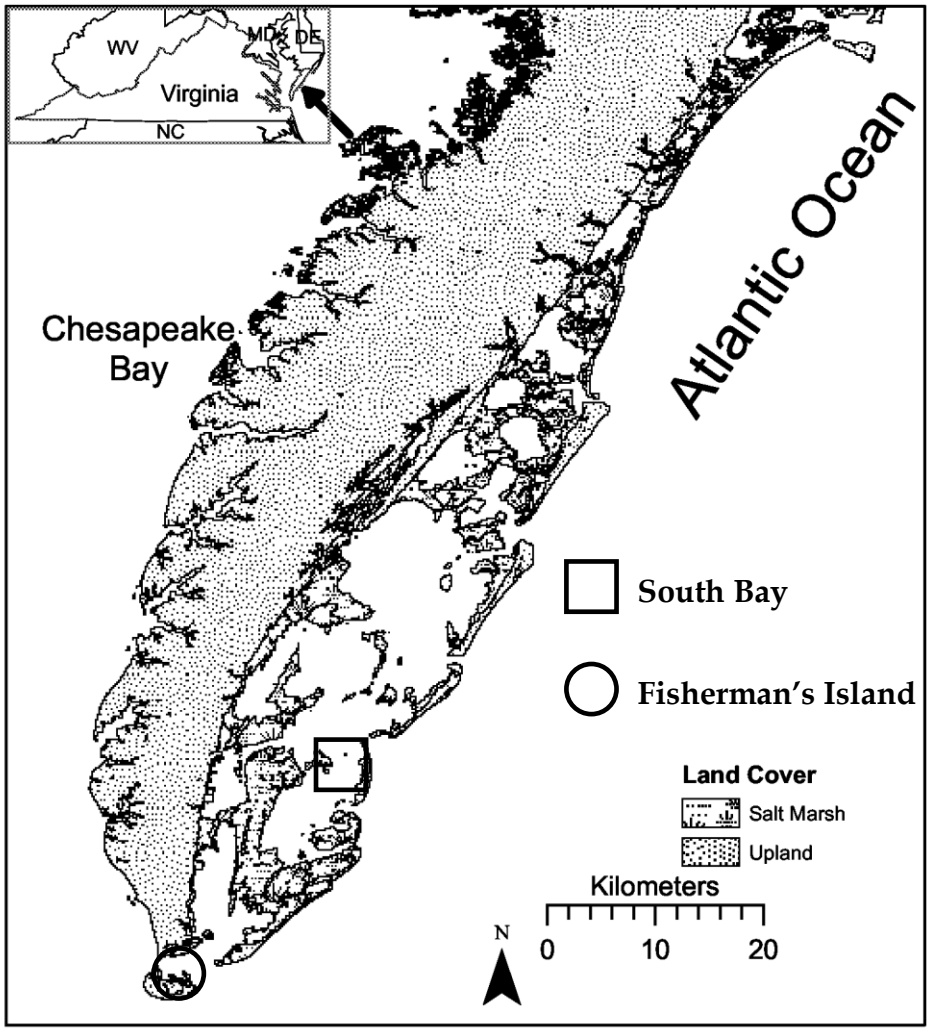


Figure 2.2. Aerial image with GIS overlay of eelgrass plots restored in 2001 (green) and 2002 (pink). Image courtesy of S. Marion and the Virginia Institute of Marine Science.

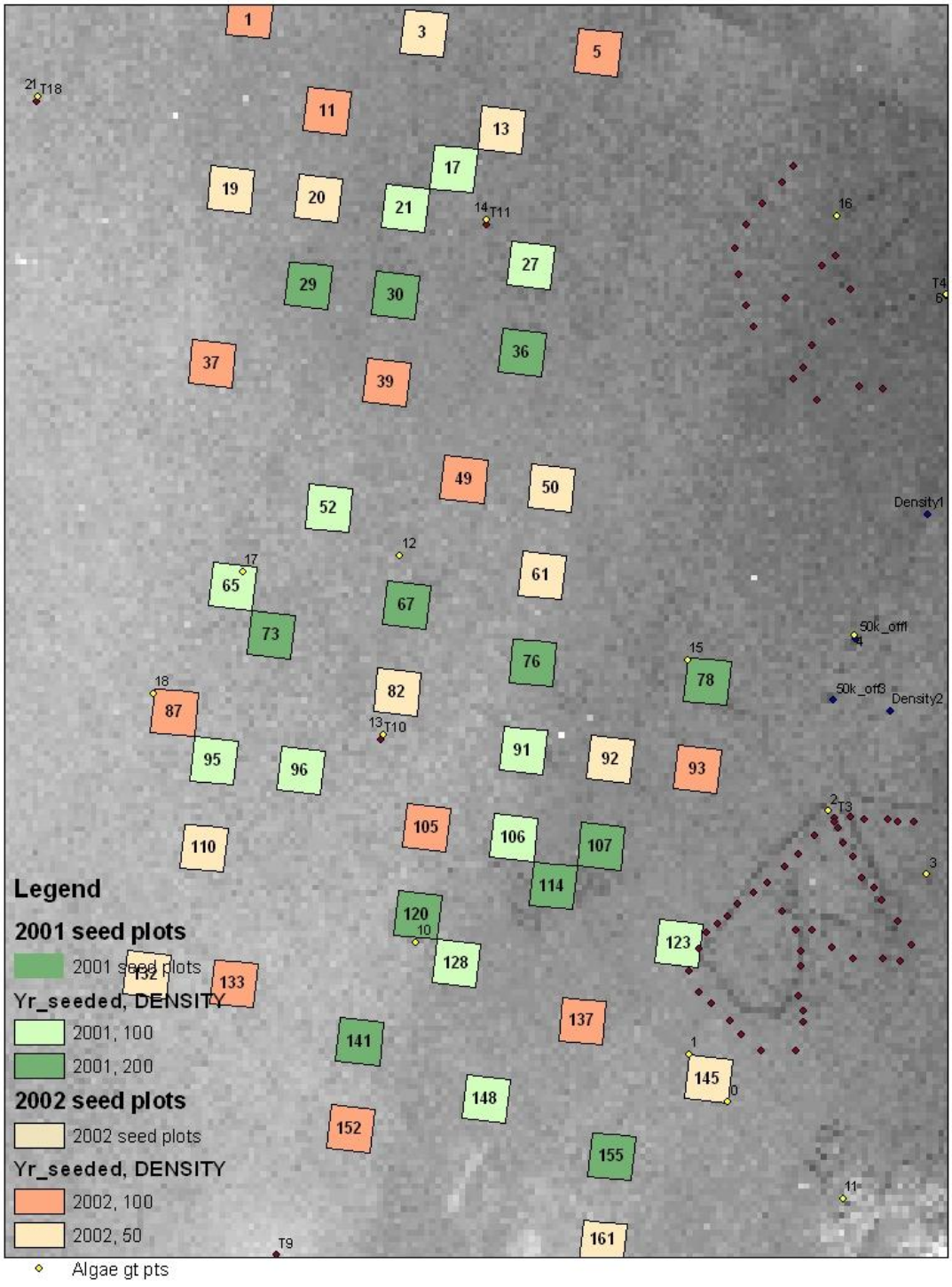


Figure 2.3a. Carbon isotope values of marine organisms and ionic species. Ranges given for organisms were collected during this study unless otherwise indicated. [a] – Mann, 2000, [b] – Peterson, 1999. Image courtesy of M. Tuite.

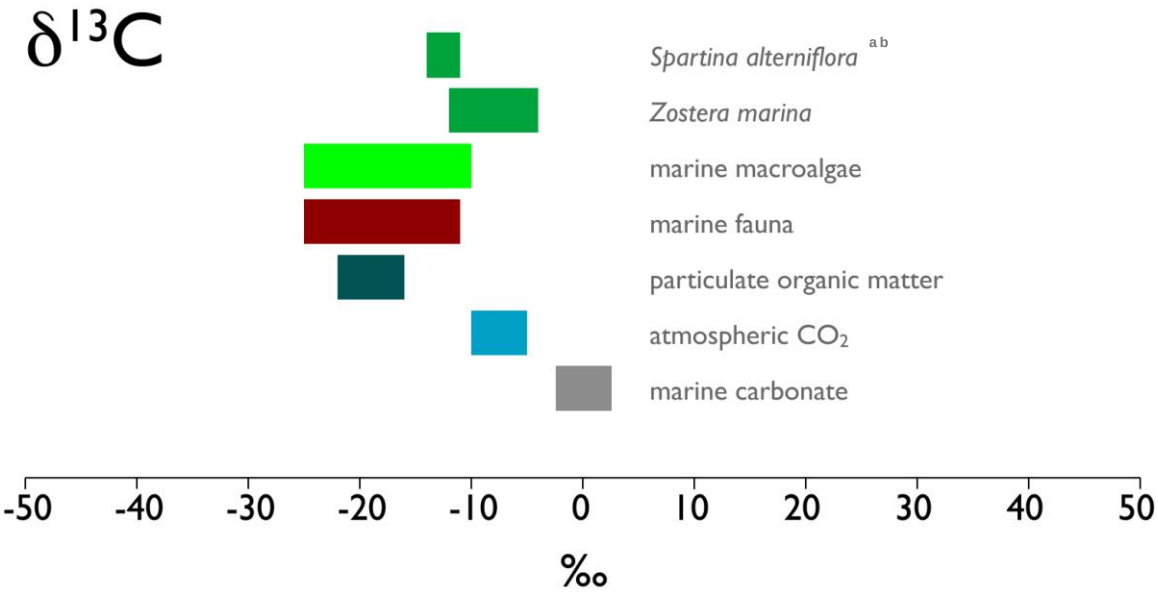


Figure 2.3b. Nitrogen isotope values of marine organisms and ionic species. Ranges indicated were collected during this study unless otherwise indicated. [a] – Wigand *et al.*, 2006; [b] – Peterson, 1999; [c] – Nadelhoffer and Fry, 1994; [d] – Kumar *et al.*, 2004. Image courtesy of M. Tuite.

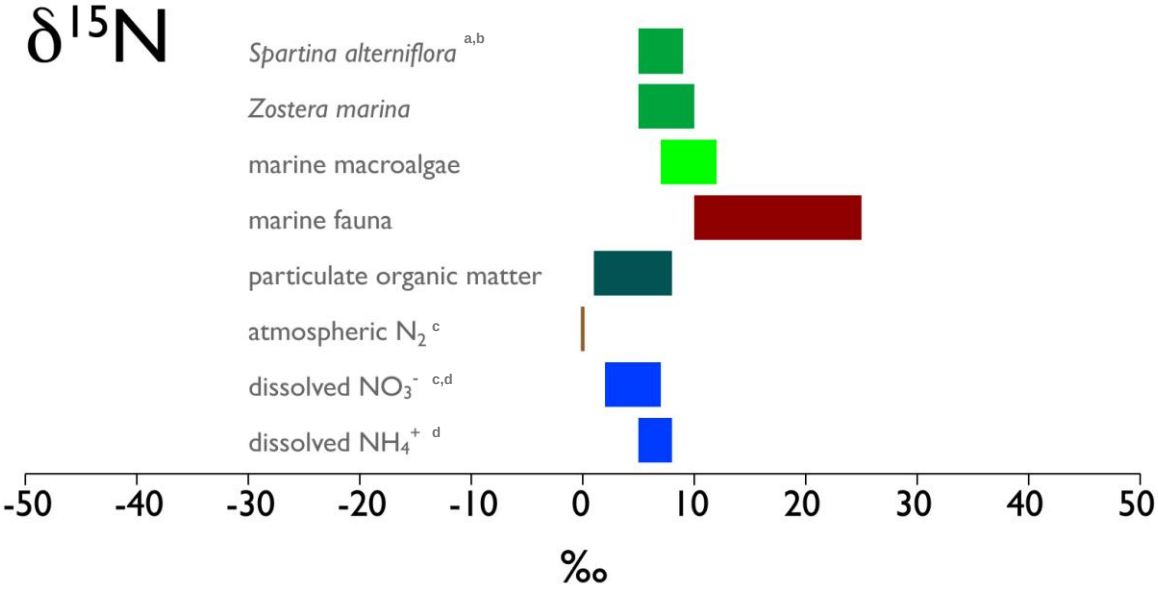


Figure 2.3c. Sulfur isotope values of marine organisms and ionic species. Ranges indicated were collected during this study unless otherwise indicated. [a] – Peterson *et al.*, 1986; [b] – Michener and Schell, 1994. Image courtesy of M. Tuite.

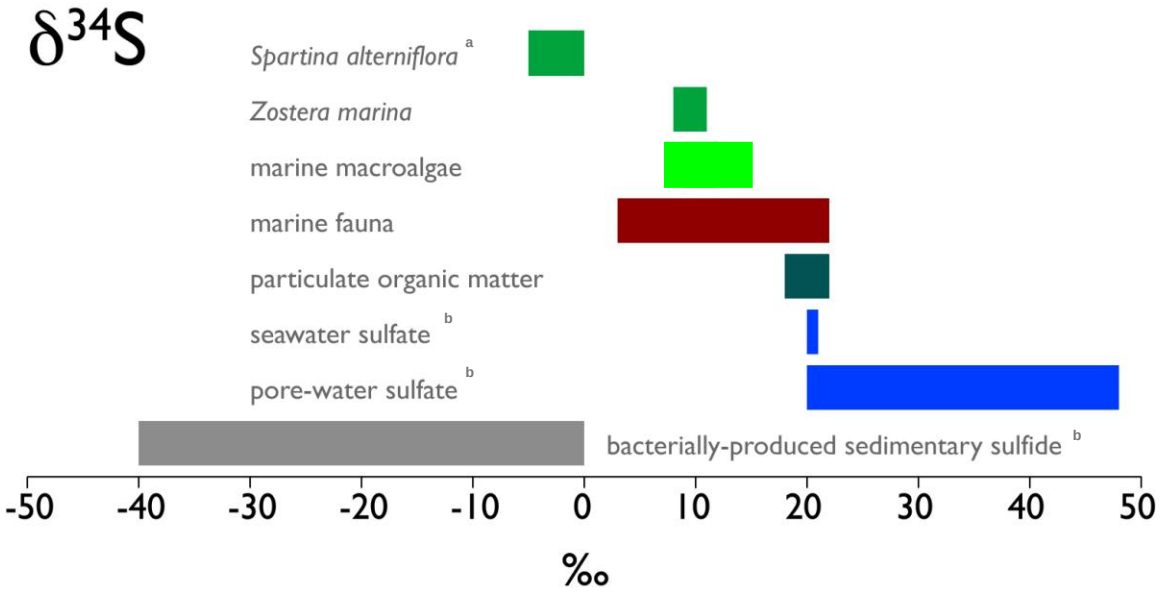
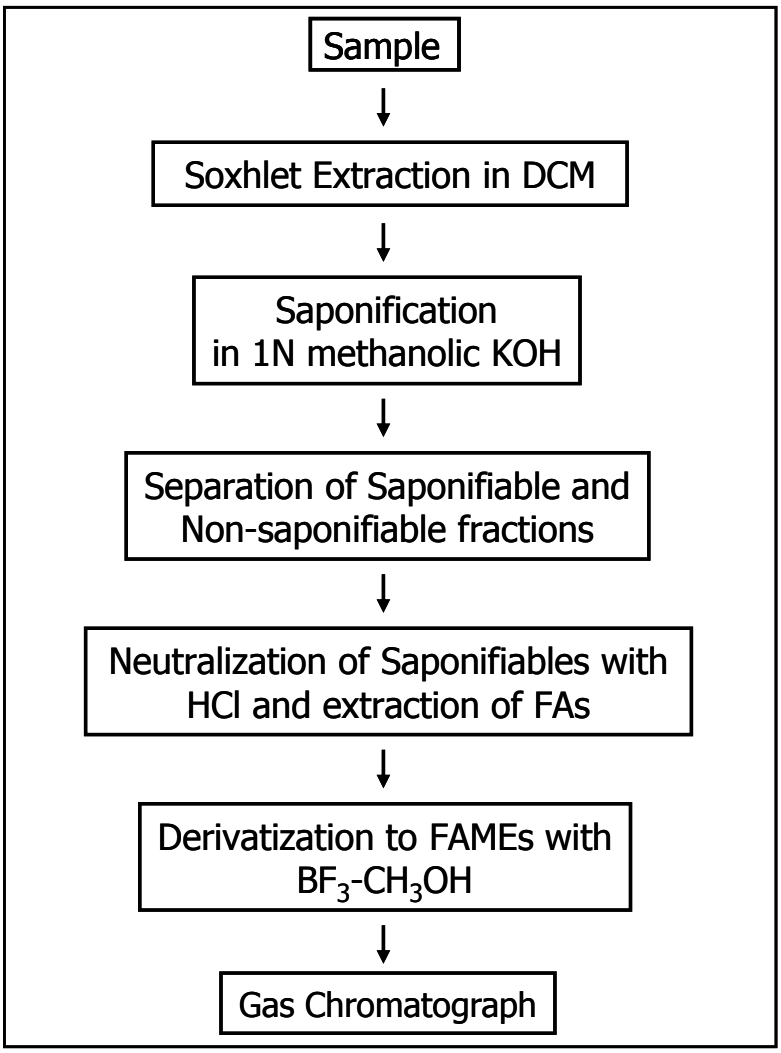


Figure 2.4. Flow chart of laboratory procedures for the extraction and derivatization of fatty acids.



III. Chapter 3: Impact of eelgrass restoration on coastal food webs: a multiple stable isotope approach.

3.1. Introduction

Seagrass meadows support diverse ecosystems within estuaries and along coastlines around the world (Hoshika *et al.*, 2006; Heck *et al.*, 2008). The meadows provide structural habitat, shelter, safety, abundant food, and protected spawning areas for many marine species that inhabit coastal or estuarine waters during one or more life stages. However, coastal waters where seagrasses were once common have suffered major grass cover losses over the last few decades (Hughes *et al.*, 2002; Orth *et al.*, 2006). Direct and indirect human activities such as dredging, coastal development, and general decreases in local water quality are responsible for recent declines in seagrass coverage in many areas (Short and Wyllie-Echeverria, 1996; Duarte, 2002; Duarte *et al.*, 2006; Short *et al.*, 2006; Waycott *et al.*, 2009; Orth *et al.*, 2010). Worldwide rates of seagrass loss have accelerated from 0.9 % per year prior to 1940 to 7 % per year since 1990, which is comparable to the habitat loss rates reported for mangroves, coral reefs, and tropical forests (Waycott *et al.*, 2009). The plight of seagrass meadows, however, has received far less media coverage compared with coral reefs and tropical forest in particular (Orth *et al.*, 2010), despite the fact that seagrass meadows have a greater geographical distribution.

Unfortunately, the loss of seagrass meadows results in more than just a loss of structural habitat. Many seagrass-associated species are also threatened (Hughes *et al.*, 2002). Evidence can be found in the literature that seagrass meadows positively affect fish and decapod survival and production via reduced predation and increased food availability (Fonseca *et al.*, 1990; Goldstein *et al.*, 1996; Minello *et al.,* 2003; Harris *et al.*, 2004). In addition, past studies have generally found that marine fish and invertebrate densities are greater in vegetated habitats than in non-vegetated habitats (e.g. Lubbers *et al*., 1990; see Heck *et al*., 2003 and Beck *et al*., 2001 for reviews). For this and other reasons, restoration of seagrass habitat has become a common choice for mitigation of the loss of marine habitats in the coastal zone (McLaughlin *et al.*, 1983; Fonseca and Fisher, 1986; Fonseca *et al*., 1990; French McCay and Rowe, 2003).

Zostera marina (eelgrass) is the dominant seagrass species found along the North Atlantic coastal regions of the United States, ranging from Canada to North Carolina (Short and Neckles, 1999). Eelgrass typically forms mono-culture meadows though it may co-occur with *Ruppia maritima* or *Halodule wrightii* in the mid-Atlantic region (Moore and Short, 2006). Historical evidence indicates that the Chesapeake Bay and nearby coastal bays once sustained far more expansive eelgrass meadows than are currently observed (Orth and Moore, 1983; Orth *et al.*, 2006); numerous instances of eelgrass decline across the Chesapeake Bay region have been well documented since the early 1930's (Rasmussen, 1977; Orth, 1976;

Kemp *et al*., 1983; Orth and Moore, 1983; Orth, 1994; Orth *et al*., 2006; Moore and Jarvis, 2008; Orth *et al*., 2010). Eelgrass restoration projects were undertaken within coastal bays of the southern Delmarva Peninsula following the severe decline of eelgrass biomass in the early part of the 20th century and the limited natural recovery that followed (Orth *et al*., 2006). Meadows restored in South Bay, Virginia, have been highly successful; the original plots have expanded in size and have recruited many fish (Orth *et al*., 2010).

The aim of this work was to evaluate the dietary effects of the reintroduction of eelgrass on consumers in South Bay, Virginia. The stable carbon, nitrogen, and sulfur isotope values of consumers and primary producers in the restored eelgrass meadows were assessed and compared with those of organisms collected from nearby eelgrass-free locations. It was hypothesized that stable isotope values of consumers from the restored meadows would differ from those of consumers from eelgrass free sites, reflecting differences in organic matter sources utilized at each site. Consumers and primary producers collected from a natural meadow recently discovered adjacent to Fisherman's Island, Virginia, were also compared with those collected from South Bay. Fisherman's Island is located at the mouth of the Chesapeake Bay, which is highly eutrophic and subject to large amounts of anthropogenic nutrient inputs. The proximity to high nutrient levels of the Chesapeake Bay likely influence the isotope values of consumers at the natural meadow. It was expected that the isotope values of

consumers at the natural meadow would differ from those of consumers in the relatively oligotrophic waters of South Bay.

3.2. Methods

Sample collections took place within a shallow subtidal section of South Bay, VA. Eelgrass was historically plentiful in the area, though the population suffered a severe decrease in the early 1930's during the widespread eelgrass decline which decimated eelgrass meadows across the North Atlantic (Orth *et al.*, 2006). South Bay was essentially devoid of eelgrass from that time through the late 1990's when a massive restoration effort was undertaken to return eelgrass to the coastal bays. Forty-eight one-acre plots were restored via reseeding within an approximately 400-acre subtidal area west of Wreck Island (Orth *et al.*, 2006). Individual plots were restored at three seed densities (50,000; 100,000; or 200,000 seeds/acre) during the summers of 2001 and 2002. 2001 plots were restored at densities of 200,000 and 100,000 seeds per acre, while 2002 plots were restored at densities of 100,000 and 50,000 seeds per acre. Seed restoration densities changed between years because fewer seeds were available in 2002.

3.2.1. Field methods

Animals and primary producers were collected during two sampling seasons from June through September 2004 and June through September 2005. The field collection parameters of the 2004 sampling season differed from the 2005 season (Tables 2.1 and 2.2). No site was revisited over the course of the

sampling season in 2004, so that in total twelve separate restored eelgrass plots and twelve separate eelgrass-free sites were visited during the course of the field season. In contrast, three restored eelgrass plots were selected prior to the start of the June 2005 field trip and three eelgrass-free sites were selected upon arrival. Subsequent samplings were repeated at the same locations for the duration of the study. In all cases the eelgrass-free sites were characterized by the visual lack of seagrass cover and were marked by GPS coordinates.

Due to the differences in sampling protocol between field seasons, eelgrass cover and plot age needed to be evaluated as potential variables affecting consumer isotope values before comparisons could be made between sampling seasons. The density of eelgrass seeds dispersed within the individual plots at the time of restoration was used as a proxy for eelgrass cover as follows – sites restored at 50,000 seeds per acre were considered low density; sites restored at 100,000 seeds per acre were medium density; sites restored at 200,000 seeds per acre were high density (see Table 2.1 and Figure 2.2).

Fish and invertebrates were collected monthly, June through September, via seine net. Primary producers were collected from the seine contents when possible and by hand when necessary. Water samples were collected at each site in two liter containers for analysis of suspended seston. Seston is here defined as the material remaining on a filter following filtration of the water sample. Invertebrates, primary producers, and water samples were transported

immediately, on ice, to the University of Virginia (UVA) for isotopic analysis. Individual fish were separated and identified by species at the Virginia Institute of Marine Science and frozen prior to transport to UVA. Up to five individuals per species collected were randomly selected for isotope analysis.

3.2.2. Isotope analyses

Samples were analyzed for carbon, nitrogen, and sulfur isotope values. Isotopic measurements are expressed using the δ notation, where:

$$\delta^{N}E\ (‰) = (R_{sam} / R_{std} - 1) * 1000.$$

In the above equation, E refers to the element analyzed, N is the atomic mass of the heavy isotope, and R is the ratio of the abundance of the heavy isotope to the light isotope (i.e. $^{13}C/^{12}C$). Isotopic values are recorded in per mil (‰). Carbon, nitrogen and sulfur isotope values are reported relative to the Pee Dee Belemnite, atmospheric nitrogen, and the Canyon Diablo Troilite standards, respectively, which are each defined as 0 ‰.

3.2.3. Statistical analyses

Carbon, nitrogen, and sulfur isotope values of organisms captured during the 2004 sampling season were analyzed using separate one-way ANOVA investigating the effect of eelgrass density. A mixed-model ANOVA was performed to test the effect of the interaction of the two variables, seed density and plot age on mean stable isotope values of organisms. One-way ANOVA were also performed addressing each individual variable when the interaction

was found to be non-significant. Mean isotope values were calculated for each species and differences between mean consumer isotope values were determined using ANOVA and Tukey's Studentized Range (HSD) test.

Student's t-tests were performed to compare the mean isotope values of all consumers as a group in eelgrass-free sites to those in the restored eelgrass sites. Individual species present in both habitat types were compared using one-way ANOVA for differences in isotope values by species among habitat types (eelgrass versus eelgrass-free). Flora and fauna were grouped by species within each habitat. The different numbers of individuals per species resulted in an unbalanced design. All data were assessed for homogeneity of variances and normality prior to analysis. Data that did not fit these assumptions were $\log_{10}(x)$ transformed and reassessed.

Sixteen species from the restored eelgrass sites were compared with those from eelgrass-free sites in 2004: Atlantic croaker (*Micropogonias undulatus*), Atlantic silversides (*Menidia menidia*), bay anchovy (*Anchoa mitchilli*), black seabass (*Centropristis striata*), blue crab (*Callinectes sapidus*), gag grouper (*Mycteroperca microlepis*), grass shrimp (*Palaemonetes vulgaris and P. pugio*), northern pipefish (*Syngnathus fuscus*), oyster toadfish (*Opsanus tau*), pigfish (*Orthopristis chrysoptera*), pinfish (*Lagodon rhomboides*), sheepshead (*Archosargus probatocephalus*), silver perch (*Bairdiella chrysoura*), spot (*Leiostomus xanthurus*), summer flounder (*Paralichthys dentatus*), and tautog (*Tautoga onitis*). *Agardhiella*

sp., epiphytic algae (likely *Ceramium rubrum*), dead man's fingers (*Codium fragile*), graceful redweed (*Gracilaria spp.*), sea lettuce (*Ulva lactuca*), and eelgrass (*Zostera marina*) were the primary producers collected for comparison. Epiphytic algae were attached to blades of eelgrass in the restored meadows and to macroalgae (usually *Gracilaria spp*.) in the eelgrass-free sites.

For the 2005 season, thirteen fish and invertebrate species were chosen for comparison between the restored eelgrass sites and the eelgrass-free sites: Atlantic croaker, Atlantic menhaden (*Brevoortia tyrannus*), Atlantic silversides, bay anchovy, grass shrimp, northern pipefish, oyster toadfish, pigfish, pinfish, silver perch, spot, summer flounder, and tautog. As with the fish from the 2004 season, these species were selected because at least three individuals were caught at each site type. Six primary producers were compared between the restored sites and the eelgrass-free sites as well: *Agardhiella sp.*, *Bryopsis sp.*, *Codium fragile*, epiphytic algae, *Gracilaria spp.*, and *Ulva lactuca*. Seston, here defined as particulate matter filtered from the water column, was also compared.

Individuals were grouped by species for comparison among the eelgrass sites in South Bay and from the natural meadow near Fisherman's Island. Only species that were represented by at least three individuals were used for statistical analysis. Seven species were collected from the natural eelgrass meadow for comparison with samples from South Bay in 2005; six of the seven were also collected in South Bay in 2004. Mean isotope values of each species

were compared using one-way ANOVA among the three collections. The sample design was unbalanced due to the varying number of individuals of each species caught each year.

3.3. Results

Approximately 900 organisms were collected from South Bay restored *Z. marina* plots and nearby eelgrass-free sites during the summer of 2004 and an additional 800 during the summer of 2005, consisting of primary producers through higher trophic level fish. Approximately 150 organisms were also collected from the natural eelgrass meadow and a nearby eelgrass-free site adjacent to Fisherman's Island, Virginia. In general, greater numbers of fish and greater species diversity were noted in the restored eelgrass beds in South Bay relative to nearby eelgrass-free areas lacking attached macrophytes (van Montfrans *et al.*, 2006; Figure 3.1).

3.3.1. Eelgrass cover and plot age in South Bay

Individuals representing nineteen species were collected in 2004 within at least two eelgrass plots restored under different combinations of the following two parameters; 1) density of seeds dispersed per acre during restoration, and 2) year restored, in sufficient numbers to allow for comparison. Table 3.2 identifies when ANOVA and Tukey's HSD test indicated that mean isotopes values were statistically different at $p < 0.05$ or $p < 0.01$. In Table 3.2, comparisons are marked as significant if the mean isotope values of fish were statistically different

between at minimum two sites restored at different levels of seed densities per acre (low density versus medium density; low versus high density; medium versus high density). Tukey's HSD test indicated that the variable 'plot age' had little influence on consumer isotope values. The mean carbon (Figure 3.2) and nitrogen (Figure 3.3) isotope values of oyster toadfish were statistically different between plots restored in 2001 and plots restored in 2002 ($p < 0.05$). The mean carbon (Figure 3.2) and sulfur (Figure 3.4) isotope values of pigfish ($p < 0.05$) and the mean sulfur (Figure 3.4) isotope values of grey trout ($p < 0.01$) were also statistically different depending on what year the plot they were collected from was restored. Table 3.3 specifies the significant comparisons for seed density as between either 1) low to medium density, 2) low to high density, and/or 3) medium to high density. No fish species investigated showed statistically significant comparisons between all three densities; only sheepshead had significantly different mean isotope values for more than one isotope between sites restored at the different densities. The mean carbon, nitrogen, and sulfur stable isotope values of 21 species collected in eelgrass sites restored at the three seed densities are given in Table 3.4. Only species represented by at least one individual in plots of at least two or more of the potential seed densities are included. Far more fish were collected in plots restored at the medium density due to the overlap in seed densities distributed within plots between the two years.

When the interaction of the two variables 'seed density' and 'plot age' was significant, the individual variables were not investigated. A significant interaction signifies that the isotope values of the species in question vary differently for each level of the second variable. For instance, the isotope values of fish captured in plots restored in 2001 will vary differently depending on the seed density at which the plot was restored compared with the isotope values of fish captured in plots restored in 2002. The mean value of one or more isotopes of three fish - bay anchovy, black seabass, and tautog – was affected by a statistically significant interaction between the two variables. First, the mean carbon isotope value of bay anchovy collected in plots restored in 2001 were less negative in eelgrass plots restored at 200,000 seeds per acre compared with plots restored at a lower density of 100,000 seeds per acre; the opposite was true for mean nitrogen isotope values. However, in plots restored in 2002, there was no difference in the mean carbon or nitrogen isotope values of bay anchovy depending on the plot restoration seed density. Similarly, the mean carbon isotope value of black seabass collected from plots restored in 2001 was relatively ^{13}C enriched in plots restored at 200,000 seeds per acre, while there was no difference in the mean carbon isotope values of black seabass collected from plots restored in 2002. Finally, for tautog, the mean nitrogen isotope values of individuals captured in eelgrass plots restored in 2001 were relatively ^{15}N enriched in plots restored at 200,000 seeds per acre compared with plots restored

at 100,000 seeds per acre; the opposite was true for mean sulfur isotope values. Again, there was no difference in the mean isotope values of tautog captured in plots restored at 50,000 compared to plots restored at 100,000 seeds per acre in 2002.

3.3.2. South Bay primary producers

Several potential primary nutritional sources can be found at this location, including eelgrass (*Zostera marina*), epiphytes, macroalgae (*Ulva lactuca*, *Codium fragile, Gracilaria spp.*, *Agardhiella sp.*, *Polysiphonia sp.*, *Cladophora sp.*), and smooth cordgrass (*Spartina alterniflora*). The average stable carbon isotope ratio of *Zostera marina* lies within the range of values from the literature (Stephenson *et al.*, 1986; Lepoint *et al.*, 2000; Kharlamenko *et al.*, 2001; Moncrieff and Sullivan, 2001) and was distinct from other local primary producers in South Bay in 2005 (Tables 3.5 and 3.7).

Due to the variability in isotope values of primary producers assessed over the course of the study, the isotope values of primary producers in eelgrass-free sites were not found to be significantly different from primary producers in the restored eelgrass sites in 2005 ($p > 0.05$), though the average values differed by up to 2 ‰ (i.e. $\delta^{13}C$ of *Codium fragile,* 2005). Only the mean $\delta^{34}S$ of seston in 2004 was significantly different ($p < 0.05$) between eelgrass-free sites and restored eelgrass sites. All other isotopic comparisons of primary producers were non-significant ($p > 0.05$).

3.3.3. South Bay consumers

Student's t-test performed on isotope values of all fauna collected in 2004 (un-weighted average) indicated that mean sulfur isotope values were significantly different between cover types ($p < 0.05$). For organisms collected in 2005, student's t-test indicated that the mean carbon and sulfur isotope values differed between cover types ($p < 0.01$ for each). The comparisons were performed with all species averaged across a season. Mean values for all three seasons are presented in Table 3.4.

Dual isotope plots of $\delta^{13}C$ vs. $\delta^{15}N$ of species collected in restored eelgrass meadows and eelgrass-free sites in 2005 are depicted in Figures 3.5 and 3.6, respectively. Average isotope values for each species are superimposed with error bars representing standard deviation. Only species represented by at least 5 individuals are included in the plots. Figures 3.7 and 3.8 depict dual isotope plots of $\delta^{13}C$ vs. $\delta^{34}S$ for the same species as the previous two figures.

Thirty-four consumer species in total were collected during the 2004 season, of which sixteen were collected in sufficient numbers for statistical comparison. The mean carbon, nitrogen and sulfur isotope values of all consumers analyzed in 2004 are listed in Table 3.5, by species within each cover type. Isotope values were compared when at least three individuals were captured in sites of each cover type. Eight species exhibited statistically different mean stable isotope values depending on collection site (Table 3.6).

Mean isotope values of individual species captured in restored eelgrass meadows and eelgrass-free sites in 2005 are listed in Table 3.7. Thirteen out of thirty-four total species were captured in both restored eelgrass meadows and eelgrass-free sites in sufficient numbers for statistical comparison (Table 3.8). Of the species that occurred in great enough numbers at sites of each cover type, only oyster toadfish and grass shrimp did not have any significantly different mean isotope values.

3.3.4. Fisherman's Island natural eelgrass meadow

Much of the surface area of the eelgrass leaves collected from Fisherman's Island was covered in epiphytic growth, unlike the eelgrass in South Bay, which had few attached macroalgal epiphytes and was free of calcareous epiphytes.

Nine primary producers and twenty-five consumer species were collected between the eelgrass meadow and the nearby eelgrass-free site near Fisherman's Island. Mean carbon, nitrogen, and sulfur stable isotope values of consumers and primary producers are given in Table 3.9. Primary producers were not collected in sufficient numbers for statistical comparison of stable isotope values. Of the seven consumer species captured in sufficient numbers at both sites, only ghost shrimp did not have at least one statistically different mean isotope value between collection sites (Table 3.10). ANOVA and Tukey's HSD tests were used to compare mean isotope values of nineteen species, eighteen of which were collected during both years in South Bay, and seven of which were collected

from the natural eelgrass meadow (Table 3.11). $\delta^{13}C$ values of four of the seven species differed between consumers collected in 2005 in South Bay and the natural meadow; all consumers from the natural site except one exhibited relatively depleted carbon isotope values.

3.4. Discussion

The vast majority of fish collected during this study were juveniles. As was the case in many previous studies (e.g. Lubbers *et al*., 1990; Heck *et al*., 2008; see Heck *et al*., 2003 and Beck *et al*., 2001 for reviews), greater numbers of fish and greater species diversity were noted in the restored eelgrass meadows in South Bay relative to nearby eelgrass-free locations lacking attached macrophytes (van Montfrans *et al*., 2006).

3.4.1. Effects of plot age and eelgrass cover

Seagrass cover and plot age were investigated as potential sources of fish isotope variation, as was the interaction of the two variables. Neither 'seed density' nor 'plot age' variables simultaneously affected the carbon, nitrogen and sulfur isotope values of any of the 19 species tested. The stable isotope values of three fish were affected by the interaction of the variables (Table 3.1).

As can be seen in Table 3.1, the mean nitrogen isotope value of only one species exhibited a statistically significant difference depending on the plot restoration seed density. The lack of variation likely results from little overall trophic variability between the seagrass plots due to similar prey species

availability. The mean $\delta^{13}C$ and $\delta^{34}S$ values of several species were found to be statistically different when collected from eelgrass plots restored at different seed densities (Tables 3.1 and 3.2). Carbon isotope values of consumers give an indication of an ecosystem's base primary production sources; therefore the variation in $\delta^{13}C$ of consumers may indicate that different primary producers are available within the sites depending on the density of seeds distributed, and thus eelgrass shoot density. For instance the more dense plots may tend to sequester more floating macroalgae and detrital material, which is then available to local consumers. However, this conclusion is not supported by the comparisons shown in Table 3.2; the mean $\delta^{13}C$ values of species captured in the lowest seed density plots were not statistically different from those captured in the highest density plots. Alternatively, the noted stable isotope variation may be attributable to natural variation between individuals within a species. Otherwise, significant differences were not consistently between two particular densities, but appeared more random. I expect that the consumer isotope differences noted between eelgrass plots restored at different seed densities are due to another factor that was not identified in this study.

There is no consistent pattern to how seed density and plot age affect the stable isotope values of animals in the restored eelgrass community, which indicates that these variables do not have a simple relationship to consumer $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values in South Bay. It cannot be ruled out with the current data

that the effects noted are in fact caused by different variables entirely. For these reasons, plot age and seed density were not considered when investigating samples collected during the 2005 season.

3.4.2. Primary producers

Stable isotope values of coexisting primary producers in South Bay vary considerably (see Tables 3.5 and 3.7, Figures 3.5 - 3.8). The broad ranges result from a combination of species-specific differences in isotope fractionation during metabolism and the utilization of different nutrient pools by primary producers. Of all organic matter sources, eelgrass had the most ^{13}C enriched values, and some of the most ^{15}N and ^{34}S depleted values measured. Mean carbon and nitrogen isotope values of eelgrass from South Bay were similar to those of eelgrass from the Fisherman's Island meadow. The mean sulfur isotope values of eelgrass did differ between eelgrass collected from South Bay and the natural meadow (2004, $p < 0.01$; 2005, $p < 0.05$), however, indicating that sedimentary sulfides were more important as a sulfur source for the Fisherman's Island eelgrass meadow than the restored eelgrass meadows.

As noted in this study and others performed within Virginia's coastal bays (i.e. McGlathery *et al.*, 2001), macroalgal abundance is highly variable in both space and time within South Bay. An early summer biomass peak is followed by a decline in the mid to late summer. Water temperatures during the summer of 2005 were warmer than usual for the Chesapeake Bay region (Orth *et al.*, 2010),

resulting in a Bay-wide dieback in primary producer cover, including macroalgae and eelgrass. The sharp decline in macroalgal cover noted in all sites towards the end of the summer of 2005 explains much of the difference in average carbon and sulfur isotope values of fish captured at eelgrass sites versus eelgrass-free sites. Though there was a mid-summer decrease in macroalgal biomass in 2004 as well, it was not as severe as in 2005, which somewhat explains why the number of isotopic differences between species captured in restored eelgrass and eelgrass-free sites was fewer than in 2005.

Macroalgae are a major primary production source throughout the region; thus the loss of live macroalgal biomass would affect the local ecosystem. First, the relative importance of the remaining primary production sources would change in response to the loss of biomass followed by a corresponding change in consumer stable isotope values reflecting the changes in dietary sources. Second, the decomposing organic matter would likely have different stable isotope values than the original living macroalgae. Decomposition can result in a shift in isotope ratios of plant detritus due to variable decay rates of individual components of the original material (Fry and Sherr, 1984; Ember *et al.*, 1987), or due to microbial activity (Mann, 1988; Peterson, 1999). Isotope values of detritivores feeding on the decaying material and microbes then shift relative to the change in diet. In addition to effects related to the direct consumption of detrital material, decomposition releases nutrients contained in decaying organic

matter. The nutrients previously bound within the plants are then recycled within the system and add into the available nutrient pool.

Previous studies in the lower Chesapeake Bay have found that the majority of the water column particulate matter is derived from autochthonous sources, specifically a combination of live and detrital phytoplankton and bacteria. Canuel and Zimmerman (1999) found a notable increase in heterotrophic biomass (bacteria and zooplankton) during summer months. The $\delta^{13}C$ value of -19 ‰ collected for seston in South Bay support this conclusion, as it is near the approximately -22 ‰ value found in the literature for phytoplankton (Peterson et al., 1985; Peterson and Fry, 1987; Michener and Schell, 1994). Sulfur isotope values were determined for only one seston sample collected in the eelgrass meadows in August 2005 and for none of the samples collected in the eelgrass-free sites due to the large amount of sample required for sulfur isotope analysis, therefore seston cannot be positively identified or ruled out as a production source. The single sulfur isotope value measured for seston from the eelgrass meadow was 18.3 ‰, which approaches that of seawater sulfate at approximately +20 to +21 ‰ (Peterson *et al.*, 1985; Michener and Schell, 1994). Phytoplankton obtain sulfur directly from seawater sulfate and so phytoplankton $\delta^{34}S$ values resemble those of seawater sulfate. The $\delta^{34}S$ values of seston collected in 2004, on the other hand, indicate a sulfur source composed primarily of sedimentary sulfides, which are highly ^{34}S depleted relative to the

sulfur isotope value of seawater sulfate. $\delta^{34}S$ of sedimentary sulfides are highly variable but generally range from -20 to - 10 ‰ (Peterson *et al*., 1986; Michener and Schell, 1994; Frederiksen *et al*., 2007). Considering the sharp distinction between $\delta^{34}S$ values of seston from 2004 and 2005 it is possible that there was contamination of the single sample analyzed for $\delta^{34}S$ in 2005, though contamination cannot be confirmed as the entire sample was used for the initial isotopic measurement. It is more likely that fine sediments were stirred up into the water column by the seining activity at the sites, which released sulfides into the water column and were then collected in the water samples. Water samples were collected prior to seining in 2005, as opposed to 2004, which were taken after seining was completed.

Benthic microalgae (mainly diatoms, cyanobacteria, and dinoflagellates) are acknowledged as important producers in eelgrass systems as a considerable portion of the available nitrogen in seagrass meadows can be provided by nitrogen fixing organisms such as cyanobacteria at the sediment surface (Touchette and Burkholder, 2000). Benthic macroalgae was not collected due to the many difficulties in obtaining sufficient biomass for isotope analysis, though stable isotope values of benthic microalgae are available from the literature. Microalgae typically have carbon isotope values ranging from -23 to -14 ‰ (Peterson and Fry, 1987; Weinstein *et al*., 2000; Sullivan and Moncrieff, 1990; Connolly, 2003; Hoshika *et al*., 2006) and $\delta^{15}N$ values range from 5 to 10 ‰

(Currin *et al*., 1995; France, 1995; Kang *et al*., 2003). Sulfur isotope values of benthic microalgae are not common in the literature, though samples collected by Sullivan and Moncrieff (1990) were found to be 14.3 ‰. Assuming that isotope values of benthic microalgae from the literature are similar to those in South Bay, benthic microalgae are important base primary producers in South Bay. $\delta^{13}C$ values of consumers in the eelgrass meadows as well as in the eelgrass-free sites were measured across much of the range of values reported for benthic microalgae.

3.4.3. Consumers

As expected, $\delta^{13}C$ values of primary producers and consumers from the restored eelgrass meadows (Figures 3.5 and 3.7) indicate that no species feed solely on live eelgrass tissues. If that were the case, that particular organism would be located on the dual isotope plot at one trophic level of enrichment, or approximately 1 ‰ for $\delta^{13}C$, 3 ‰ for $\delta^{15}N$, and 0 ‰ for $\delta^{34}S$. Specifically, the organism would have mean carbon, nitrogen, and sulfur isotope values of approximately -8 ‰, 11 ‰, and 11 ‰, respectively; however, no organism fits those criteria. Rather, a mixture of food sources that includes a small proportion of eelgrass tissue is possible such that multiple combinations of primary producers in a range of proportions are positioned on a mixing line representing fractions of consumer diets.

The main objective of the 2004 study was to investigate whether seed density and plot age of the restored eelgrass meadows influenced the stable isotope values of consumers in this ecosystem. However, isotope differences related to the presence or lack of eelgrass at the sites were also investigated. Fewer species showed statistically significant isotopic differences between restored eelgrass meadows and eelgrass-free sites in 2004 (eight of sixteen species; Table 3.6) compared to 2005 (eleven of thirteen species; Table 3.8). Also, only three of those species had multiple isotope differences between sites in 2004, while the values of at least two of the three measured stable isotopes were statistically different between site types for seven of the species from 2005. No species had isotope compositions that were statistically different for carbon, nitrogen, and sulfur depending on the collection site type in 2004, while the average $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of spot and Atlantic silversides collected in 2005 were statistically different between site types (Table 3.8).

Table 3.8 shows that fish species captured in 2005 in the restored eelgrass meadows and the nearby macroalgal-dominated eelgrass-free sites are well differentiated based on sulfur isotope values. Mean sulfur isotope values of all but four of the thirteen species compared between restored eelgrass meadows and eelgrass-free sites were statistically different and in all cases the average sulfur isotope values of fish in the eelgrass meadows were higher than the eelgrass-free sites. Similarly, for all statistically significant comparisons, the

average carbon isotope values of fish in the eelgrass meadows were higher than in eelgrass-free sites. Approximately half of the significant comparisons for fish nitrogen isotope values showed an increase between restored eelgrass and eelgrass-free sites, while the other half showed a decrease. Nitrogen isotopes are strongly affected by trophic position and since many species are opportunistic feeders, the available food sources define which fish occupy different trophic levels dependent upon the available food sources. On the other hand, consumer $\delta^{13}C$ and $\delta^{34}S$ values more closely resemble those of dietary sources (Peterson and Fry, 1987).

A large decrease in carbon and sulfur isotope values of several fish species was observed in eelgrass-free sites late in the 2005 season. The differences were large enough such that the average isotope values of fish at the eelgrass-free sites were shown to be statistically different from fish at the eelgrass meadows even when consumer isotope values were averaged over the course of the entire sampling season. A visual comparison of Figures 3.4 and 3.5 reveal the strong distinction in the mean carbon and/or the sulfur isotope values of several species between restored eelgrass and eelgrass-free sites. The difference in average sulfur isotope values of consumers between eelgrass and eelgrass-free habitats sheds light on the sulfur pools utilized by the primary producers supporting the food web. There is generally little fractionation between the sulfur isotope values of macro-consumers and the food consumed. However, sulfate-reducing bacteria

produce sulfides in marine sediments that are up to 50 ‰ depleted (Frederikson *et al*., 2006) relative to seawater sulfate at +21 ‰. Sulfide uptake through root systems anchored in reducing sediments has been observed in eelgrass (Fry *et al*., 1982; Fredericksen *et al*., 2006) as well as *Spartina alterniflora* (Carlson and Forrest, 1982; Fry *et al*., 1982), with the ^{34}S-depleted sulfur gradually redistributing from the roots to the rhizomes and eventually to the leaves. Thus the relatively lower $\delta^{34}S$ values of consumers from the eelgrass-free sites results from the dominance of an organic matter source that relies at least partly on sedimentary sulfides.

Two possible explanations for consumer isotopic distinction depending on habitat are; 1) the organic matter sources supporting the food web in the eelgrass meadows have distinct isotope values from those supporting the food web in the eelgrass-free sites; and 2) The fish collected were cohorts recently settled at the sites, each group having come from a population with distinct average isotope compositions. In the first scenario, the difference in isotope values can be explained by a change in the major organic matter source or sources utilized by primary consumers, which would then filter up through the food web to higher level consumers. A shift to decomposing macroalgal matter as the main source for primary consumers, and therefore the prevalence of the detrital pathway, would fit this scenario. In the second case, the fish would have arrived from elsewhere, likely within the Chesapeake Bay, having already acquired an isotopic signature similar to dietary sources at the previous location. In either

case, the distinct isotope compositions of the species between the site types indicate that individuals tend not to move between the sites once settled.

Due to the close proximity of Fisherman's Island to the mouth of the eutrophic Chesapeake Bay, it was expected that anthropogenic nutrient loading would strongly affect the $\delta^{15}N$ values of organisms in the natural eelgrass meadow adjacent to the island. $\delta^{15}N$ values of inorganic nitrogen (nitrate and ammonium) from industrial and domestic sewage effluent, particularly human and animal waste, are relatively enriched compared with natural marine sources of nitrogen (McClelland *et al.*, 1997; Tucker *et al.*, 1999). In addition, periods of oxygen depletion in the Chesapeake Bay promote intense denitrification and nutrient recycling, resulting in relatively enriched nitrogen isotope values of plants and surficial sediments throughout the region (Bratton *et al.*, 2003). However, stable isotope analysis of consumers and primary producers collected within the eelgrass meadow shows little evidence of the influence of such anthropogenic nutrient inputs.

3.5. Summary

The isotope values of consumer species in the restored eelgrass meadows were expected to be somewhat similar to those of the same species collected from eelgrass-free locations. Rather, there were distinct differences between the isotope values of consumers depending on the habitat from which they were collected, which indicates that these consumers tend to remain within one or the

other habitat and tend not to move between the sites. This conclusion is supported by the findings from an earlier part of this study in which stomach content analysis showed that the material ingested by several species differed in restored seagrass meadows and eelgrass-free sites (van Montfrans *et al.*, 2006).

The results of this study emphasize the importance of microbial decomposition of organic matter and the general recycling of nutrients within South Bay. Unlike in tropical seagrass systems, there are few large consumers in temperate regions that feed on seagrass directly. Therefore, the importance of seagrass to the local food web is mainly limited to indirect influence via decomposition and the detrital pathway, not unlike the fate of *Spartina spp.* primary production in temperate salt marshes (Peterson *et al.*, 1985; Peterson *et al.*, 1986; Peterson and Howarth, 1987; Currin *et al.*, 1995; Kwak and Zedler, 1997). Higher than normal temperatures in the region increased environmental stress on the system, which explains why the differences in mean isotope values of consumers collected in eelgrass meadows and eelgrass-free sites in 2005 were more distinct than those collected in 2004. Temperature stress resulted in widespread death and decay of primary producers throughout South Bay. Nutrients bound in the organic matter were released and made available to the remaining primary producers as bacteria modified the dead material. The high productivity rates observed in temperate seagrass meadows would not be sustainable without nutrient recycling.

3.6. References

Beck, M.W., Heck, K.L. Jr., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., and Weinstein, M.P. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. Bioscience 51 (8): 633-641.

Bratton, J.F., Colman, S.M., Seal, R.R., II. 2003. Eutrophication and carbon sources in Chesapeake Bay over the last 2700 yr: human impacts in context. Geochimica et Cosmochimica Acta 67 (18): 3385-3402.

Canuel, E.A., and Zimmerman, A.R. 1999. Composition of particulate organic matter in the southern Chesapeake Bay: sources and reactivity. Estuaries 22 (4): 980-994.

Carlson, P.R., and Forrest, J. 1982. Uptake of dissolved sulfide by *Spartina alterniflora* – evidence from natural sulfur isotope abundance ratios. Science 216: 633-635.

Connolly, R.M. 2003. Differences in trophodynamics of commercially important fish between artificial waterways and natural coastal wetlands. Estuarine Coastal and Shelf Science 58: 929-936.

Currin, C.A., Newell, S.Y., Paerl, H.W. 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. Marine Ecology Progress Series 121: 99-116.

Duarte, C.M. 2002. The future of seagrass meadows. Environmental Conservation 29 (2): 192-206.

Duarte, C.M., Fourqurean, J.W., Krause-Jensen, D., and Olesen, B. 2006. Dynamics of seagrass stability and change. Pages 271-294 in: Larkum, A.W.D., R.J. Orth, and C.M. Duarte (eds), *Seagrasses: Biology, ecology and conservation*. Dordrecht (Netherlands): Springer. 691 pages.

Ember, L.M., Williams, D.F., and Morris, J.T. 1987. Processes that influence carbon isotope variations in salt marsh sediments. Marine Ecology Progress Series 36: 33-42.

Fonseca, M.S., and Fisher, J.S. 1986. A comparison of canopy friction and sediment movement between four species of seagrass with reference to their ecology and restoration. Marine Ecology Progress Series 29: 15-22.

Fonseca, M.S., Kenworthy, W.J., Colby, D.R., Rittmaster, K.A., and Thayer, G.W. 1990. Comparisons of fauna among natural and transplanted eelgrass *Zostera marina* meadows: criteria for mitigation. Marine Ecology Progress Series 65: 251-264.

France, R.L. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: food web implications. Marine Ecology Progress Series 124: 307-312.

Frederiksen, M.S., Holmer, M., Borum, J., and Kennedy, H. 2006. Temporal and spatial variation of sulfide invasion in eelgrass (*Zostera marina*) as reflected by its sulfur isotopic composition. Limnology and Oceanography 51 (5): 2308-2318.

Frederiksen, M.S., Holmer, M., Díaz-Almela, E., Marba, N., and Duarte, C.M. 2007. Sulfide invasion in the seagrass *Posidonia oceanica* at Mediterranean fish farms: assessment using stable sulfur isotopes. Marine Ecology Progress Series 345: 93-104.

French McKay, D.P., and Rowe, J.J. 2003. Habitat restoration as mitigation for lost production at multiple trophic levels. Marine Ecology Progress Series 264: 233-247.

Fry, B., Scalan, R.S., Winters, K., and Parker, P.L. 1982. Sulphur uptake by salt grasses, mangroves, and seagrasses in anaerobic sediments. Geochimica et Cosmochimica Acta 46: 1121-1124.

Fry, B., and Sherr, E. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions in Marine Science 27: 15-47.

Goldstein, B.A., Perkins-Visser, E., Wolcott, T.G., and Wolcott, D.L. 1996. Nursery role of seagrass beds: enhanced growth of juvenile blue crabs (*Callinectes sapidus* Rathburn). Journal of Experimental Marine Biology and Ecology 198 (2): 155-173.

Harris, L.A., Buckley, B., Nixon, S.W., and Allen, B.T. 2004. Experimental studies of predation by bluefish *Pomatomus saltatrix* in varying densities of seagrass and macroalgae. Marine Ecology Progress Series 281: 233-239.

Heck, K.L. Jr., Hays, G., and Orth, R.J. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series 253: 123-136.

Heck, K.L. Jr., Carruthers, T.J.B., Duarte, C.M., Hughes, A.R., Kendrick, G., Orth, R.J., and Williams, S.J. 2008. Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. Ecosystems 11: 1198-1210.

Hoshika, A., Sarker, M.J., Ishida, S., Mishima, Y., and Takai, N. 2006. Food web analysis of an eelgrass (*Zostera marina* L.) meadow and neighboring sites in Mitsukuchi Bay (Seto Inland Sea, Japan) using carbon and nitrogen stable isotope ratios. Aquatic Botany 85: 191-197.

Hughes, J.E., Deegan, L.A., Wyda, J.C., Weaver, M.J., and Wright, A. 2002. The effects of eelgrass habitat loss on estuarine fish communities of southern New England. Estuaries 25 (2): 235-249.

Kang, C.-K., Kim, J.B., Lee, K.-S., Kim, J.B., Lee, P.-L., and Hong, J.-S. 2003. Trophic importance of benthic microalgae to macrozoobenthos in coastal bay systems in Korea: dual stable C and N isotope analyses. Marine Ecology Progress Series 259: 79-92.

Kemp, W.M., Twilley, R.R., Stevenson, J.C., Boynton, W.R., and Means, J.C. 1983. The decline of submerged vascular plants in upper Chesapeake Bay: Summary of results concerning possible causes. Marine Technology Society Journal 17 (2): 78-89.

Kharlamenko, V.I., Kiyashko, S.I., Imbs, A.B., and Vyshkvartzev, D.I. 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. Marine Ecology Progress Series 220: 103-117.

Kwak, T.J., and Zedler, J.B. 1997. Food web analysis of southern California coastal wetlands using multiple stable isotopes. Oecologia 110: 262-277.

Lepoint, G., Nyssen, F., Gobert, S., Dauby, P., and Bouquegneau, J.-M. 2000. Relative impact of a seagrass bed and its adjacent epilithic algal community on consumer diets. Marine Biology 136: 513-518.

Lubbers, L., Boynton, W.R., and Kemp, W.M. 1990. Variations in structure of estuarine fish communities in relation to abundance of submersed vascular plants. Marine Ecology Progress Series 65: 1-14.

Mann, K.H. 1988. Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnology and Oceanography 33 (4, part 2): 910-930.

McClelland, J.W., Valiela, I., and Michener, R.H. 1997. Nitrogen stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. Limnology and Oceanography 42 (5): 930-937.

McGlathery, K.J., Anderson, I.C., and Tyler, A.C. 2001. Magnitude and variability of benthic and pelagic metabolism in a temperate coastal lagoon. Marine Ecology Progress Series 216: 1-15.

McLaughlin, P.A., Treat, S.F., Thorhaug, A., and Lemaitre, R. 1983. A restored seagrass (*Thalassia*) bed and its animal community. Environmental Conservation 10 (3): 247-254.

Michener, R.H., and Schell, D.M. 1994. Stable isotope ratios as tracers in marine aquatic food webs. Pages 138-157 in: Lajthe, K. and Michener, R.M. (eds.), *Methods in Ecology: Stable Isotopes in Ecology and Environmental Science.* Oxford (UK): Blackwell Scientific Publications. 316 pages.

Minello, T.J., Able, K.W., Weinstein, M.P., and Hays, C.G. 2003. Salt marshes as nurseries for nekton: testing hypotheses on density, growth and survival through meta-analysis. Marine Ecology Progress Series 246: 39-59.

Moncrieff, C.A., and Sullivan, M.J. 2001. Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. Marine Ecology Progress Series 215: 93-106.

Moore, K.A., and Jarvis, J.C. 2008. Environmental factors affecting recent summertime eelgrass diebacks in the lower Chesapeake Bay: Implications for long-term persistence. Journal of Coastal Research SI 55: 135-147.

Moore, K.A., and Short, F.T. 2006. *Zostera*: biology, ecology, and management. Pages 361-386 in: Larkum, A.W.D., Orth, R.J., and Duarte, C.M. (eds.), *Seagrasses: biology, ecology, and conservation*. Dordrecht (Netherlands): Springer. 691 pages.

Orth, R.J. 1976. The demise and recovery of eelgrass, *Zostera marina*, in the Chesapeake Bay, Virginia. Aquatic Botany 2: 141-159.

Orth, R.J., and Moore, K.A. 1983. Chesapeake Bay: An unprecedented decline in submerged aquatic vegetation. Science 222: 51-53.

Orth, R.J. 1994. Chesapeake Bay submersed aquatic vegetation: Water quality relationships. Lake and Reservoir Management 10 (1): 49-52.

Orth, R.J., Luckenbach, M.L., Marion, S.M., Moore, K.A., and Wilcox, D.J. 2006. Seagrass recovery in the Delmarva coastal bays, USA. Aquatic Botany 84: 26-36.

Orth, R.J., Marion, S.R., Moore, K.A., and Wilcox, D.J. 2010. Eelgrass (*Zostera marina* L.) in the Chesapeake Bay region of mid-Atlantic coast of the USA: challenges in conservation and restoration. Estuaries and Coasts 33: 139-150.

Peterson, B.J., Howarth, R.W., and Garritt, R.H. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science 227: 1361-1363.

Peterson, B.J., Howarth, R.W., and Garritt, R.H. 1986. Sulfur and carbon isotope as tracers of salt-marsh organic matter flow. Ecology 67 (4): 865-874.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18: 293-320.

Peterson, B.J., and Howarth, R.W. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnology and Oceanography 32 (6): 1195-1213.

Peterson, B.J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. Acta Oecologia 20 (4): 479-487.

Rasmussen, E. 1977. The wasting disease of eelgrass (*Zostera marina*) and its effects on environmental factors and fauna. Pages 1-51 in: McRoy, C.P. and C. Helfferich (eds.), *Seagrass Ecosystems: A Scientific Perspective*. New York: Marcel Dekker. 314 pages.

Short, F.T., Koch, E.M., Creed, J.C., Magalhaes, K.M., Fernandes, E., and Gaeckle, J.L. 2006. SeagrassNet monitoring across the Americas: case studies of seagrass decline. Marine Ecology 27 (4): 277-289.

Short, F.T., and Neckles, H.A. 1999. The effects of global change on seagrasses. Aquatic Botany 63: 169-196.

Short, F.T., and Wyllie-Echeverria, S. 1996. Natural and human-induced disturbance of seagrasses. Environmental Conservation 23: 17-27.

Stephenson, R.L., Tan, F.C., and Mann, K.H. 1986. Use of stable carbon isotope ratios to compare plant material and potential consumers in a seagrass bed and a kelp bed in Nova Scotia, Canada. Marine Ecology Progress Series 30: 1-7.

Sullivan, M.J., and Moncrieff, C.A. 1990. Edaphic algae are an important component of salt marsh food-webs: evidence from multiple stable isotope analysis. Marine Ecology Progress Series 62: 149-159.

Touchette, B.W., and Burkholder, J.M. 2000. Review of nitrogen and phosphorus metabolism in seagrasses. Journal of Experimental Marine Biology and Ecology 250: 133-167.

Tucker, J., Sheats, N., Giblin, A.E., Hopkinson, C.S., Montoya, J.P. 1999. Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. Marine Environmental Research 48 (4-5): 353-375.

van Montfrans, J., Macko, S.A., Harbeson, S.A., and Combs, D. 2006. Trophic Interactions of fish predators utilizing restored seagrass beds in Virginia's Coastal Bays. Final Report for FY 2003, Task 12.05. 7 pages.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L. Jr., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., and Williams, S.L. 2009. Accelerating loss of seagrass across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences 106: 12377-12381.

Weinstein, M.P., Litvin, S.Y., Bosley, K.L., Fuller, C.M., and Wainright, S.C. 2000. The role of tidal salt marsh as an energy source for marine transient and resident finfishes: A stable isotope approach. Transactions of the American Fisheries Society 129: 797-810.

Table 3.1. Shows significant comparisons for ANOVA and Tukey's HSD tests investigating two variables; 1) density of seeds dispersed, and 2) plot age, as well as the interaction of the variables on stable isotope values of fish captured in 2004. Significant comparisons are indicated as follows: * $p < 0.05$; ** $p < 0.01$.

Species name	Common Name	Seed Density			Plot Age			Interaction		
		$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Menidia menidia	Atlantic silversides		**							
Anchoa mitchilli	Bay anchovy			*				*	*	
Centropristis striata	Black seabass			*				*		
Callinectes sapidus	Blue crab									
Mycteroperca microlepis	Gag grouper									
Palaemonetes spp.	Grass shrimp									
Cynoscion regalis	Grey trout (weakfish)						**			
Syngnathus fuscus	Northern pipefish	*								
Sphyraena borealis	Northern sennet									
Opsanus tau	Oyster toadfish	*			*	*				
Orthopristis chrysoptera	Pigfish				*		*			
Lagodon rhomboides	Pinfish									
Archosargus probatocephalus	Sheepshead	**		*						
Bairdiella chrysoura	Silver perch			**						
Leiostomus xanthurus	Spot	**								
Cynoscion nebulosus	Spotted seatrout									
Paralichthys dentatus	Summer flounder	*								
Tautoga onitis	Tautog	**							*	*
Merlangius merlangus	Whiting									

Table 3.2. Results of ANOVA and Tukey's HSD test of differences of mean isotope values of consumers focusing on variable 'seed density.' [1] Low and medium density sites were significantly different; [2] Low and high density sites were significantly different; [3] Medium and high density sites were significantly different.

Species name	Common name	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Menidia menidia	Atlantic silversides		$p < 0.01^{1,2}$	
Anchoa mitchilli	Bay anchovy			$p < 0.05^{1}$
Centropristis striata	Black seabass			$p < 0.05^{2}$
Callinectes sapidus	Blue crab			
Mycteroperca microlepis	Gag grouper			
Palaemonetes spp.	Grass shrimp			
Cynoscion regalis	Grey trout (weakfish)			
Syngnathus fuscus	Northern pipefish	$p < 0.05^{3}$		
Sphyraena borealis	Northern sennet			
Opsanus tau	Oyster toadfish	$p < 0.05^{3}$		
Orthopristis chrysoptera	Pigfish			
Lagodon rhomboides	Pinfish			
Archosargus probatocephalus	Sheepshead	$p < 0.01^{1,3}$		$p < 0.05^{1,3}$
Bairdiella chrysoura	Silver perch			$p < 0.01^{1,2}$
Leiostomus xanthurus	Spot	$p < 0.01^{1}$		
Cynoscion nebulosus	Spotted seatrout			
Paralichthys dentatus	Summer flounder	$p < 0.05^{3}$		
Tautoga onitis	Tautog	$p < 0.01^{1}$		
Merlangius merlangus	Whiting			

Table 3.3. Mean and standard deviation of stable carbon, nitrogen, and sulfur isotope values of consumers captured in plots restored at the three different seed densities in 2004. 'n' refers to the number of samples. Greater numbers of fish were captured in the medium density plots due to the overlap in seed densities distributed within plots between 2001 and 2002.

	Low density (50,000 seeds/acre)				**Medium density (100,000 seeds/acre)**				**High density (200,000 seeds/acre)**			
Common name	n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Atlantic croaker					6	-16.0 ± 1.5	13.2 ± 0.5	14.9 ± 0.6	1	-14.7	12.7	15.0
Atlantic silversides	5	-17.6 ± 0.4	12.3 ± 0.9	15.3 ± 1.0	16	-17.4 ± 1.0	14.0 ± 0.7	15.7 ± 1.2	14	-16.4 ± 1.4	13.7 ± 0.4	15.0 ± 4.4
Bay anchovy	10	-17.7 ± 1.1	13.6 ± 1.4	15.1 ± 1.9	20	-18.4 ± 1.5	14.2 ± 1.1	16.6 ± 0.9	3	-15.3 ± 0.7	13.2 ± 0.4	16.4 ± 0.5
Black seabass	7	-15.1 ± 0.7	13.6 ± 0.7	14.2 ± 1.6	12	-15.2 ± 1.0	13.7 ± 0.7	15.5 ± 0.9	11	-14.8 ± 0.8	13.3 ± 0.4	15.8 ± 0.8
Blue crab	3	-14.2 ± 0.6	12.9 ± 0.7	15.9 ± 0.1	4	-14.3 ± 3.2	11.5 ± 2.9	14.8 ± 2.1	4	-14.5 ± 0.9	13.8 ± 3.0	15.6 ± 2.6
Gag grouper					10	-14.4 ± 0.7	14.0 ± 0.7	16.0 ± 1.0	4	-14.1 ± 0.7	13.9 ± 0.1	15.7 ± 0.4
Grass shrimp					6	-14.5 ± 0.9	12.7 ± 1.4	13.9 ± 2.0	2	-14.0	11.7	19.2
Grey trout (weakfish)	2	-15.7	13.7	16.7	8	-16.7 ± 0.7	13.0 ± 1.0	13.6 ± 2.3	3	-16.3 ± 0.2	13.4 ± 0.3	16.1 ± 0.4
Northern pipefish	15	-14.7 ± 0.8	12.7 ± 0.8	13.6 ± 1.2	30	-15.4 ± 0.8	12.5 ± 0.7	14.0 ± 1.9	15	-14.6 ± 0.9	12.7 ± 0.6	14.9 ± 0.9
Northern puffer					3	-15.5 ± 0.3	12.9 ± 0.5	14.2 ± 0.6	1	-14.6	12.5	15.5
Northern sennet	2	-16.2	13.7	16.7	3	-17.2 ± 0.5	13.6 ± 1.4	15.9 ± 0.4				
Oyster toadfish					6	-14.9 ± 0.8	13.3 ± 0.9	13.7 ± 1.6	3	-13.2 ± 0.9	12.8 ± 0.5	15.3 ± 1.7
Pigfish	6	-15.1 ± 0.3	13.4 ± 0.8	14.2 ± 0.4	27	-15.8 ± 0.8	13.2 ± 0.5	14.6 ± 1.6	12	-15.9 ± 0.9	12.9 ± 0.7	14.9 ± 2.0
Pinfish					13	-15.0 ± 0.8	12.7 ± 0.5	15.5 ± 1.4	5	-14.6 ± 0.8	13.1 ± 0.3	16.7 ± 0.7
Sheepshead	5	-14.2 ± 0.4	13.0 ± 0.3	16.1 ± 1.8	4	-15.9 ± 0.2	12.5 ± 0.9	13.1 ± 1.7	3	-13.3 ± 0.9	13.4 ± 0.2	16.9 ± 1.2
Silver perch	15	-15.4 ± 0.8	14.1 ± 1.0	14.8 ± 1.6	36	-15.4 ± 0.8	14.2 ± 1.0	15.7 ± 0.6	15	-14.9 ± 0.8	14.3 ± 0.9	15.7 ± 0.6
Spot	5	-13.4 ± 0.3	12.7 ± 1.3	12.3 ± 0.8	4	-14.9 ± 0.5	12.1 ± 0.4	12.3 ± 1.2				
Spotted seatrout	1	-14.5	14.0	15.6	5	-16.2 ± 0.7	14.0 ± 0.8	15.7 ± 1.1				
Summer flounder	1	-14.5	11.5	12.9	13	-15.6 ± 0.8	13.1 ± 2.1	14.3 ± 0.7	6	-14.3 ± 0.8	13.4 ± 1.0	14.5 ± 0.9
Tautog	7	-13.2 ± 1.1	13.6 ± 0.5	15.8 ± 0.6	24	-14.9 ± 0.8	13.1 ± 0.6	15.3 ± 2.2	10	-14.3 ± 1.1	13.9 ± 0.5	15.5 ± 0.8
Whiting	5	-14.8 ± 0.4	13.4 ± 0.3	13.7 ± 1.0	5	-14.4 ± 0.7	13.2 ± 0.4	14.1 ± 0.2				

Table 3.4. Mean, minimum, and maximum $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values (± standard deviation) of all consumers analyzed, separated by collection year and location.

Year	Site		$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
South Bay 2004	Restored eelgrass meadows	Min	-21.68	7.3	9.6
		Max	-9.5	19.7	19.4
		Mean	-15.4 ± 1.5	13.3 ± 1.1	15.1 ± 1.6
	Eelgrass-free sites	Min	-20.9	5.3	9.4
		Max	-11.3	16.7	21.4
		Mean	-15.2 ± 1.6	13.1 ± 1.4	14.7 ± 1.5
South Bay 2005	Restored eelgrass meadows	Min	-19.5	10.5	7.8
		Max	-10.5	23.0	21.0
		Mean	-15.5 ± 1.4	14.3 ± 1.8	14.3 ± 2.5
	Eelgrass-free sites	Min	-26.8	9.8	4.8
		Max	-10.6	19.3	17.2
		Mean	-16.6 ± 2.5	14.4 ± 1.9	12.4 ± 2.5
Fisherman's Island 2006	Natural eelgrass meadow	Min	-20.3	8.4	6.6
		Max	-9.8	15.4	18.3
		Mean	-15.6 ± 1.9	12.9 ± 1.7	13.7 ± 2.3
	Eelgrass-free site	Min	-19.0	11.2	9.2
		Max	-11.0	19.1	18.5
		Mean	-15.4 ± 1.8	14.0 ± 2.1	13.3 ± 2.0

Table 3.5. Mean carbon, nitrogen and sulfur stable isotope values (± standard deviation) of organisms collected in South Bay restored eelgrass meadows and eelgrass-free sites in 2004. 'n' indicates number individuals analyzed. [a] tentative identification. [b] insufficient sample remained for sulfur analysis.

Scientific name	Common name	Restored eelgrass meadows				Eelgrass-free sites			
		n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Agardhiella sp.		1	-17.2	8.7	19.7				
Ceramium rubrum[a]	Epiphytic algae	4	-15.6 ± 1.3	9.9 ± 0.3	14.2 ± 3.3	2	-14.8	9.4	16.8
Codium fragile	Dead man's fingers	1	-13.0	11.7	18.9	2	-13.5	9.6	20.7
Gracilaria spp.	Graceful redweed	2	-15.3	10.4	18.0	3	-17.3 ± 1.2	10.0 ± 1.0	18.5 ± 1.0
	Seston	9	-20.2 ± 1.8	10.0 ± 3.9	-0.8 ± 1.8	9	-20.2 ± 2.9	10.9 ± 4.1	-4.5 ± 2.5
Ulva lactuca	Sea lettuce	3	-12.4 ± 2.3	10.1 ± 0.8	17.0 ± 0.9	2	-11.7	9.8	17.7
Zostera marina	Live eelgrass	6	-9.7 ± 0.8	8.1 ± 2.0	13.3 ± 2.6				
Z. marina	Standing dead eelgrass	3	-10.4 ± 0.5	7.6 ± 0.3	10.0 ± 2.3				
Z. marina	Detrital eelgrass	1	-15.5	10.2	15.7				
Micropogonias undulatus	Atlantic croaker	7	-15.8 ± 1.4	13.1 ± 0.5	14.9 ± 0.6	7	-15.6 ± 1.1	12.7 ± 1.1	13.3 ± 0.8
Menidia menidia	Atlantic silversides	35	-17.1 ± 1.2	13.6 ± 0.8	15.9 ± 1.1	47	-16.7 ± 1.0	14.0 ± 0.8	15.2 ± 0.9
Anchoa mitchilli	Bay anchovy	33	-17.9 ± 1.6	13.9 ± 1.2	16.1 ± 1.4	12	-17.8 ± 1.9	14.8 ± 1.2	16.1 ± 1.5
Centropristis striata	Black seabass	30	-14.9 ± 0.9	13.5 ± 0.6	15.3 ± 1.2	3	-14.1 ± 1.0	13.5 ± 0.1	15.2 ± 1.0
Callinectes sapidus	Blue crab	11	-14.3 ± 1.8	12.7 ± 2.5	15.1 ± 1.9	11	-15.3 ± 1.5	11.4 ± 0.8	14.3 ± 1.6
Pomatomus saltatrix	Bluefish	1	-17.1	14.1	14.9	6	-17.3 ± 2.4	14.2 ± 1.7	14.6 ± 2.9
Hypsoblennius hentzi	Feather blenny	2	-15.0	13.6	13.0	2	-14.7	14.2	16.1
Mycteroperca microlepis	Gag grouper	14	-14.3 ± 0.7	14.0 ± 0.6	15.9 ± 0.8	3	-12.8 ± 0.3	13.8 ± 0.9	15.8 ± 1.6
Palaemonetes spp.	Grass shrimp	8	-14.4 ± 1.0	12.5 ± 1.6	15.7 ± 3.2	10	-13.9 ± 1.0	12.2 ± 1.9	15.0 ± 1.8
Cynoscion regalis	Grey trout (weakfish)	13	-16.4 ± 0.6	13.2 ± 0.8	14.7 ± 2.3	2	-15.8	12.6	15.4
Stomatopod	Mantis shrimp					2	-14.2	11.8	12.9
Eucinostomus argenteus	Spotfin mojarra					4	-13.9 ± 0.2	13.4 ± 0.2	13.1 ± 0.2
	Mud crab					1	-16.6	11.6	15.7
Gobiosoma bosci	Naked goby	1	-15.8	13.3	16.1	16	-14.5 ± 0.6	13.1 ± 1.1	13.2 ± 0.7

Syngnathus fuscus	Northern pipefish	64	-15.0 ± 0.9	12.6 ± 0.7	14.1 ± 1.6	33	-14.5 ± 1.3	12.5 ± 0.8	14.2 ± 1.3
Sphoeroides maculatus	Northern puffer	4	-15.2 ± 0.5	12.8 ± 0.4	14.5 ± 0.8	1	-15.0	12.3	14.1
Sphyraena borealis	Northern sennet	5	-16.8 ± 0.7	13.6 ± 1.0	16.2 ± 0.6	2	-15.4	15.4	15.3
Opsanus tau	Oyster toadfish	9	-14.3 ± 1.2	13.1 ± 0.8	14.2 ± 1.7	15	-14.3 ± 1.0	12.1 ± 1.1	15.3 ± 2.2
Penaeus sp.	Prawn	1	-13.5	12.7	21.3	4	-12.9 ± 0.4	12.0 ± 0.3	19.1 ± 4.7
Orthopristis chrysoptera	Pigfish	45	-15.7 ± 0.8	13.1 ± 0.6	14.6 ± 1.6	29	-15.1 ± 1.2	13.4 ± 0.7	13.7 ± 1.3
Lagodon rhomboides	Pinfish	18	-14.9 ± 0.8	12.8 ± 0.5	15.8 ± 1.3	14	-15.2 ± 0.5	13.1 ± 1.0	15.3 ± 1.2
Prionotus sp.	Searobin	2	-16.0	12.3	13.8	1	-13.3	13.7	13.3
Hippocampus erectus	Seahorse	1	-14.2	13.7	15.6				
Archosargus probatocephalus	Sheepshead	12	-14.5 ± 1.2	13.0 ± 0.7	15.3 ± 2.2	11	-14.4 ± 1.7	13.2 ± 0.6	14.8 ± 0.8
Bairdiella chrysoura	Silver perch	67	-15.3 ± 0.8	14.2 ± 0.9	15.5 ± 1.0	33	-15.0 ± 1.0	13.5 ± 1.0	15.0 ± 1.1
Gobiesox strumosus	Skilletfish	1	-15.5	11.8	9.7				
Chilomycterus schoepfii	Spiny boxfish	1	-13.5	13.3	12.7				
Leiostomus xanthurus	Spot	9	-14.1 ± 0.9	12.4 ± 1.0	12.3 ± 0.9	7	-13.2 ± 1.0	10.8 ± 3.7	11.8 ± 1.6
Cynoscion nebulosus	Spotted seatrout	6	-16.0 ± 0.9	14.0 ± 0.7	15.7 ± 1.0	3	-13.9 ± 1.4	13.1 ± 0.4	12.6 ± 0.8
	Squid	1	-15	13.9	[b]				
Paralichthys dentatus	Summer flounder	20	-15.1 ± 0.9	13.1 ± 0.7	14.3 ± 0.8	7	-14.7 ± 1.3	12.8 ± 0.8	14.5 ± 0.8
Tautoga onitis	Tautog	40	-14.4 ± 1.1	13.4 ± 0.6	15.4 ± 1.7	13	-14.3 ± 1.1	13.6 ± 0.6	15.2 ± 0.7
Symphurus sp.	Tonguefish	2	-14.9	12.2	14.4	5	-14.7 ± 0.9	12.0 ± 0.8	13.2 ± 0.5
Merlangius merlangus	Whiting	10	-14.6 ± 0.6	13.3 ± 0.4	13.9 ± 0.7				

Table 3.5. cont'd.

Table 3.6. Results of ANOVA and Tukey's HSD test of differences in mean isotope values of organisms. Comparisons are of organisms collected in restored eelgrass sites and adjacent eelgrass-free sites in 2004. Different letters indicate significantly different mean isotope values at $p < 0.05$ unless otherwise noted.

		$\delta^{13}C$		$\delta^{15}N$		$\delta^{34}S$	
Scientific name	Common name	Restored	Eelgrass-free	Restored	Eelgrass-free	Restored	Eelgrass-free
Seston	Particulate organic matter	a	a	a	a	a	b
Micropogonias undulatus	Atlantic croaker	a	a	a	a	a	b
Menidia menidia	Atlantic silversides	a	a	a	b	a	b
Anchoa mitchilli	Bay anchovy	a	a	a	b	a	a
Centropristis striata	Black seabass	a	a	a	a	a	a
Callinectes sapidus	Blue crab	a	a	a	a	a	a
Mycteroperca microlepis	Gag grouper	a	b ($p < 0.01$)	a	a	a	a
Palaemonetes spp.	Grass shrimp	a	a	a	a	a	a
Syngnathus fuscus	Northern pipefish	a	b	a	a	a	a
Opsanus tau	Oyster toadfish	a	a	a	b ($p < 0.01$)	a	a
Orthopristis chrysoptera	Pigfish	a	b	a	a	a	b
Lagodon rhomboides	Pinfish	a	a	a	a	a	a
Archosargus probatocephalus	Sheepshead	a	a	a	a	a	a
Bairdiella chrysoura	Silver perch	a	a	a	b	a	b
Leiostomus xanthurus	Spot	a	a	a	a	a	a
Paralichthys dentatus	Summer flounder	a	a	a	a	a	a
Tautoga onitis	Tautog	a	a	a	a	a	a

Table 3.7. Mean carbon, nitrogen and sulfur stable isotope values (± standard deviation) of organisms collected in South Bay restored eelgrass meadows and eelgrass-free sites in 2005. 'n' indicates number individuals analyzed. [a] tentative identification. [b] insufficient sample remained for sulfur analysis.

Scientific name	Common name	Eelgrass meadows				Eelgrass-free sites			
		n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Agardhiella sp.		4	-17.7 ± 1.9	11.1 ± 0.8	20.2 ± 0.6	8	-18.2 ± 1.9	10.7 ± 0.9	19.3 ± 2.8
Bryopsis sp.		3	-17.3 ± 1.5	12.9 ± 2.2	12.7 ± 0.4	3	-18.5 ± 0.9	11.1 ± 2.0	14.3 ± 1.4
Ceramium rubrum[a]	Epiphytic algae	9	-16.5 ± 1.9	10.7 ± 1.0	13.6 ± 3.1	4	-14.8 ± 0.4	9.9 ± 1.5	15.7 ± 2.0
Cladophora sp.		5	-18.7 ± 1.7	10.6 ± 1.2	15.9 ± 1.1	1	-18.9	11.3	15.5
Codium fragile	Dead man's fingers	6	-12.5 ± 3.3	10.0 ± 1.8	17.6 ± 1.2	7	-14.5 ± 2.5	8.8 ± 0.6	18.6 ± 1.4
Enteromorpha sp.		1	-22.8	10.6	16.9	1	-17.8	9.8	20.3
Gracilaria spp.	Graceful redweed	10	-17.2 ± 1.3	10.7 ± 1.6	17.2 ± 1.8	8	-17.5 ± 1.4	11.2 ± 0.9	16.1 ± 0.8
	Seston	12	-19.5 ± 1.2	7.7 ± 1.2	18.3	8	-19.8 ± 0.9	6.3 ± 2.3	[b]
Polysiphonia sp.		1	-20.4	9.6	14.6	2	-21.1	11.7	15.9
Ulva lactuca	Sea lettuce	11	-14.6 ± 3.7	10.3 ± 1.1	18.1 ± 1.6	5	-13.9 ± 2.1	9.5 ± 0.8	19.7 ± 1.2
Zostera marina	Live eelgrass	10	-9 ± 1.7	8.0 ± 1.1	11.7 ± 2.9				
Z. marina	Standing dead eelgrass	1	-11.2	7.2	[b]				
Z. marina	Detrital eelgrass	1	-8.5	7.3	[b]				
Micropogonias undulatus	Atlantic croaker	15	-13.7 ± 1.6	12.8 ± 0.4	15.3 ± 1.6	15	-14.2 ± 1.1	13.6 ± 0.7	13.5 ± 2.5
Brevoortia tyrannus	Atlantic menhaden	5	-18.7 ± 0.4	12.6 ± 0.7	16.1 ± 0.7	11	-18.3 ± 1.1	13.1 ± 1.3	12.6 ± 1.0
Menidia menidia	Atlantic silversides	52	-16.9 ± 0.7	14.6 ± 2.2	14.8 ± 2.2	31	-19.4 ± 3.1	15.7 ± 1.8	13.8 ± 1.7
Anchoa mitchilli	Bay anchovy	38	-17.3 ± 1.0	15.6 ± 2.1	16.0 ± 2.3	25	-18.7 ± 1.5	15.5 ± 1.8	14.7 ± 1.4
Centropristis striata	Black seabass	14	-14.9 ± 0.7	14.4 ± 0.7	14.2 ± 2.2	1	-15.7	15.9	15.1
Fundulus sp.	Mummichog					1	-15.2	12.0	10.7
Callinectes sapidus	Blue crab	8	-15.0 ± 0.3	12.0 ± 0.8	10.7 ± 1.4	1	-15.5	12.9	13.2
Hypsoblennius hentzi	Feather blenny	5	-15.6 ± 0.8	15.5 ± 1.6	11.9 ± 1.6				
Mycteroperca microlepis	Gag grouper	4	-14.6 ± 0.4	14.3 ± 0.4	13.6 ± 1.4				
Palaemonetes spp.	Grass shrimp	10	-14.0 ± 1.1	12.6 ± 0.6	12.4 ± 1.6	12	-13.8 ± 1.1	13.0 ± 1.4	13.0 ± 1.7

Lutjanus griseus	Grey snapper	1	-14.3	13.8	13.2				
Cynoscion regalis	Grey trout (weakfish)	3	-16.6 ± 0.4	13.7 ± 0.9	15.6 ± 1.5	1	-18.2	13.4	14.6
Hippolyte spp.	Ghost shrimp	2	-14.4	11.4	9.2				
(Stomatopod)	Mantis shrimp	1	-14.4	11.6	10.1				
	Mud crab	1	-14.7	11.3	9.4				
Gobiosoma bosci	Naked goby	3	-15.6 ± 0.2	15.3 ± 0.5	11.2 ± 0.8	8	-15.5 ± 1.7	13.8 ± 1.8	10.9 ± 1.8
Syngnathus fuscus	Northern pipefish	59	-14.3 ± 1.1	13.5 ± 1.0	14.0 ± 2.8	16	-15.3 ± 1.6	12.8 ± 1.2	12.6 ± 1.7
Sphyraena borealis	Northern sennet	11	-17.4 ± 0.7	13.8 ± 0.8	16.7 ± 2.1				
Opsanus tau	Oyster toadfish	9	-15.0 ± 0.6	14.1 ± 1.0	12.8 ± 0.9	8	-15.8 ± 1.4	14.1 ± 1.9	13.1 ± 1.0
Orthopristis chrysoptera	Pigfish	34	-15.7 ± 0.8	15.0 ± 2.3	12.9 ± 2.1	3	-15.9 ± 0.6	14.0 ± 0.2	7.4 ± 0.3
Lagodon rhomboides	Pinfish	16	-15.0 ± 1.2	13.6 ± 0.5	16.2 ± 1.8	7	-15.7 ± 0.4	13.3 ± 1.5	12.1 ± 1.5
Penaeus sp.	Prawn	1	-14.1	13	10.8	3	-14.0 ± 0.2	14.2 ± 0.7	14.3 ± 1.1
Sciaenops ocellata	Red drum	5	-14.3 ± 0.3	13.0 ± 0.2	12.3 ± 0.4				
Prionotus sp.	Searobin	2	-14.5	13.4	15				
Hippocampus erectus	Seahorse	3	-13.9 ± 0.5	13.9 ± 0.1	13.4 ± 0.2				
Archosargus probatocephalus	Sheepshead	1	-15.7	15.9	15	1	-14.9	11.9	7.5
Bairdiella chrysoura	Silver perch	58	-16.0 ± 0.9	15.1 ± 1.8	15.2 ± 1.8	35	-16.2 ± 1.1	14.1 ± 1.2	13.0 ± 2.0
Chilomycterus schoepfii	Spiny boxfish	5	-15.1 ± 0.8	13.0 ± 0.4	14.5 ± 2.0				
Leiostomus xanthurus	Spot	41	-14.8 ± 0.7	14.3 ± 1.7	12.7 ± 2.7	40	-17.0 ± 2.5	15.2 ± 1.9	9.7 ± 2.5
Cynoscion nebulosus	Spotted seatrout	9	-15.6 ± 0.5	14.6 ± 0.2	14.1 ± 0.9	1	-16	12.7	12.8
Paralichthys dentatus	Summer flounder	12	-14.9 ± 0.8	13.7 ± 1.8	14.0 ± 1.2	12	-15.3 ± 1.1	15.6 ± 1.6	11.6 ± 2.1
Tautoga onitis	Tautog	32	-14.7 ± 1.0	15.3 ± 1.7	15.2 ± 1.9	5	-14.4 ± 0.5	12.9 ± 0.1	13.5 ± 0.6
Symphurus sp.	Tonguefish	1	-15.2	14.1	9.9				
Merlangius merlangus	Whiting	1	-14.6	16.4	13.4				

Table 3.7. continued.

Table 3.8. Results of ANOVA and Tukey's HSD test of differences in mean isotope values of consumers. Comparisons are of consumers collected in restored eelgrass sites and adjacent eelgrass-free sites in 2005. Different letters indicate significantly different mean isotope values at $p < 0.05$ unless otherwise noted.

		$\delta^{13}C$		$\delta^{15}N$		$\delta^{34}S$	
Scientific name	Common name	Restored	Eelgrass-free	Restored	Eelgrass-free	Restored	Eelgrass-free
Anchoa mitchilli	Bay anchovy	a	b ($p < 0.01$)	a	a	a	b
Bairdiella chrysoura	Silver perch	a	a	a	b ($p < 0.01$)	a	b ($p < 0.01$)
Brevoortia tyrannus	Atlantic menhaden	a	a	a	a	a	b ($p < 0.01$)
Lagodon rhomboides	Pinfish	a	a	a	a	a	b ($p < 0.01$)
Leiostomus xanthurus	Spot	a	b ($p < 0.01$)	a	b	a	b ($p < 0.01$)
Menidia menidia	Atlantic silversides	a	b ($p < 0.01$)	a	b	a	b
Micropogonias undulatus	Atlantic croaker	a	a	a	b ($p < 0.01$)	a	b
Opsanus tau	Oyster toadfish	a	a	a	a	a	a
Orthopristis chrysoptera	Pigfish	a	a	a	a	a	b ($p < 0.01$)
Palaemonetes spp.	Grass shrimp	a	a	a	a	a	a
Paralichthys dentatus	Summer flounder	a	a	a	b ($p < 0.01$)	a	b ($p < 0.01$)
Syngnathus fuscus	Northern pipefish	a	b ($p < 0.01$)	a	b	a	a
Tautoga onitis	Tautog	a	a	a	b ($p < 0.01$)	a	a

Table 3.9. Mean carbon, nitrogen and sulfur isotope values (± standard deviation) of organisms collected from the natural eelgrass meadow at Fisherman's Island and nearby eelgrass-free site. 'n' indicates number individuals analyzed. [a] tentative identification. [b] insufficient sample remained for sulfur analysis.

Scientific name	Common name	Eelgrass meadows				Eelgrass-free sites			
		n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	n	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Agardhiella sp.		1	-14.7	11.3	17.5	1	-11.8	11.6	17.9
Cladophora sp.		1	-21.2	9.9	16.9	1	-19.8	9.8	18.3
Ceramium rubrum[a]	Epiphytic algae	1	-14.6	12.7	13.7	1	-15.8	10.1	12.3
Gracilaria spp.	Graceful redweed	1	-15.8	12.4	17.5	3	-13.7 ± 1.3	10.2 ± 1.2	16.5 ± 1.6
	Unknown red alga					1	-22.0	10.0	12.7
Ruppia maritima	Widgeongrass	2	-12.4	3.1	[b]				
Spartina alterniflora (standing dead)	Saltmarsh cordgrass					1	-10.6	1.1	4.9
Ulva lactuca	Sea lettuce	1	-13.4	10.2	11.4	2	-13.1	14.5	12
Zostera marina	Live eelgrass	3	-10.8 ± 0.6	7.2 ± 0.2	8.1				
Z. marina	Standing dead eelgrass	1	-9.4	7.2	4.2				
Z. marina	Detrital eelgrass	1	-4.8	3.6	8.7	1	-8.1	8.4	7.6
Micropogonias undulatus	Atlantic croaker	1	-17.7	15.0	12.8	1	-18.6	14.2	13.2
Brevoortia tyrannus	Atlantic menhaden	5	-14.2 ± 1.9	11.7 ± 0.6	13.4 ± 0.6	3	-14.9 ± 0.8	17.3 ± 1.6	18.0 ± 0.5
Menidia menidia	Atlantic silversides	15	-17.8 ± 0.7	14.5 ± 0.3	16.3 ± 1.2	8	-17.0 ± 0.7	15.4 ± 1.7	13.6 ± 1.6
Anchoa mitchilli	Bay anchovy	3	-18.2 ± 0.4	14.8 ± 0.6	16.3 ± 1.8	2	-18.6	13.4	14.5
Callinectes sapidus	Blue crab	12	-16.0 ± 1.0	12.5 ± 0.6	12.7 ± 0.8	4	-13.5 ± 1.9	12.7 ± 0.8	13.8 ± 0.7
Syngnathus floridae	Dusky pipefish	4	-15.6 ± 1.5	13.4 ± 0.8	12.3 ± 1.1	3	-14.7 ± 0.3	16.5 ± 0.4	12.2 ± 0.2
Mycteroperca microlepis	Gag grouper	1	-15.3	13.8	11.3				
Palaemonetes spp.	Grass shrimp					1	-15.7	12.3	11.9
	Hermit crab	3	-12.9 ± 2.8	10.5 ± 0.5	14.7 ± 2.8	4	-14.4 ± 1.8	12.3 ± 0.7	12.4 ± 1.4
Hippolyte sp.	Ghost shrimp	4	-14.0 ± 0.3	11.9 ± 1.0	11.4 ± 0.9	3	-14.1 ± 1.3	12.9 ± 1.3	10.3 ± 1.1
Erichsonella sp.	Isopod	2	-11.6	10.6	10.5				
Hippocampus erectus	Lined seahorse	3	-16.1 ± 0.2	12.1 ± 0.6	12.7 ± 0.8				

Syngnathus fuscus	Northern pipefish	12	-15.0 ± 1.6	13.3 ± 0.7	12.5 ± 1.3	1	-13.2	11.2	14.3
Opsanus tau	Oyster toadfish	1	-16.7	11.6	14.4				
Orthopristis chrysoptera	Pigfish	2	-16.8	13.7	8.4				
Lagodon rhomboides	Pinfish	1	-15.2	13.6	14.0				
Penaeus sp.	Prawn	6	-14.3 ± 2.2	11.6 ± 1.2	16.1 ± 1.5	3	-14.9 ± 0.8	17.3 ± 1.6	18.8 ± 0.5
	Sea cucumber	1	-16.2	10.0	13.9				
Bairdiella chrysoura	Silver perch	16	-15.4 ± 1.3	13.4 ± 0.9	14.8 ± 2.0				
Mitrella sp.	Snail	6	-13.8 ± 1.2	9.7 ± 0.9	11.4 ± 1.7				
Leiostomus xanthurus	Spot					2	-14.7	13.4	13.3
Paralichthys dentatus	Summer flounder					3	-15.4 ± 0.5	12.0 ± 0.2	12.1 ± 0.5
Tautoga onitis	Tautog	5	-15.6 ± 0.3	14.0 ± 0.5	14.1 ± 0.1				
	Whelk	1	-20.3	12.1	15.6				
Merlangius merlangus	Whiting					2	-14.7	13.4	14.3

Table 3.9. continued.

Table 3.10. Results of ANOVA and Tukey's HSD test of differences in mean isotope values of consumers. Comparisons are of consumers collected in the natural eelgrass meadow and eelgrass-free site near Fisherman's Island in 2006. Different letters indicate significantly different mean isotope values at $p < 0.05$ unless otherwise noted.

		$\delta^{13}C$		$\delta^{15}N$		$\delta^{34}S$	
Scientific name	Common name	Natural	Eelgrass-free	Natural	Eelgrass-free	Natural	Eelgrass-free
Brevoortia tyrannus	Atlantic menhaden	a	a	a	b ($p < 0.01$)	a	b ($p < 0.01$)
Callinectes sapidus	Blue crab	a	b ($p < 0.01$)	a	a	a	b
Hippolyte sp.	Ghost shrimp	a	a	a	a	a	a
Menidia menidia	Atlantic silversides	a	b	a	a	a	b ($p < 0.01$)
Penaeus sp.	Prawn	a	a	a	b ($p < 0.01$)	a	b
Syngnathus floridae	Dusky pipefish	a	a	a	b ($p < 0.01$)	a	a
	Hermit crab	a	a	a	b	a	a

Table 3.11. Results of ANOVA and Tukey's HSD tests for significant differences in mean isotope values of species between sampling seasons. 1 – 2004 versus 2005 was significantly different at $p < 0.05$. 2 – 2004 versus Natural meadow (2006) was significantly different at $p < 0.05$. 3 – 2005 versus Natural meadow was significantly different at $p < 0.05$. ** Indicates significant difference in consumer isotope values at $p < 0.01$.

Common Name	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Atlantic croaker	1**		
Atlantic silversides	2, 3**	1**, 2**	1, 3
Bay anchovy		1**	
Black seabass		1**	1
Blue crab	2**, 3		1**, 2**, 3**
Gag grouper			1**
Grass shrimp			1
Grey trout (weakfish)			
Lined seahorse	3**	3**	
Northern pipefish	1**	1**, 2**	2
Northern sennet			
Oyster toadfish		1	1
Pigfish		1**	1**
Pinfish		1**	
Silver perch	1**, 3	1**, 2**, 3**	
Spot	1**	1**	
Spotted seatrout		1	1**
Summer flounder			
Tautog	2	1**, 2	

Figure 3.1. Monthly totals of all fish collected, separated by year, in eelgrass meadows and eelgrass-free sites.

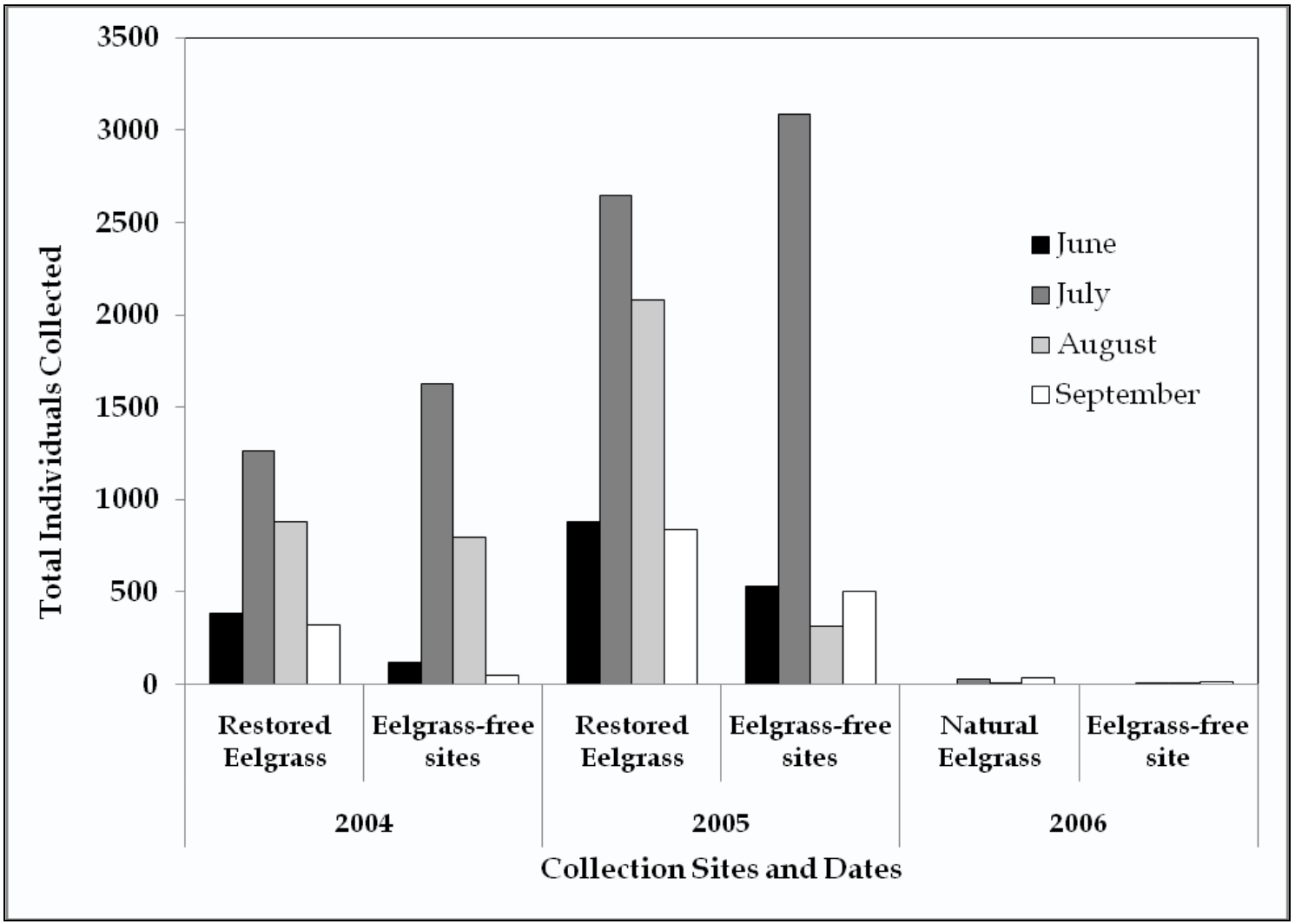


Figure 3.2. Average $\delta^{13}C$ values of two fish species in plots restored in 2001 and 2002. ♦ - oyster toadfish (*Opsanus tau*); ■ – pigfish (*Orthopristis chrysoptera*). Error bars depict standard error.

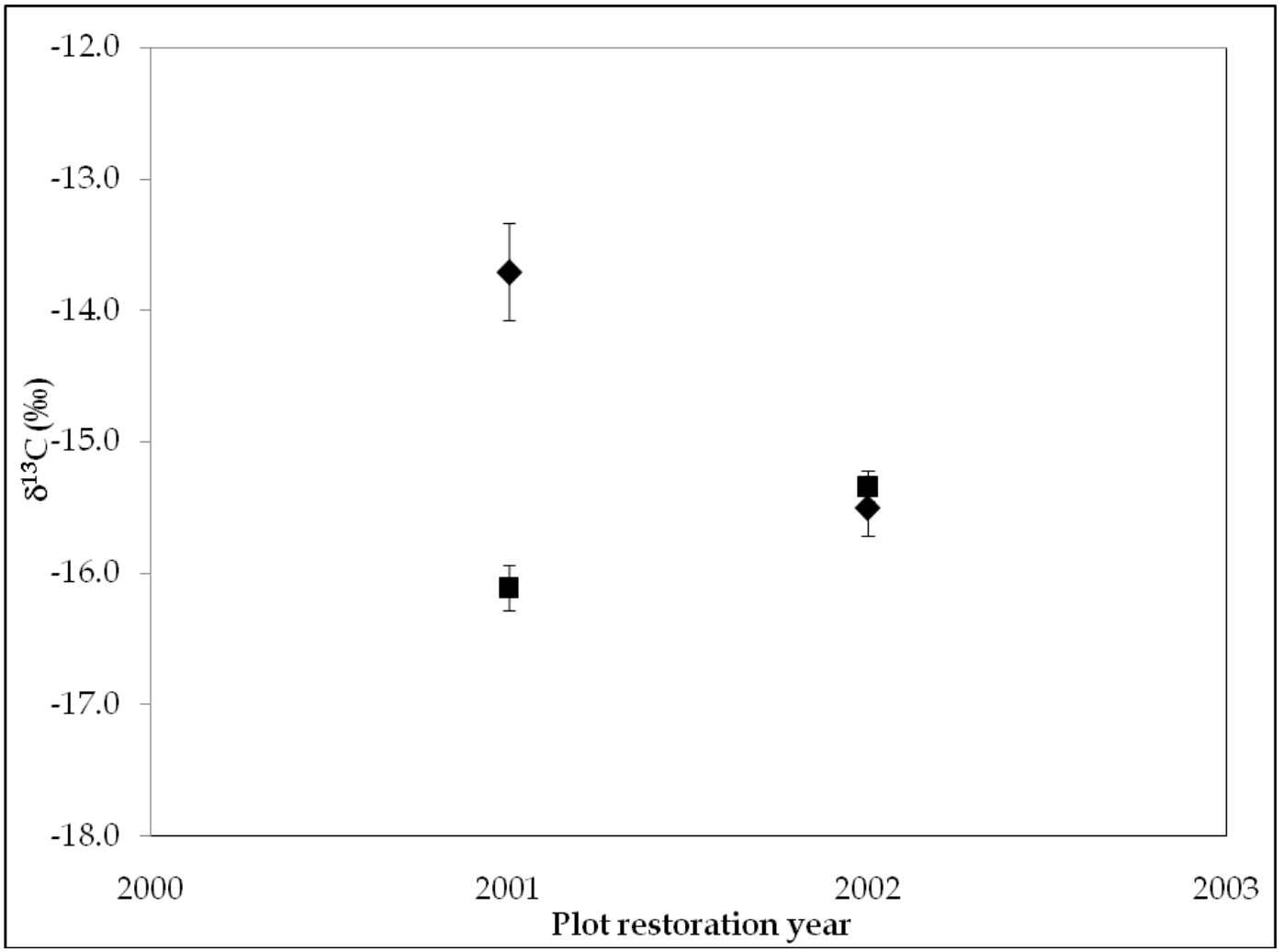


Figure 3.3. Average $\delta^{15}N$ values of oyster toadfish (*Opsanus tau*) in plots restored in 2001 and 2002. Error bars depict standard error.

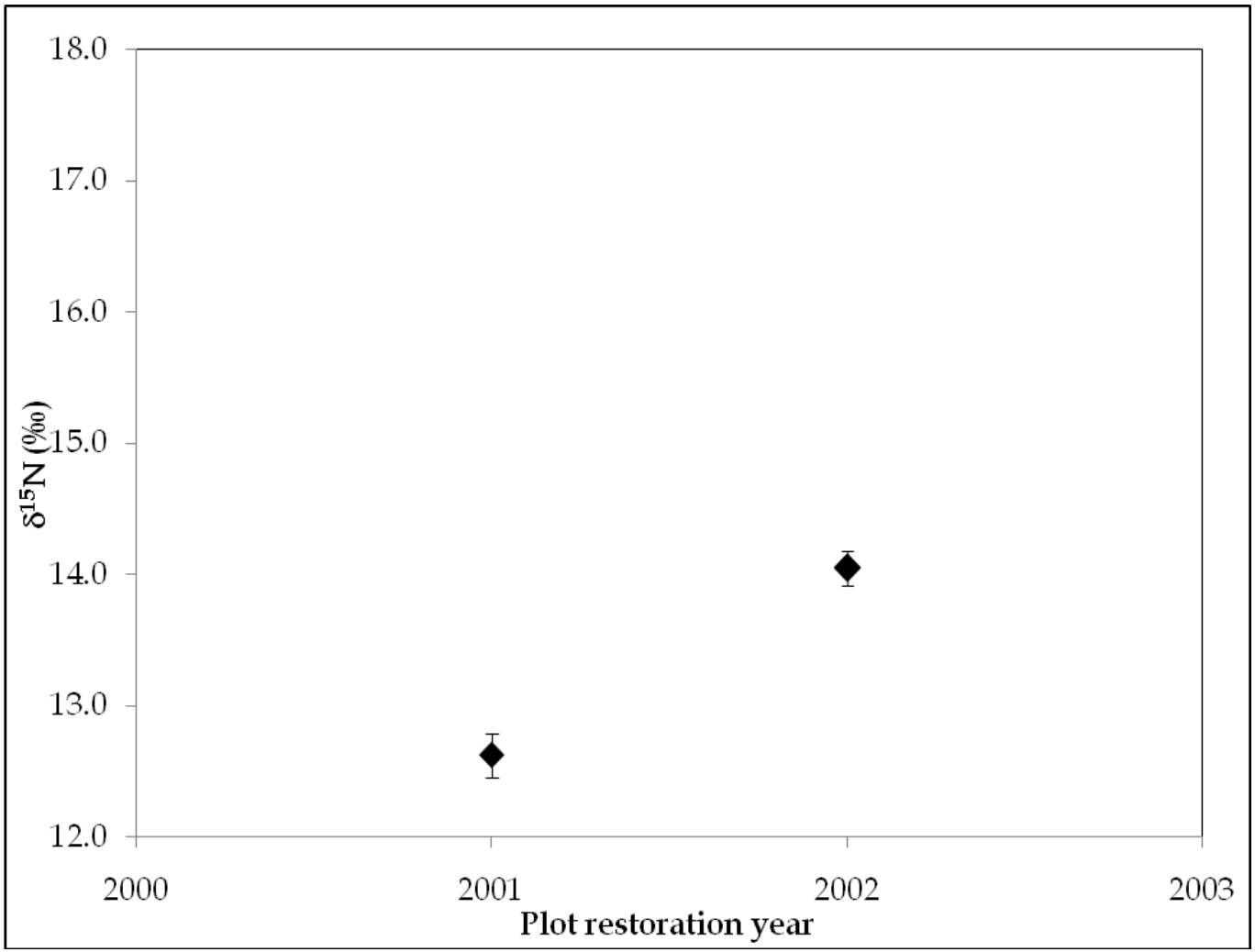


Figure 3.4. Average $\delta^{34}S$ values of two fish species in plots restored in 2001 and 2002. ■ - pigfish (*Orthopristis chrysoptera*); • - weakfish (*Cynoscion regalis*). Error bars depict standard error.

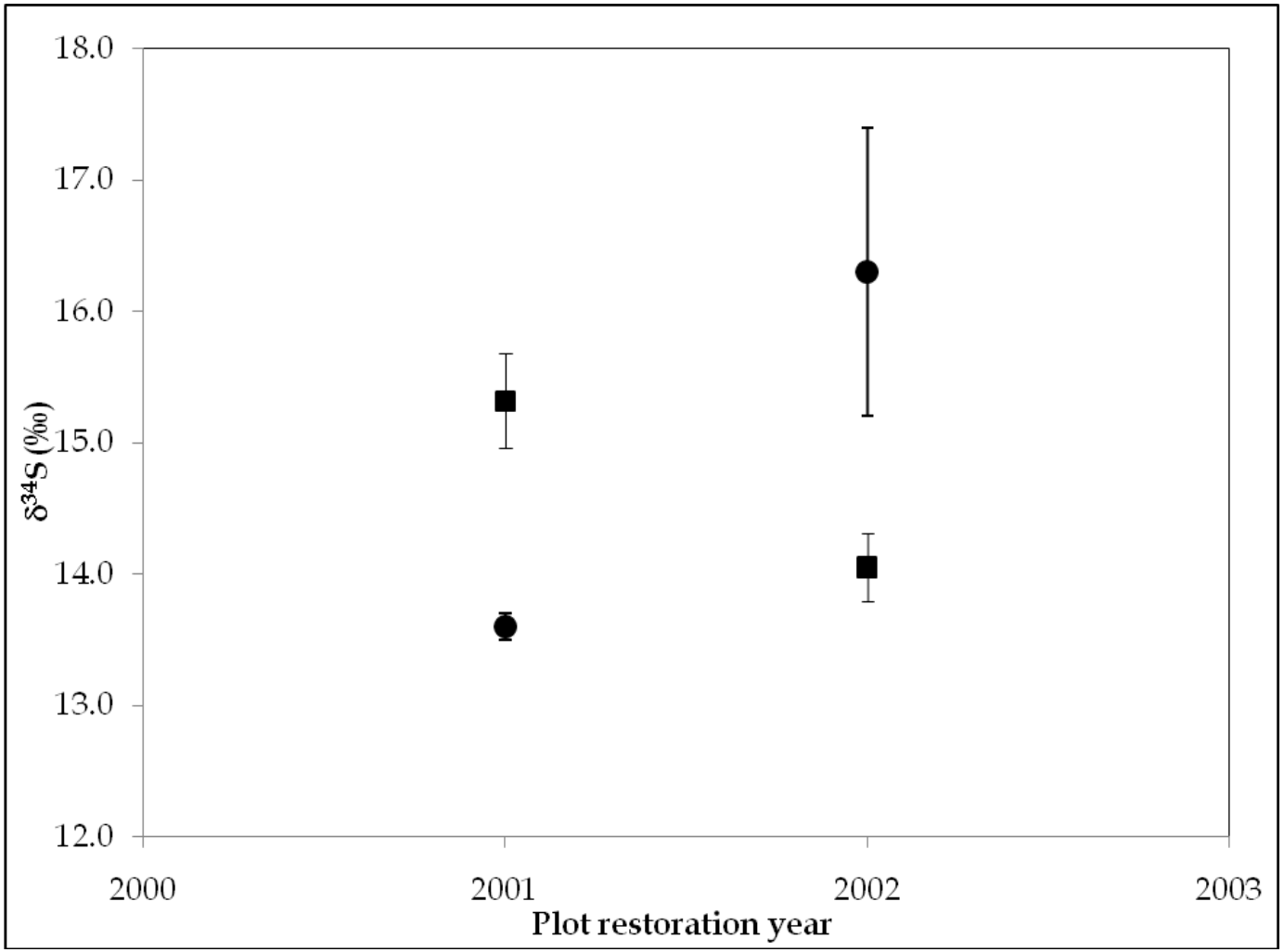


Figure 3.5. $\delta^{13}C$ vs. $\delta^{15}N$ of consumers and primary producers collected in South Bay restored seagrass sites, 2005. Species included were represented by at least 5 individuals. Isotope values are mean values ± one standard deviation.

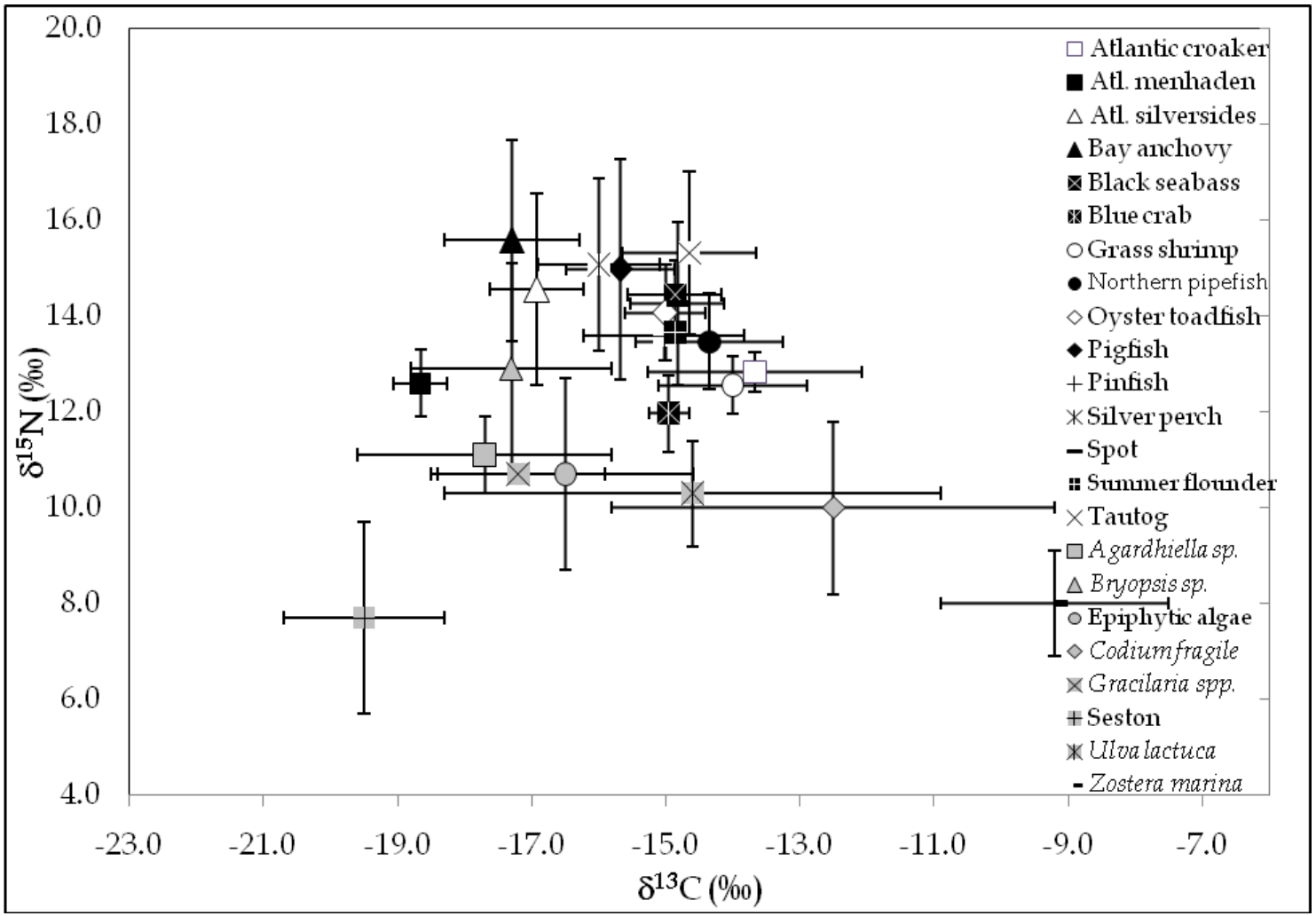


Figure 3.6. $\delta^{13}C$ vs. $\delta^{15}N$ of consumers and primary producers collected in South Bay eelgrass-free sites, 2005. Species included were represented by at least 5 individuals. Isotope values are mean values ± one standard deviation.

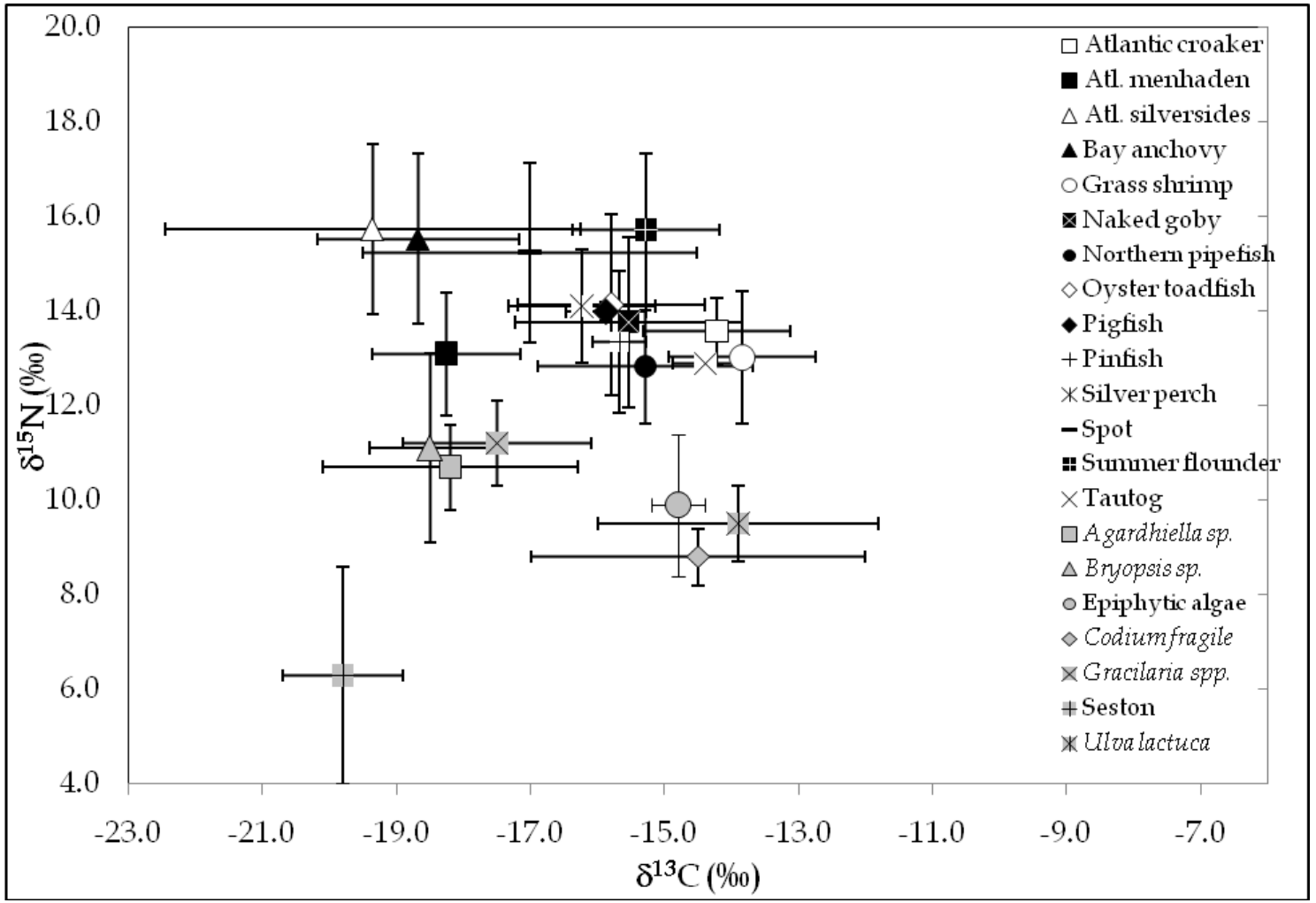


Figure 3.7. $\delta^{13}C$ vs. $\delta^{34}S$ of consumers and primary producers collected in South Bay restored seagrass sites, 2005. Species included were represented by at least 5 individuals. Isotope values are mean values ± one standard deviation.

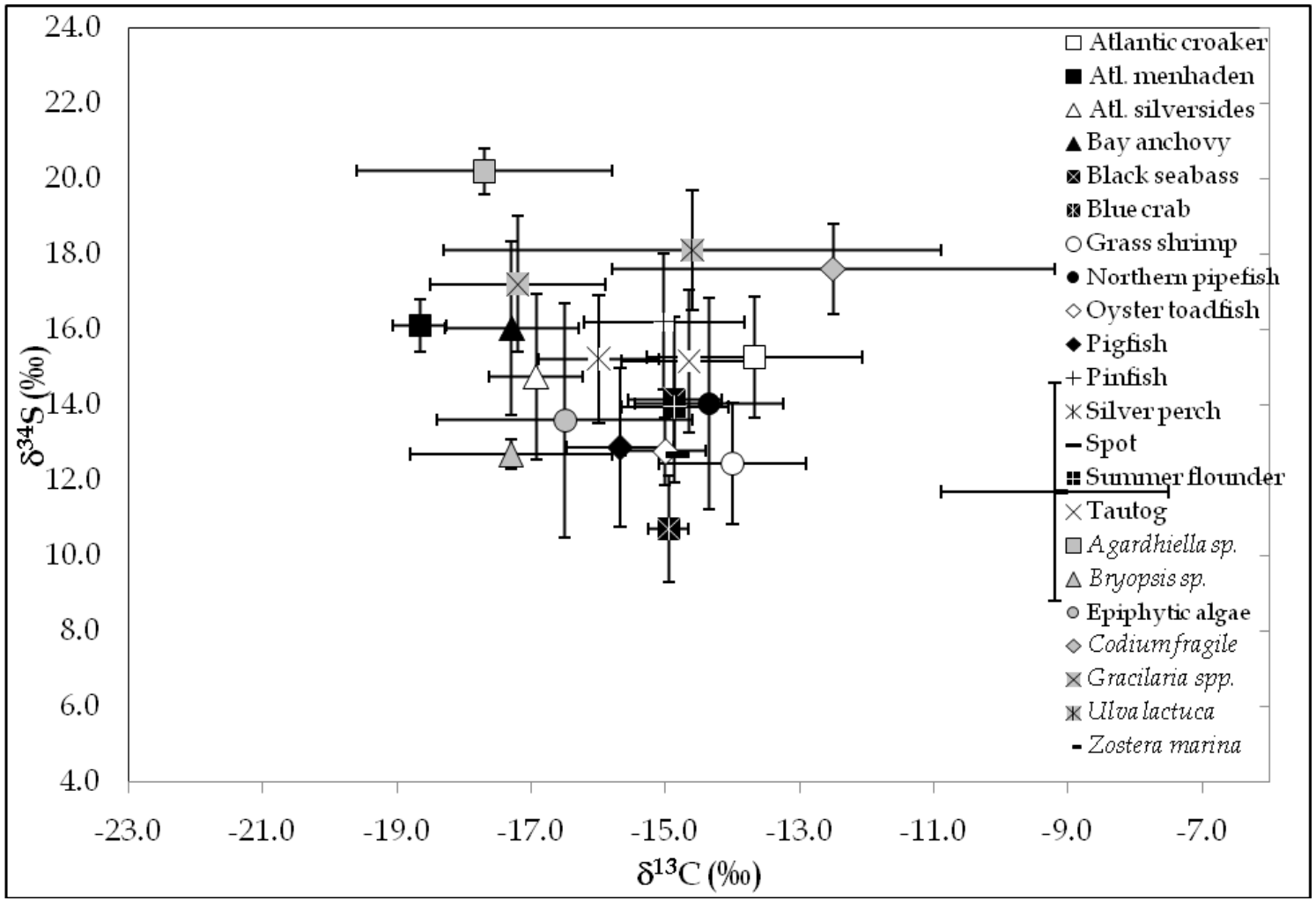
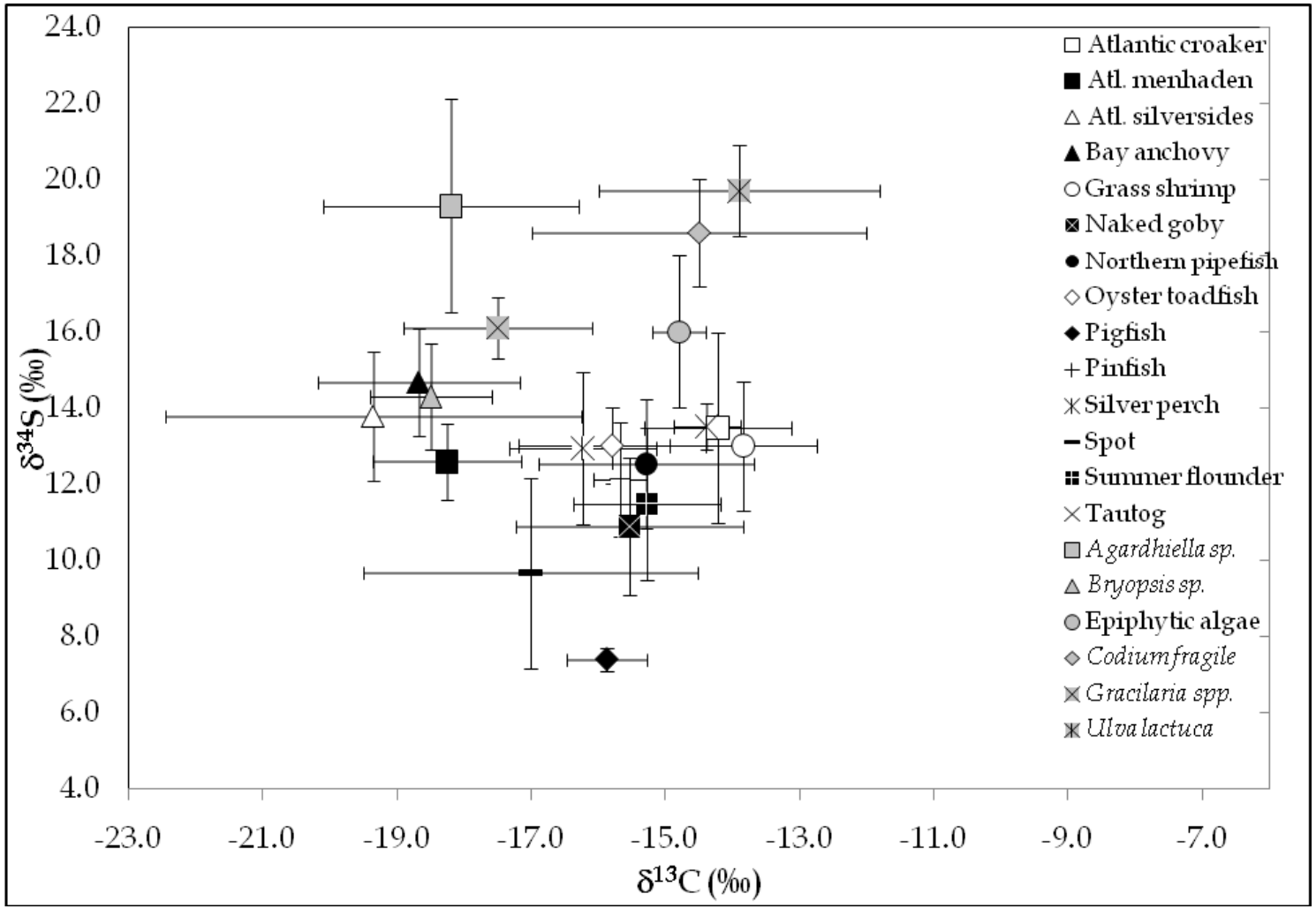


Figure 3.8. $\delta^{13}C$ vs. $\delta^{34}S$ of consumers and primary producers collected in South Bay eelgrass-free sites, 2005. Species included were represented by at least 5 individuals. Isotope values are mean values ± one standard deviation.



IV. Chapter 4: Stable isotope evidence of short term seasonal changes in primary production utilization in a coastal lagoon.

4.1. Introduction

Seagrasses are the main structural habitat for shallow marine communities that can be found all over the world. These communities are among the most productive in the marine environment (Zieman and Wetzel, 1980; Duarte, 1989; Mateo *et al*., 2006). However, recent decades have brought extensive declines in seagrass cover along coasts around the world (Hughes *et al*., 2002; Orth *et al*., 2006; Waycott *et al*., 2009). Human activities continue to contribute heavily to seagrass loss. Heavy inputs of nitrogen and phosphorus from agricultural runoff have result in widespread algal blooms, which compete with seagrasses for nutrient and light resources. Boating activities result in propeller scars and increased water column turbidity, which similar to the effect of algal blooms, causes seagrasses to be shaded out.

Evidence can be found in the literature that seagrass meadows positively affect fish and decapod survival and production via reduced predation and increased food availability (Fonseca *et al*., 1990; Goldstein *et al*., 1996; Minello *et al*., 2003; Harris *et al*., 2004). Consequently, the loss of seagrass meadows threatens numerous seagrass-associated species (Hughes *et al*., 2002). Eelgrass loss is particularly detrimental to the Chesapeake Bay and the coastal bays of the

Delmarva Peninsula in particular as there are no substitute organisms that provide the same habitat structure and functions and that can grow in the same salinity range and persist on subtidal sand and mudflats.

The Chesapeake Bay and the nearby coastal bays of the southern Delmarva Peninsula, such as South Bay, are near the southern limit of eelgrass distribution on the eastern coast of the United States (Heck *et al*., 1989; Koch and Orth, 2003; Moore and Short, 2006). Eelgrass was abundant in the region in the early 20th century; however a combination of a major storm in August 1933 and a wasting disease resulted in the disappearance of eelgrass from the southern Delmarva coastal bays and a considerable reduction in eelgrass abundance within the Chesapeake Bay (Orth *et al.*, 2006, 2010). Little natural recovery was since reported in the lower Delmarva coastal bays until the discovery of a small natural patch in South Bay, Virginia, in the late 1990s. Small-scale experiments with eelgrass seeds and adult plants and later large-scale, seed-based restoration in 2001 and 2002 resulted in the establishment of a thriving eelgrass population in a shallow sub-tidal section of South Bay by 2004.

The Chesapeake Bay region is subject to seasonal temperature and weather patterns typical of the temperate zone; environmental conditions can change drastically within and among seasonal timeframes. Ephemeral primary producers such as floating macroalgae are subject to environmental stresses associated with seasonal variation. Even eelgrass and other rooted plants that

persist year-round undergo cyclic changes in growth and biomass, though rooted macrophytes provide relatively more habitat stability. As a result, varying patterns of primary production and growth, senescence, and death affect food availability for local consumers.

The aim of this work was to evaluate the dietary implications of the continuous structural presence of eelgrass meadows by quantitatively verifying observations noted from stable isotope analyses of consumers in South Bay, Virginia. It was clear from results of early analyses in South Bay that the mean stable isotope values of many consumer species collected from the restored eelgrass meadows differed from the same species collected from nearby eelgrass-free sites. Carbon and sulfur isotope values of consumers indicated differences in nutritional sources for species depending on the presence or absence of eelgrass cover at the location in which the consumer was collected, possibly resulting from the utilization of different base primary producers at the two site types. Two general observations made from the comparison of stable isotope values of consumers collected from restored eelgrass meadows and eelgrass-free sites led to the current analyses. First, a greater number of species exhibited very different stable isotope values depending on the habitat from which each was collected in 2005 compared with 2004. Second, there was some evidence that at least for a few species, the disparity in isotope values depending on habitat varied greatly over the four-month sampling season. The purpose of the following analyses was to

more closely investigate the observed differences by focusing on the seasonal component. I hypothesized that the seasonal persistence of eelgrass provided stability to consumer nutritional sources relative to nearby eelgrass-free sites that were dominated by ephemeral macrophyte primary production sources. In order to test the hypothesis, I sought to verify quantitatively whether the observed differences between isotope values of species at the two sites varied over the course of the 2005 sampling season. I expected that the disparity in consumer isotope values would be more pronounced during the late summer and early fall when environmental stresses such as high water temperatures and lower nutrient availability would have the greatest effect on primary producers at the base of the food web.

The summer of 2005 proved unseasonably warm for the mid-Atlantic coast of the United States. Unusually high water temperatures throughout the Chesapeake Bay coupled with poor water quality contributed to diebacks of eelgrass populations that had recently begun to recover from earlier population losses (Moore and Jarvis, 2008; Orth *et al.*, 2010). Eelgrass meadows in Virginia coastal bays were less adversely affected; eelgrass populations within the lower Chesapeake Bay followed a declining trend through much of the current decade, while coastal populations increased (Orth *et al.*, 2010). Coastal populations were believed to have benefitted from slightly cooler water temperatures due to proximity to the Atlantic Ocean (Orth *et al.*, 2010).

Previous studies have used stable isotopic methods to study seasonal changes in isotope values of consumers and producers in order to describe the food web in aquatic systems (e.g. Buskey *et al*., 1999; Vizzini and Mazzola, 2003; Gustafson *et al*., 2007; Hill *et al*., 2008), commonly by comparing results from summer and winter as representing the temperature extremes within one or more years. Seasonal variation was investigated in the present study within a shorter timeframe. Sample collection dates encompassed the majority of the growing season as well as a short period following the observed decline in primary producer biomass.

Stable carbon, nitrogen and sulfur isotope values of consumer tissues were utilized to investigate consumer diets South Bay through much of the growing season and into the early fall of 2005. Fish were collected from the restored eelgrass meadows for comparison with fish removed from several nearby eelgrass-free sites. The restored eelgrass meadows maintain a constant presence in South Bay, while the eelgrass-free sites are dominated by ephemeral floating macroalgae such as *Ulva lactuca* (sea lettuce) and *Gracilaria spp.* (graceful redweed). Ephemeral algae experienced a sharp decline in total biomass during the mid summer, beginning in late July and resulting in comparatively low abundances during August and September; eelgrass above-ground biomass has also been noted to decrease during the late summer in the region (McGlathery *et*

al., 2001) though the full extent of the decline was unknown for the restored meadows in 2005.

4.2. Methods

The field site is located in South Bay, a sheltered lagoon along the southern coast of Virginia on the Delmarva Peninsula. Approximately 50 one-acre plots were restored, via reseeding, in a shallow sub-tidal section of South Bay in 2001 and 2002. Restoration parameters were as follows; individual eelgrass plots were restored at three different seed densities (50000, 100000, or 200000 seeds/acre) and over a span of two years (2001 and 2002). 2001 plots were restored at densities of 200,000 and 100,000 seeds per acre, while 2002 plots were restored at densities of 100,000 and 50,000 seeds per acre. Fewer seeds were available for restoration efforts in 2002, thus the differences in seeds distributed per acre between years (Orth, *personal communication*). Three specific combinations of plot age and seed density disbursed during restoration were chosen prior to the start of the first field trip; one site was selected at random from within each set of parameters for a total of three eelgrass plots. Three eelgrass-free sites were selected upon arrival in South Bay. Eelgrass-free sites were characterized by the visual lack of eelgrass cover. Subsequent samplings were repeated at the same locations for the duration of the study. Fish and invertebrates were collected monthly, June through September of 2005, via seine net. Primary producers were collected from the seine contents when possible and

by hand when necessary at each site. Individuals were identified and separated by species at VIMS and frozen prior to transport to the University of Virginia (UVA) for isotope analysis. Up to five individuals per species collected were selected at random for isotope analysis.

Fish and invertebrates and primary producer tissues were freeze-dried upon arrival at UVA in preparation for isotope analysis. White muscle tissue of animals was used when possible. Animals that were too small to discriminate between tissue types were homogenized with care taken to avoid skin, bone or shell fragments. Epiphytes were carefully removed from eelgrass and macroalgae by scraping with the flat end of a scalpel and were set aside for separate analysis. Plants and epiphytes were washed with tap water to remove sediment and debris and then with deionized water to remove salt and other surface accumulations prior to freeze-drying.

4.2.1. Stable isotopes

Samples were analyzed for carbon, nitrogen, and sulfur isotope values. Isotope measurements are expressed using the δ notation, where:

$$\delta^{N}E\ (‰) = (R_{sam} / R_{std} - 1) * 1000.$$

In the above equation, E refers to the element analyzed, N is the atomic mass of the heavy isotope, and R is the ratio of the abundance of the heavy isotope to the light isotope (i.e. $^{13}C/^{12}C$). Isotopic values are recorded in per mil (‰). Carbon, nitrogen and sulfur isotope values are reported relative to the Pee

Dee Belemnite, atmospheric nitrogen, and the Canyon Diablo Troilite standards, respectively, which are each defined as 0 ‰.

4.2.2. Statistical analyses

Three variables were evaluated as sources of variation in consumer isotope values at the two sites and so were included in statistical analyses; species type (SPECIES), habitat (COVER), and sampling date (MONTH). Differences in nutritional sources and metabolism between species were expected to exist and to manifest in consumer isotope values. Habitat in this case specifically refers to the designation of the collection site as eelgrass or eelgrass free. Consumer isotope values were also compared across each sampling period in order to assess temporal variation. Organisms collected during the June and July field trips were combined for repeated measures analysis; the two field excursions were treated as a single collection period in order to attain sufficient sample sizes for statistical analyses.

SAS version 9.1.3 statistical software (SAS Institute Inc., Cary, NC) was used to perform statistical analyses. The effects of cover, species, and the interaction of these two variables on consumer isotope compositions were explored using factorial multivariate analysis of variance (MANOVA) within each month. If the MANOVA null hypothesis of no significant differences in isotope values was rejected at an alpha (α) of 0.05, analysis of variance (ANOVA) was used to test for significant differences in isotope values of species and to

quantify $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ variation both within and among sites. Due to the unbalanced design, adjusted mean isotope values were obtained via least squared means. Tukey's Studentized Range (HSD) test was used to identify significant differences between consumers in eelgrass-free sites and eelgrass-vegetated sites as well as differences between individual species. Significant interactions were tested for simple effects, which were considered significant at $p < 0.05$.

When MANOVA indicated statistically significant differences, fish isotope measurements were analyzed using a three-way ANOVA - SPECIES x COVER x MONTH, 4 x 2 x 3 - with repeated measures on the factor MONTH. The data fit the ANOVA assumption of homogeneity of variances and was normally distributed. The data for all repeated measures analyses met the assumption of normality. However, an adjusted univariate F-test was used to account for deviation from sphericity based on the Greenhouse-Geisser epsilon correction. A Bonferroni correction was applied to tests for simple effects to control for experiment-wise error rates. Simple effects were considered significant at $\alpha = 0.025$. The α was set as follows: the initial α of 0.05 was divided by 2, which was the number of tests for simple effects (Looney and Stanley, 1989).

Four fish species were selected for repeated measures testing; Atlantic silversides (*Menidia menidia*), northern pipefish (*Syngnathus fuscus*), silver perch (*Bairdiella chrysoura*), and spot (*Leiostomus xanthurus*). Atlantic silversides are one

of the most abundant fishes found in shallow coastal waters and estuaries and are typically found in large schools not far from shore and have been known to seek shelter in seagrass beds (Fay *et al.*, 1983). Silversides are opportunistically omnivorous, with copepods, mysids, shrimp and other small crustaceans serving as common food sources along with algae and detrital fragments. Silversides are important forage fish for many larger species including bluefish (*Pomatomus saltatrix*) and striped bass (*Morone saxatilis*) (Fay *et al.*, 1983). Northern pipefish in the Chesapeake Bay region reside in seagrass beds during the spring and summer, avoiding predators by aligning their bodies vertically within the meadows to imitate grass blades. The prey items of pipefish are limited by the mouth shape and relatively small size; pipefish feed mainly on small crustaceans, and fish larvae and eggs (Teixeira and Musick, 1995). Silver perch are found in shallow areas along much of the Atlantic coast of the United States, with the highest abundances in the Mid-Atlantic region observed in late summer and declining through early fall (Murphy, 2005). Stomach contents of silver perch are dominated by crustaceans, penaeids, fish, and organic matter (Ayala-Pérez *et al*., 2006). Spot are abundant in estuarine and coastal waters, particularly in late spring through early fall. Spawning occurs offshore with larvae moving inshore from December through April. Tidal creeks and seagrass meadows are important habitats for juveniles in the Mid-Atlantic region, where they may constitute upwards of 90 percent of the total fish present (Weinstein and Brooks, 1983).

Juvenile spot are generalists that feed mainly on benthic infauna, including benthic copepods, polychaetes, mysids and amphipods (Phillips *et al.*, 1989). The reasons for choosing these fish for analysis are twofold. First, individuals from each of these species were captured at all sites in August and September and were captured in either June or July sampling periods. Second, these species represent a range of feeding strategies from benthopelagic to planktivorous and piscivorous.

4.3. Results

Approximately 800 samples were collected during the summer of 2005 consisting of primary producers through higher trophic level fish. The vast majority of fish collected were juveniles, with few exceptions. Consumer captures in both site types reached maximum levels in July. In eelgrass sites, captures decreased steadily to minimum levels for the study in September. Consumer capture numbers plummeted in eelgrass-free sites during August with a slight increase in September, though the total for both months together was less than one third of the total from July alone (see Table 4.1).

Greater numbers of fish and greater species diversity were noted in restored eelgrass beds relative to eelgrass-free sites (Table 4.1; van Montfrans *et al.*, 2005). Overall totals as well as totals within each sampling month were larger in eelgrass meadows (with the exception of July 2005), which is consistent with previous studies in many natural marine systems (e.g. Heck *et al.*, 1989; Lubbers

et al., 1990; Minello *et al.,* 2003; Nagelkerken and van der Velde, 2004). The large numbers encountered in the eelgrass-free sites in July 2005 were due to a large school of Atlantic menhaden at a single site from which nearly 2000 individuals were captured. The school comprised greater than fifty percent of the entire catch in eelgrass-free sites for that year. Excluding the menhaden, just over 1200 fish were collected from the eelgrass-free sites during July, compared to the greater than 2600 fish from the restored eelgrass meadows.

4.3.1. Isotope variation between site types by month

Analyses revealed significant multivariate effects within each month. COVER and SPECIES level effects resulted in significant differences in fish isotope values. Figures 4.1a-c depict the average carbon, nitrogen, and sulfur isotope values of all fish grouped together by site within each month.

Collectively, tissue samples of all fish captured in eelgrass plots were enriched in ^{13}C relative to fish captured in eelgrass-free sites for all months excluding July (for June $F = 7.35$, $p < 0.01$; for August $F = 9.20$, $p < 0.01$; for September $F = 180.58$, $p < 0.01$) (Figures 4.2a-b). Nitrogen isotope values of fish in the two cover types differed significantly during the month of June only ($F = 6.41$, $p < 0.05$), where fish from eelgrass-free plots exhibited greater average values (Figure 4.2a). Mean sulfur isotope values of fish from eelgrass sites were significantly greater than those from eelgrass-free sites for all months (for June F

= 26.99, $p < 0.01$; for July $F = 44.28$, $p < 0.01$; for August $F = 13.99$, $p < 0.01$; for September $F = 22.37$, $p < 0.01$) as well (Figure 4.2b).

As was expected, species-level differences in isotope values were important factors influencing fish isotope values within each month. SPECIES was the variable that explained the greatest variation in carbon isotope values of fish collected in June ($F = 43.42$, $p < 0.01$), July ($F = 32.42$, $p < 0.01$) and August ($F = 10.82$, $p < 0.01$). Fish nitrogen values varied significantly between species from both cover types in June ($F = 24.52$, $p < 0.01$) and September ($F = 4.28$, $p < 0.01$). Similarly, average sulfur isotope values were significantly different among species during the month of June regardless of the sampling site ($F = 7.71$, $p < 0.01$).

The interaction between the independent variables COVER and SPECIES had statistically significant effects on the isotope values of fish collected during July, August, and September (Table 4.2). Subsequent analyses showed that there were simple effects of COVER on the nitrogen and sulfur isotope values of several species captured in both July and August. The average nitrogen isotope values of spot from eelgrass-free sites were greater than that for spot from eelgrass sites in July and August, while the values were nearly identical in September (Figure 4.3). The average nitrogen isotope values of summer flounder captured in eelgrass-free sites was greater than for flounder in restored eelgrass plots within each month the fish were collected with one exception (Figure 4.4).

A single flounder captured in September in the restored eelgrass meadows exhibited a much higher nitrogen isotope value than the flounder in eelgrass-free sites. This flounder was fully mature and the largest sized flounder captured during the 2005 season. The high nitrogen value suggests a diet containing a greater proportion of higher trophic level fish.

Additionally, there were simple effects of COVER on the carbon and sulfur isotope values of spot and summer flounder. When each month is considered individually, the average carbon and sulfur isotope values of spot and summer flounder were enriched for fish captured in restored eelgrass meadows for all months though the difference was significant for September only (Figures 4.5 and 4.6, respectively). Silver perch showed similar trends. The sulfur isotope values of silver perch from the restored eelgrass meadows were relatively enriched for all months except June, when only one perch was captured in the eelgrass-free sites. While the average carbon isotope value of silver perch from July was enriched in eelgrass-free sites, the opposite was true for all other months (Figure 4.7).

4.3.2. Repeated measures analysis of isotope variation

The average carbon isotope values of the four species selected for repeated measures analysis – Atlantic silversides (*Menidia menidia*), northern pipefish (*Syngnathus fuscus*), silver perch (*Bairdiella chrysoura*), and spot (*Leiostomus xanthurus*) – changed over the course of the sampling season at the eelgrass-free

sites. $\delta^{13}C$ values increased somewhat for northern pipefish from June/July into August before dropping in September to approximately the same values as June/July, while silver perch and spot maintained approximately the same values through August before dropping to a minimum in September, and finally, Atlantic silversides decreased steadily throughout the sampling season. Carbon isotope values of fish from restored eelgrass plots showed little variation over the same period. Overall, during August and September, fish captured in eelgrass-free sites had significantly different carbon isotope values than fish from restored eelgrass meadows.

For fish carbon isotope values, the within groups interaction of the independent variables MONTH, COVER, and SPECIES was statistically significant, $F = 10.88$, $p < 0.01$. The interaction was further investigated by testing the simple effects of the variable MONTH on SPECIES at each site. Tests for simple effects of SPECIES indicated that mean carbon isotope values for individual species captured in eelgrass beds were not significantly different between months $F = 5.07$, *ns,* while in eelgrass-free sites species were significantly different over time, $F = 40.27$, $p < 0.01$ (Figure 4.8). Post-hoc contrasts showed that fish in eelgrass-free beds had more negative mean carbon isotope values for both June/July $F = 46.57$, $p < 0.01$ and August $F = 45.27$, $p < 0.01$ compared to September. No statistically significant difference was found between June/July and August. Tests for simple effects of COVER were also

performed for each month. COVER was found to have a significant effect on carbon isotope values of fish during both August ($F = 8.03$, $p < 0.01$) and September ($F = 71.92$, $p < 0.01$) at the 0.025 level. COVER was not a significant factor for samples collected June/July ($F = 1.42$, *ns*).

SPECIES differences accounted for fish nitrogen isotope variation across all levels of MONTH, $F = 31.24$, $p < 0.01$. Neither the effect of COVER nor the interaction between COVER and SPECIES was statistically significant at the 0.05 level. The repeated measures term MONTH did have a statistically significant effect, $F = 68.43$, $p < 0.01$. Post-hoc contrasts found that the mean nitrogen isotope values for fish captured in September were significantly greater than those from June/July ($F = 72.35$, $p < 0.01$) as well as those in August ($F = 132.66$, $p < 0.01$).

In general for species in restored eelgrass beds, sulfur isotopes started at a relatively higher value in June/July, then decreased to the minimum value in August, and finally rose again in September for all species except Atlantic Silversides, which did not change significantly from August to September (Figure 4.9). Sulfur isotope values of fish in eelgrass-free sites also dropped from a maximum in June/July to a lower value in August, however, the values decreased again in September for three of the four species. Overall, fish sulfur isotope values in eelgrass-free sites were depleted relative to those in restored eelgrass beds during the June/July and September sampling periods.

Similar to the results for carbon, the interaction of the variables MONTH and COVER and SPECIES was statistically significant for sulfur isotope values at the 0.05 level ($F = 5.53$, $p < 0.01$). Tests for simple effects of the variable MONTH indicated that the mean isotope values of individual species captured in both eelgrass beds and eelgrass-free sites varied over time ($F = 27.12$, $p < 0.01$, and $F = 19.54$, $p < 0.01$, respectively). See Figure 4.9 for the charted interaction. Post hoc contrasts were performed for all levels of MONTH against September: June/July fish isotope values were not statistically different from September at the 0.025 level ($F = 5.54$; *ns*), though in August the isotope values were less than those from September, $F = 16.16$, $p < 0.01$. Fish in eelgrass-free sites exhibited a different pattern, such that isotope values from June/July were greater ($F = 42.46$, $p < 0.01$) than August and September. There was no difference from August to September ($F = 0.21$, *ns*).

Simple effects due to the variable COVER were also tested within each month. Sulfur isotope values of fish in eelgrass-free sites differed from those in restored eelgrass beds in both June/July ($F = 22.38$, $p < 0.01$) and September ($F = 25.37$, $p < 0.01$) sampling periods.

Large differences were found in the isotope values of several species captured in September in restored eelgrass meadows versus eelgrass-free sites. Figures 4.10 through 4.14 show the differences in $\delta^{13}C$ vs. $\delta^{15}N$ of Atlantic silversides, bay anchovy, silver perch, spot, and northern pipefish. In all cases the

carbon isotope values of the fish in restored eelgrass meadows was enriched relative to the fish in eelgrass-free sites. The range of carbon isotope values of silversides, and spot was much larger for fish in eelgrass-free sites, though for the other three species the ranges were comparable. For Atlantic silversides, bay anchovy, and silver perch, the range of fish nitrogen isotope values was greater for fish captured in restored eelgrass meadows. Figures 4.15 through 4.19 are dual isotope plots of $\delta^{13}C$ vs. $\delta^{34}S$ for the same species as Figures 4.10 to 4.14. The range of sulfur isotope values of Atlantic silversides, bay anchovy, spot, and northern pipefish was much larger in the restored eelgrass meadows than the eelgrass-free sites. The sulfur isotope values of silver perch, spot and northern pipefish were higher on average for fish in restored eelgrass meadows.

4.4. Discussion

The repeated removal of fish from the same sites on a monthly basis is not expected to have caused a sampling bias. A related study at the same location found that while nearly twice as many fish were captured during this study, the pattern of total numbers collected approximated that of the previous study (van Montfrans *et al.*, 2005). Fish abundance in eelgrass meadows was greatest in July with slightly fewer in August, while June and September had similar values and the lowest abundances. The number of species captured for each month was also comparable between the two studies.

While fish are relatively mobile creatures, it is believed that the fish involved are unlikely to have left the relative safety of the eelgrass meadows and to have traversed the open water to the relatively barren eelgrass-free sites during the timeframe of this study. None of the plots selected were immediately adjacent to other restored plots; at least one open acre of sediment devoid of rooted macrophytes separated each eelgrass plot from the nearest adjacent plot on all sides. Furthermore, each of the selected eelgrass plots was separated by a straight-line distance of at least 1500 feet. I expect that the juvenile fish tended to remain within the eelgrass meadow in which they originally settled.

4.4.1. Individual species differences

For several sampling months, the carbon, nitrogen, and sulfur isotope values of fish species in eelgrass-free sites differed from those of the same species captured in restored eelgrass plots on the same date. Carbon isotope values of fish captured in September were significantly more negative in eelgrass-free sites than in restored eelgrass meadows. Though the magnitude of the effect on sulfur isotope values due to the variable COVER was different between species, the isotopic shift was in the same direction for all significant comparisons – in all cases, sulfur isotope values were higher in restored eelgrass beds than in eelgrass-free sites. When the isotope values of all fish collected at each site type were averaged as a group (unweighted) the trends remain intact. Within eelgrass-free sites the average carbon isotope values of fish collected in June,

August and September were more negative (Figure 4.1a), while average nitrogen isotope values from June and August were more positive than in restored eelgrass (Figure 4.1b). Fish in restored eelgrass plots had consistently greater sulfur isotope values for all months (Figure 4.1c).

In general, the patterns of carbon and sulfur isotopic change over time for individual species followed the overall patterns of isotopic variation for all species as a group. In the restored eelgrass meadows, the carbon isotope values of the four individual species remained relatively constant, with less than 1 ‰ change for each from June through September (Figures 4.3 and 4.8). Carbon isotope values of Atlantic silversides, silver perch, and spot from eelgrass-free sites changed little from the start of sampling through August, but displayed a sharp drop in $\delta^{13}C$ in September, while northern pipefish $\delta^{13}C$ values changed less than 1 ‰ throughout the study period. For all species except Atlantic silversides, there was a distinct pattern of decreasing $\delta^{34}S$ values from June through to a minimum value in August, with an increase to a median value in September in restored eelgrass meadows (Figures 4.5 and 4.9). In contrast, in eelgrass-free sites, the $\delta^{34}S$ values of three of the four species continued to decrease from August to September (Figure 4.9). The exception was again, Atlantic silversides. Atlantic silversides are generally schooling pelagic feeders (Fay *et al*., 1983), and so may be more likely than other three species to feed outside of the safety of the eelgrass habitat in a large school. Meanwhile, spot

and silver perch, which are members of Sciaenidae, are generally considered benthic feeders as juveniles (Miltner *et al.*, 1995) and may exploit eelgrass habitat early in life (Rooker *et al.*, 1998). Northern pipefish belong to a family of fish (Syngnathidae) that strongly associates with vegetated habitats such as eelgrass (Kendrick and Hyndes, 2003).

Figures 4.3 through 4.7 depict average $\delta^{13}C$ versus $\delta^{15}N$ for two individual species, spot and summer flounder, and $\delta^{13}C$ versus $\delta^{34}S$ for the previous two species plus silver perch, across the four-month sampling period. Average $\delta^{13}C$ values for summer flounder in restored eelgrass plots changed little during the four months while in eelgrass-free sites the average value dropped in September, similar to the general trend for all fish (Figures 4.1a and 4.4). Greater variation in $\delta^{15}N$ values were noted for summer flounder in restored eelgrass meadows where there was a general increase in $\delta^{15}N$ values over the course of the season topped by a large departure in September; only a single large adult fish was captured in September. The higher nitrogen isotope value is understandable for this particular individual, as it would likely feed on larger and higher trophic level prey due to its size. There was little change in $\delta^{15}N$ values for summer flounder in eelgrass-free sites, though on average (and excluding the adult individual from September), the values were higher than for fish captured in restored eelgrass meadows. Average sulfur isotope values of summer flounder from eelgrass-free sites were similar for all months except August, which were

approximately 2 ‰ lower; the $\delta^{34}S$ of the single large flounder collected in September resembled that of flounder collected in June and July. A similar departure in $\delta^{34}S$ values occurred in eelgrass-free sites, though values remained low in September. The carbon and sulfur stable isotope values of silver perch were somewhat different between sites, though there was a high degree of variability in isotope values of individuals.

4.4.2. Seasonal patterns

Temporal variation in stable isotope ratios of primary producers and associated fauna has been observed in other seagrass systems (Stephenson *et al.*, 1984; Fourqurean *et al*., 1997; Buskey *et al*., 1999; Vizzini and Mazzola, 2003 and 2006). Previous studies in temperate systems have found that, in general, consumers tend to have more negative carbon isotope values in fall and winter compared to summer; nitrogen isotopes do not tend to yield a consistent pattern (Buskey *et al*., 1999; Vizzini and Mazzola, 2003). This study also noted a decrease in carbon isotope values of fish during the early fall, but only in eelgrass-free meadows. The average carbon isotope values of all fish collected in the restored eelgrass plots remained relatively constant for the duration of the study, with overall average values staying between approximately 15 ‰ and -16 ‰ (Figure 4.1a). In contrast, there was a distinct change in the average $\delta^{13}C$ values of fish collected in eelgrass-free sites at the end of sampling season. During the first three sampling months, the average stable carbon isotope values of fish from

eelgrass-free sites varied little, with values remaining from approximately -16 ‰ to -16.5 ‰ (Figure 4.1a). However, there was a sharp drop of nearly 4 ‰ to an average of -20 ‰ in September. It is possible that if sampling continued through the winter a similar trend would emerge for eelgrass meadows, but has yet to be investigated in South Bay.

4.4.3. Habitat-related differences

The detrital pathway is generally accepted as the most important link between seagrass production and the food web (Klumpp *et al.*, 1989; Vizzini *et al.*, 2002) in temperate systems. Though seagrass meadows contribute a large proportion of the primary productivity of coastal ecosystems worldwide (Thayer *et al.*, 1984, Duarte & Cebrián, 1996; Duarte, 2002; Orth *et al.*, 2006), relatively little of the live primary productivity is grazed directly. Comparatively few macroorganisms are known to graze on live seagrass, unlike in tropical systems (Thayer *et al.*, 1984) where many species including dugongs (Yamamuro and Chirapart, 2005), turtles (Bjorndal, 1980), and numerous fish species (McAfee and Morgan, 1996; Mariani and Alcoverro, 1999; Cebrián and Duarte, 1998) are known to graze seagrass leaves. Rather, much of the excess primary production enters the detrital pathway, which is believed to be the main conduit for the transfer of eelgrass primary production to consumers in temperate meadows. Seagrass production is incorporated into the local food web through microbial decomposition of detrital material and eventually utilization by higher

consumers (Vizzini *et al.*, 2002). Hence, the different isotope values of consumers at the two sites reflect different sources of organic matter contributing to the detritus; eelgrass and seston were the main sources in the eelgrass meadows, while seston, macroalgae, and perhaps wrack from marsh plants dominated organic matter at the eelgrass-free sites.

The average nitrogen isotope values of consumers in both site types increased dramatically from summer to early fall. An overall average increase of approximately 2.7 ‰ and 3.0 ‰ was found for fish in eelgrass-free sites and eelgrass sites, respectively (Figure 4.1b). Such an abrupt and large increase in $\delta^{15}N$ values suggests considerable changes in the South Bay ecosystem, as the increases for both sites are near the generally accepted value of isotopic enrichment for one trophic level (DeNiro and Epstein, 1981; Peterson and Fry, 1987; Fry, 1988). The shift in nitrogen isotopes is believed to result from natural nitrogen cycling processes within the system. A widespread die-off of macroalgae was noted throughout the South Bay experimental sites during the mid-summer of 2005 as well as in an earlier study in nearby Hog Island Bay (McGlathery *et al.*, 2001). Decomposition of the macroalgae is a likely cause of the observed change in isotope values, via alteration of available nitrogen throughout the system. It is expected that bacterially-mediated decomposition accounts for the noted shift in nitrogen isotope values. The pathway of nitrogen isotope variation resulting from bacterial modification of decomposing tissues is

complicated by many factors including what forms of nitrogen are available, bacterial growth and population turnover rates, and the species composition (Hoch *et al*., 1996). A simplified mechanism for the shift is as follows. During early decomposition, hydrolysis of organic matter results in the preferential release of ^{14}N (Lehmann *et al*., 2002); the remaining material is relatively ^{15}N enriched. In addition, bacterially-mediated organic matter decomposition can preferentially remove ^{14}N from decomposing organic matter, again resulting in the relative increase in ^{15}N content of the remaining material (Cifuentes *et al*., 1988). In each case, the remaining material becomes available to detritivores, affecting a shift in nitrogen isotope values at the base of the food web, which would eventually filter up through the food web. The lag time between algal senescence and death and the subsequent shift in fish isotope values can be explained by isotope turnover time following a dietary change (Vander Zanden *et al*., 1998; Herzka *et al*., 2001). Isotope values of young fish with higher growth rates such as those captured in this study have been shown adjust to reflect a new diet on the order of days to weeks (Herzka *et al*., 2005).

The sharp decline in macroalgal presence in eelgrass-free sites towards the end of the summer could also explain the large difference in carbon and sulfur isotope values between fish captured in eelgrass plots and eelgrass-free sites during September 2005. First, macroalgae may be the major primary production sources to consumers in the eelgrass-free sites. If so, loss of algal biomass could

result in a wide change in the base primary production sources and thus the stable isotope values at the base of the food chain. Decaying algae and associated bacterial decomposers could potentially fill the source void, at least in the short term. The addition of decomposing material to the local pool of base primary production sources could also help to explain the isotopic shift late in the season. Variable decay rates of the individual components of a material can cause a shift in the isotope ratios of plant detritus during decomposition (Fry and Sherr, 1984; Ember *et al*., 1987) or due to selectivity by microbial decomposers (Mann, 1988; Peterson, 1999).

Overall average sulfur isotope values of consumers differed between habitat cover types for all months. The average isotope values of fish at each followed a similar pattern of change, though for all months the average values of fish from restored eelgrass plots was between 1 and 2.5 ‰ higher than fish from eelgrass-free sites. The decreasing trend noted in eelgrass meadows can be attributed to increasing sulfide intrusion from sulfide-rich porewaters into eelgrass roots; plants rooted in sediments with high sulfide concentrations tend to have lower $\delta^{34}S$ values (Oakes and Connolly, 2004). The sediments beneath eelgrass meadows are typically low in oxygen or anoxic (Holmer *et al*., 2005). Sulfate-reducing bacteria thrive in anoxic sediments and produce high concentrations of ^{34}S-depleted sulfides via dissimilatory sulfate reduction (Habicht and Canfield, 1997), which are generally toxic to plants. Seagrasses have

developed a lacunal system that allows for the transport of oxygen produced during photosynthesis to below-ground tissues to survive living in such a reducing environment (Oakes and Connolly, 2004; Holmer *et al*., 2005; Orth *et al.*, 2006).

Photosynthesis provides the oxygen distributed to below-ground tissues during the day, but oxygen must be obtained by diffusion from the water column at night (Moore and Jarvis, 2008). Sulfide intrusion rates in eelgrass are controlled by the internal oxygen concentration; lower concentrations result in higher sulfide intrusion (Pederson *et al.*, 2004). During the summer of 2005, increasing temperatures through South Bay coupled with decreasing water-column oxygen concentrations would have decreased the capacity of eelgrass to stop sulfide intrusion. The increasing exposure to ^{34}S-depleted porewater sulfides caused the formation of eelgrass tissues with gradually decreasing $\delta^{34}S$ values and also increased eelgrass mortality; the resulting eelgrass detritus would also bear relatively ^{34}S depleted values. Eventually, the sulfur isotope values of higher organisms at these sites would decrease similar to the nutritional source.

The similar decreasing sulfur isotope trend noted in eelgrass-free sites is also expected to be caused by predominance of plants rooted in sulfide-rich sediments. The eelgrass-free sites selected for this study were adjacent to Wreck Island, a barrier island lined by salt marshes dominated by *Spartina alterniflora*

(smooth cordgrass). The sediments in marshes in which *Spartina alterniflora* grows are also generally low in oxygen or anoxic. *Spartina* thrives in such environments and likely uses sulfides preferentially to produce sulfur-containing compounds (Fry *et al.*, 1982).

The change in direction of the trend of average sulfur isotope values (towards more positive values) of consumers from both eelgrass and eelgrass-free sites can again be attributed, in part, to the mass die-off of macroalgae in South Bay. The macroalgae prevalent in South Bay is unattached to the sediment; therefore the only sulfur source available to floating macroalgae is seawater sulfate, which the macroalgae obtains directly from the water column. $\delta^{34}S$ of seawater sulfate is approximately 20-21 ‰ (Peterson *et al.*, 1986; Michener and Schell, 1994). The increasing $\delta^{34}S$ values of consumers indicate greater sulfate utilization, most likely through assimilation of a proportion of decaying macroalgal biomass and the associated microbiota.

An alternative explanation for the differences in average isotope values of fish collected in eelgrass sites versus eelgrass-free sites is that the fish at the two sites were cohorts that came from separate populations with distinct isotopic compositions. The juvenile fish would have arrived from elsewhere to settle in their respective habitats, having already acquired isotope signatures similar to dietary sources from the previous location. Regardless of whether the noted differences are due to a change in dietary sources or due to fish settlement

history, the fact that fish at the two sites have distinct isotope compositions indicates that once settled, individuals do not tend to move between the eelgrass meadows and the eelgrass-free sites.

4.5. Summary

Average carbon and sulfur isotope values of fish species captured in the restored eelgrass meadows differed from the same species captured in eelgrass-free sites over the course of the collection period. Sulfur isotopes appear to be the most useful indicator of general site-related differences in South Bay as the differences in values between sites are more consistent than for carbon and nitrogen. The magnitude of consumer isotope differences due to the presence or absence of eelgrass cover was unexpected and suggests strong habitat fidelity within the study timeframe. The pattern of variation in consumer isotope values over time was different for organisms among the two cover types as well as between species within the sites. The most pronounced division between fish depending on the habitat in which each was captured occurred during September. An assortment of feeding strategies and life-histories among the fish collected explains the differences in the habitat effect between species.

Carbon isotope values gave useful information regarding organic matter cycling and availability in South Bay. $\delta^{13}C$ values indicate a late-summer to early fall divergence in available organic matter sources utilized by consumers in eelgrass meadows and nearby sites devoid of eelgrass. The relatively consistent

average $\delta^{13}C$ values of consumers in the eelgrass meadows throughout the study suggests that the continuous presence of eelgrass provided stability to consumer nutritional sources relative to eelgrass-free sites. Isotope values of consumers in eelgrass-free sites, on the other hand, show a sharp decline in $\delta^{13}C$ values of many species following the widespread loss of macroalgal biomass. It is believed that the widespread loss of macroalgal biomass throughout South Bay in 2005 resulted in a shift in dietary carbon sources utilized in eelgrass-free sites; the mass of decaying macroalgae became a major carbon source to consumers via the detrital pathway as the main organic matter source to bacterial decomposers.

4.6. References

Ayala-Pérez, L.A., Ramos-Miranda, J., Tapia-García, M., Salgado-Ugarte, I.H., and Miranda, G. 2006. Distribution, abundance and population parameters of the silver perch *Bairdiella chrysoura* (Lacepede, 1802) (*Pisces*, *Sciaenidae*) in Terminos Lagoon, Campeche, Mexico. Thalassas 22(1): 9-18.

Bjorndal, K.A. 1980. Nutrition and grazing behavior of the green turtle *Chelonia midas*. Marine Biology 56: 147-156.

Buskey, E.J., Dunton, K.H., and Parker, P.L. 1999. Variations in stable carbon isotope ratio of the copepod Acartia tonsa during the onset of the Texas brown tide. Estuaries 22 (4): 995-1003.

Cebrián, J., and Duarte, C.M. 1998. Patterns of leaf herbivory on seagrasses. Aquatic Botany 60: 67-82.

Cifuentes, L.A., Sharp, J.H., and Fogel, M.L. 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. Limnology and Oceanography 33 (5): 1102-1115.

Connolly, R.M., Guest, M.A., Melville, A.J., and Oakes, J.M. 2004. Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia 138: 161-167.

DeNiro, M.J., and Epstein, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45: 341-351.

Duarte, C.M. 1989. Temporal biomass variability and production biomass relationship of seagrass communities. Marine Ecology Progress Series 51: 269-276.

Duarte, C.M. 2002. The future of seagrass meadows. Environmental Conservation 29 (2): 192-206.

Duarte, C.M. and Cebrián, J. 1996. The fate of marine autotrophic production. Limnology and Oceanography 41 (8): 1758-1766.

Ember, L.M., Williams, D.F., and Morris, J.T. 1987. Processes that influence carbon isotope variations in salt marsh sediments. Marine Ecology Progress Series 36: 33-42.

Fay, C.W., Neves, R.J., and Pardue, G.B. 1983. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic) - Atlantic silverside. U.S. Fish and Wildlife Service, Division of Biological Services, FWS/OBS-82/11.10. U. S. Army Corps of Engineers, TR EL-82-4. 15 pages.

Fonseca, M.S., Kenworthy, W.J., Colby, D.R., Rittmaster, K.A., and Thayer, G.W. 1990. Comparisons of fauna among natural and transplanted eelgrass *Zostera marina* meadows: criteria for mitigation. Marine Ecology Progress Series 65: 251-264.

Fourqurean, J.W., Moore, T.O., Fry, B., and Hollibaugh, J.T. 1997. Spatial and temporal variation in C:N:P ratios, $\delta^{15}N$, and $\delta^{13}C$ of eelgrass Zostera marina as indicators of ecosystem processes, Tomales Bay, California, USA. Marine Ecology Progress Series 157: 147-157.

Fry, B., Scanlan, R.B., Winters, J.K., and Parker, P.L., 1982. Sulfur uptake by salt grasses, mangroves, and seagrasses in anaerobic sediments. Geochimica et Cosmochimica Acta 46: 1121-1124.

Fry, B., and Scherr, E. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions in Marine Science 27: 15-47.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnology and Oceanography 33 (5): 1182-1190.

Goldstein, B.A., Perkins-Visser, E., Wolcott, T.G., and Wolcott, D.L. 1996. Nursery role of seagrass beds: enhanced growth of juvenile blue crabs (*Callinectes sapidus* Rathburn). Journal of Experimental Marine Biology and Ecology 198 (2): 155-173.

Gustafson, L., Showers, W., Kwak, T., Levine, J., and Stoskopf, M. 2007. Temporal and spatial variability in stable isotope compositions of a freshwater mussel: implications for biomonitoring and ecological studies. Oecologia 152: 140-150.

Habicht, K.S., and Canfield, D.E. 1997. Sulfur isotope fractionation during bacterial sulfate reduction in organic-rich sediments. Geochimica et Cosmochimica Acta 61 (24): 5351-5361.

Harris, L.A., Buckley, B., Nixon, S.W., and Allen, B.T. 2004. Experimental studies of predation by bluefish *Pomatomus saltatrix* in varying densities of seagrass and macroalgae. Marine Ecology Progress Series 281: 233-239.

Heck, K.L. Jr., Able, K.W., Fahay, M.P., and Roman, C.P. 1989. Fishes and decapods crustaceans of Cape Cod eelgrass meadows: species composition, seasonal abundance patterns and comparison with unvegetated substrates. Estuaries 12 (2): 59-65.

Herzka, S.Z., Holt, S.A., and Holt, G.J. 2001. Documenting the settlement history of individual fish larvae using stable isotope ratios: model development and validation. Journal of Experimental Marine Biology and Ecology 265 (1): 49-74.

Herzka, S.Z., Rooker, J.R., and Secor, D.H. 2005. Assessing connectivity of estuarine fishes based on stable isotope ratio analysis. Estuarine, Coastal and Shelf Science 64 (1): 58-69.

Hill, J.M., McQuaid, C.D., and Kaehler, S. 2008. Temporal and spatial variability in stable isotope ratios of SPM link to local hydrography and longer term SPM averages suggest heavy dependence of mussels on nearshore production. Marine Biology 154 (5): 899-909.

Hoch, M.P., Snyder, R.A., Cifuentes, L.A., and Coffin, R.B. 1996. Stable isotope dynamics of nitrogen recycled during interactions among marine bacteria and protists. Marine Ecology Progress Series 132: 229-239.

Holmer, M., Frederiksen, M., and Møllegaard, H. 2005. Sulfur accumulation in eelgrass (*Zostera marina*) and effect of sulfur on eelgrass growth. Aquatic Botany 81: 367-379.

Hughes, J.E., Deegan, L.A., Wyda, J.C., Weaver, M.J., and Wright, A. 2002. The effects of eelgrass habitat loss on estuarine fish communities of southern New England. Estuaries 25 (2): 235-249.

Kendrick, A.J., and Hyndes, G.A. 2003. Patterns in the abundance and size-distribution of syngnathid fishes among habitats in a seagrass-dominated marine environment. Estuarine, Coastal and Shelf Science 57: 631-640.

Klumpp, D.W., Howards, R.K., and Pollard, D.A. 1989. Trophodynamics and nutritional ecology of seagrass communities. Pages 394-457 in: A.W.D. Larkum, A.J. McComb and S.A. Shepherd (eds.) *Biology of Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region.* Amsterdam: Elsevier Science Publishing. 841 pages.

Koch, E.W., and Orth, R.J. 2003. Seagrasses of the Mid-Atlantic Coast of the United States. Pages 216-223 in: Green, E.P. and Short, F.T. (eds.), *World Atlas of Seagrasses.* Berkeley, California: University of California Press. 310 pages.

Lehmann, M.F., Bernasconi, S.M., Barbieri, A., and McKenzie, J.A. 2002. Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sediment diagenesis. Geochimica et Cosmochimica Acta 66 (20): 3573-3584.

Looney, S., and Stanley, W. 1989. Exploratory repeated measures analysis for two or more groups. The American Statistician 43: 220-225.

Lubbers, L., Boynton, W.R., and Kemp, W.M. 1990. Variations in structure of estuarine fish communities in relation to abundance of submersed vascular plants. Marine Ecology Progress Series 65: 1-14.

Mann, K.H. 1988. Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnology and Oceanography 33 (4, part 2): 910-930.

Mariani, S., and Alcoverro, T. 1999. A multiple-choice feeding-preference experiment utilising seagrasses with a natural population of herbivorous fishes. Marine Ecology Progress Series 189: 295-299.

Mateo, M.A., Cebrián, J. Dunton, K., and Mutchler, T. 2006. Carbon flux in seagrass ecosystems. Pages 159-192 in: A.W.D. Larkum, R.J. Orth, and C.M. Duarte (eds.), *Seagrasses: Biology, Ecology and Conservation*. Dordrecht (Netherlands): Springer. 691 pages.

McAfee, S.T., and Morgan, S.G. 1996. Resource use by five sympatric parrotfishes in the San Blas Archipelago, Panama. Marine Biology 125: 427-437.

McGlathery, K.J., Anderson, I.C, and Tyler, A.C. 2001. Magnitude and variability of benthic and pelagic metabolism in a temperate coastal lagoon. Marine Ecology Progress Series 216: 1-15.

Michener, R.H., and Schell, D.M. 1994. Stable isotope ratios as tracers in marine aquatic food webs. Pages 138-157 in: Lajthe, K. and Michener, R.M. (eds.), *Methods in Ecology: Stable Isotopes in Ecology and Environmental Science.* Oxford (UK): Blackwell Scientific Publications. 316 pages.

Miltner, R.J., Ross, S.W., and Posey, M.H. 1995. Influence of food and predation on the depth distribution of juvenile spot (Leiostomus xanthurus) in tidal nurseries. Canadian Journal of Fisheries and Aquatic Sciences 52 (5): 971-982.

Minello, T.J., Able, K.W., Weinstein, M.P., and Hays, C.G. 2003. Salt marshes as nurseries for nekton: testing hypotheses on density, growth and survival through meta-analysis. Marine Ecology Progress Series 246: 39-59.

Moore, K.A., and Short, F.T. 2006. *Zostera*: Biology, ecology, and management. Pages 361–386 in: A.W.D. Larkum, R.J. Orth, and C.M. Duarte (eds.), *Seagrasses: Biology, Ecology and Conservation*. Dordrecht (Netherlands): Springer. 691 pages.

Moore, K.A., and Jarvis, J.C. 2008. Environmental factors affecting recent summertime eelgrass diebacks in the lower Chesapeake Bay: implications for long-term persistence. Journal of Coastal Research Special Issue 55: 135-147.

Murphy, R.F. 2005. Fish assemblage structure in Maryland's coastal lagoon complex. M.S. Thesis, Department of Marine Estuarine and Environmental Sciences, University of Maryland. 102 pages.

Nagelkerken, I., and van der Velde, G. 2004. A comparison of fish communities of subtidal seagrass beds and sandy seabeds in 13 marine embayments of a Caribbean island, based on species, families, size distribution and functional groups. Journal of Sea Research 52: 127-147.

Oakes, J.M., and Connolly, R.M. 2004. Causes of sulfur isotope variability in the seagrass, *Zostera capricorni*. Journal of Experimental Marine Biology and Ecology 302: 153-164.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Jr., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., and Williams, S.L. 2006. A global crisis for seagrass ecosystems. Bioscience 56 (12): 987-996.

Orth, R.J., Marion, S.R., Moore, K.A., Wilcox, D.J. 2010. Eelgrass (*Zostera marina* L.) in the Chesapeake Bay region of mid-Atlantic coast of the USA: Challenges in conservation and restoration. Estuaries and Coasts 33: 139-150.

Pederson, O., Binzer, T., and Borum, J. 2004. Sulphide intrusion in eelgrass (*Zostera marina* L.). Plant, Cell and Environment 27 (5): 595-602.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18: 293-320.

Peterson, B.J., Howarth, R.W., and Garritt, R.H. 1986. Sulfur and carbon isotopes as tracers of salt-marsh organic matter flow. Ecology 67 (4): 865-874.

Peterson, B.J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. Acta Oecologia 20 (4): 479-487.

Phillips, J.M., Huish, M.T., Kerby, J.H., and Moran, D.P. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic) – spot. U.S. Fish and Wildlife Service Biological Report 82(11.98). U.S. Army Corps of Engineers. TR EL-82-4. 13 pages.

Rooker, J.R., Holt, S.A., Soto, M.A., Holt, G.J. 1998. Postsettlement patterns of habitat use by sciaenid fishes in subtropical seagrass meadows. Estuaries 21 (2): 318-327.

Stephenson, R.L., Tan, F.C., and Mann, K.H. 1984. Stable carbon isotope variability in marine macrophytes and its implications for food web studies. Marine Biology 81: 223-230.

Teixeira, R.L., and Musick, J.A. 1995. Trophic ecology of two congeneric pipefishes (Syngnathidae) of the lower York River, Virginia. Environmental Biology of Fishes 43 (3): 295-309.

Thayer, G.W., Bjorndal, K.A., Ogden, J.C., Williams, S.L., and Zieman, J.C. 1984. Role of larger herbivores in seagrass communities. Estuaries and Coasts 7 (4): 351-376.

van Montfrans, J., Macko, S.A., Harbeson, S.A., and Combs, D. 2005. Trophic interactions of fish predators utilizing restored seagrass beds in Virginia's Coastal Bays. Final Report for FY 2003, Task 12.05. 7 pages.

Vander Zanden, M.J., Hulshof, M., Ridgway, M.S., and Rasmussen, J.B. 1998. Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. Transactions of the American Fisheries Society 127: 729-739.

Vizzini, S., Sarà, G., Michener, R.H., and Mazzola, A. 2002. The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter to secondary consumers as revealed by carbon and nitrogen stable isotope analysis. Acta Oecologia 23: 277–285.

Vizzini, S., and Mazzola A. 2003. Seasonal variations in the stable carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of primary producers and consumers in a western Mediterranean coastal lagoon. Marine Biology 142: 1009-1018.

Vizzini, S., and Mazzola, A. 2006. Sources and transfer of organic matter in food webs of a Mediterranean coastal environment: Evidence for spatial variability. Estuarine, Coastal and Shelf Science 66: 459-467.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L. Jr., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., and Williams, S.L. 2009. Accelerating loss of seagrass across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences 106: 12377-12381.

Weinstein, M.P. and Brooks, H.A. 1983. Comparative ecology of nekton residing in a tidal creek and adjacent seagrass meadow: community composition and structure. Marine Ecology Progress Series 12: 15-28.

Yamamuro, M., and Chirapart, A. 2005. Quality of the seagrass *Halophila ovalis* on a Thai intertidal flat as food for the dugong. Journal of Oceanography 61: 183-186.

Zieman, J.C., and Wetzel, R.G. 1980. Productivity in seagrasses: methods and rates. Pages 87-116 in: Phillips, R.C. and McRoy, C.P. (eds.), *Handbook of seagrass biology: an ecosystem perspective*. New York, NY: Garland STPM. 353 pages.

Table 4.1. Total numbers of collected fish, individual species, and individuals analyzed for stable isotopes, 2005.

Sampling Date	Site Type	Total Species Collected	Total Fish Collected	Total Individuals selected for Isotope Analysis
6/16/2005	Restored eelgrass plots	20	881	78
	Eelgrass-free sites	12	531	44
7/14/2005	Restored eelgrass plots	42	2643	146
	Eelgrass-free sites	24	3088	54
8/15/2005	Restored eelgrass plots	36	2084	137
	Eelgrass-free sites	25	311	88
9/12/2005	Restored eelgrass plots	31	837	101
	Eelgrass-free sites	15	505	51

Table 4.2. Effects of independent variables COVER and SPECIES and the interaction of the variables on fish mean isotope values during July, August, and September. The interaction did not have a statistically significant effect on any fish isotope values in June 2005 ($p > 0.05$). ns – difference between mean values was not statistically significant at $p < 0.05$.

Species	Month	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)			$\delta^{34}S$ (‰)		
		Eelgrass-free	Eelgrass	F @ p<0.05	Eelgrass-free	Eelgrass	F @ p<0.05	Eelgrass-free	Eelgrass	F @ p<0.05
Bairdiella chrysoura	July	-16.3	-16.5	ns	14.2	14.1	Ns	14.6	15.8	7.8
Leiostomus xanthurus		-15.2	-15.0	ns	14.9	13.6	13.2	11.7	13.1	11.2
Paralichthys dentatus		-15.1	-15.0	ns	16.2	13.5	25.7	13.5	14.6	ns
Tautoga onitis		-15.0	-14.4	ns	12.8	14.7	12.5	13.8	17.2	29.4
Brevoortia tyrannus		-18.0	-18.7	ns	12.9	12.6	Ns	12.9	16.3	35.2
	August	Eelgrass-free	Eelgrass	F @ p<0.05	Eelgrass-free	Eelgrass	F @ p<0.05	Eelgrass-free	Eelgrass	F @ p<0.05
Menidia menidia		-18.0	-17.0	ns	14.5	13.5	7.5	12.2	13.6	4.5
Syngnathus fuscus		-14.9	-14.0	ns	12.2	12.9	Ns	12.8	11.3	ns
Orthopristis chrysoptera		-15.9	-15.5	ns	14.0	13.8	Ns	7.4	11.9	16.7
Bairdiella chrysoura		-15.6	-15.2	ns	13.5	13.8	Ns	11.8	13.8	9.8
Leiostomus xanthurus		-15.7	-14.6	ns	14.0	13.2	4.4	8.8	10.9	9.9
	September	Eelgrass-free	Eelgrass	F @ p<0.05	Eelgrass-free	Eelgrass	F @ p<0.05	Eelgrass-free	Eelgrass	F @ p<0.05
Menidia menidia		-22.7	-16.8	162.3	17.7	17.3	Ns	14.4	13.4	ns
Anchoa mitchilli		-20.1	-16.9	32.1	17.1	17.6	Ns	13.8	14.3	ns
Syngnathus fuscus		-16.2	-14.9	ns	14.1	14.8	Ns	11.0	13.9	4.8
Bairdiella chrysoura		-18.1	-16.3	8.9	15.9	16.8	Ns	12.3	15.4	6.3
Leiostomus xanthurus		-19.5	-14.9	91.5	16.6	16.3	Ns	8.6	14.8	39.0

Figure 4.1a. Average $\delta^{13}C$ values (± standard error) of all fish captured in eelgrass plots vs. eelgrass-free sites by sampling month.

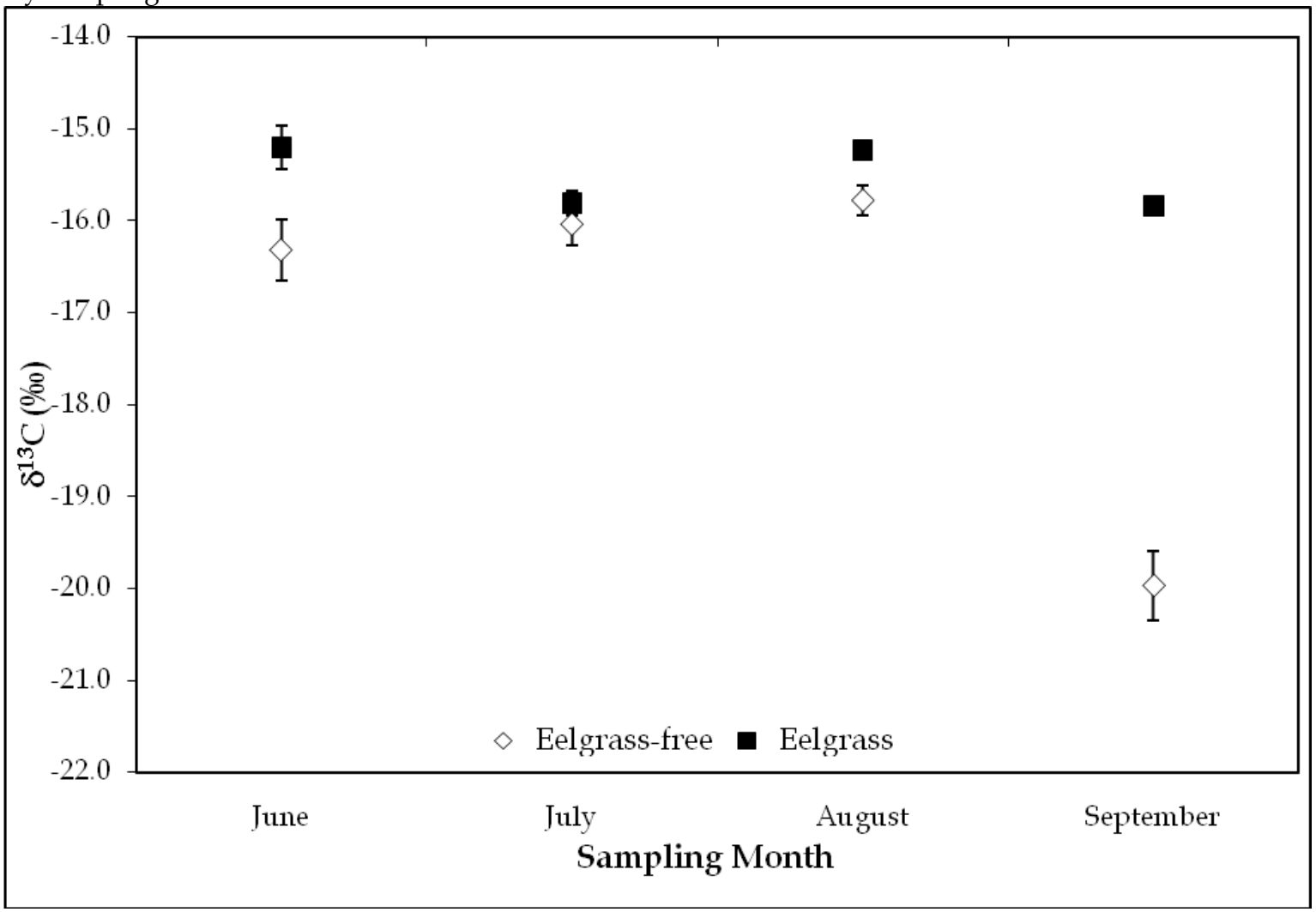


Figure 4.1b. Average $\delta^{15}N$ values (± standard error) of all fish in eelgrass plots vs. eelgrass-free sites by month.

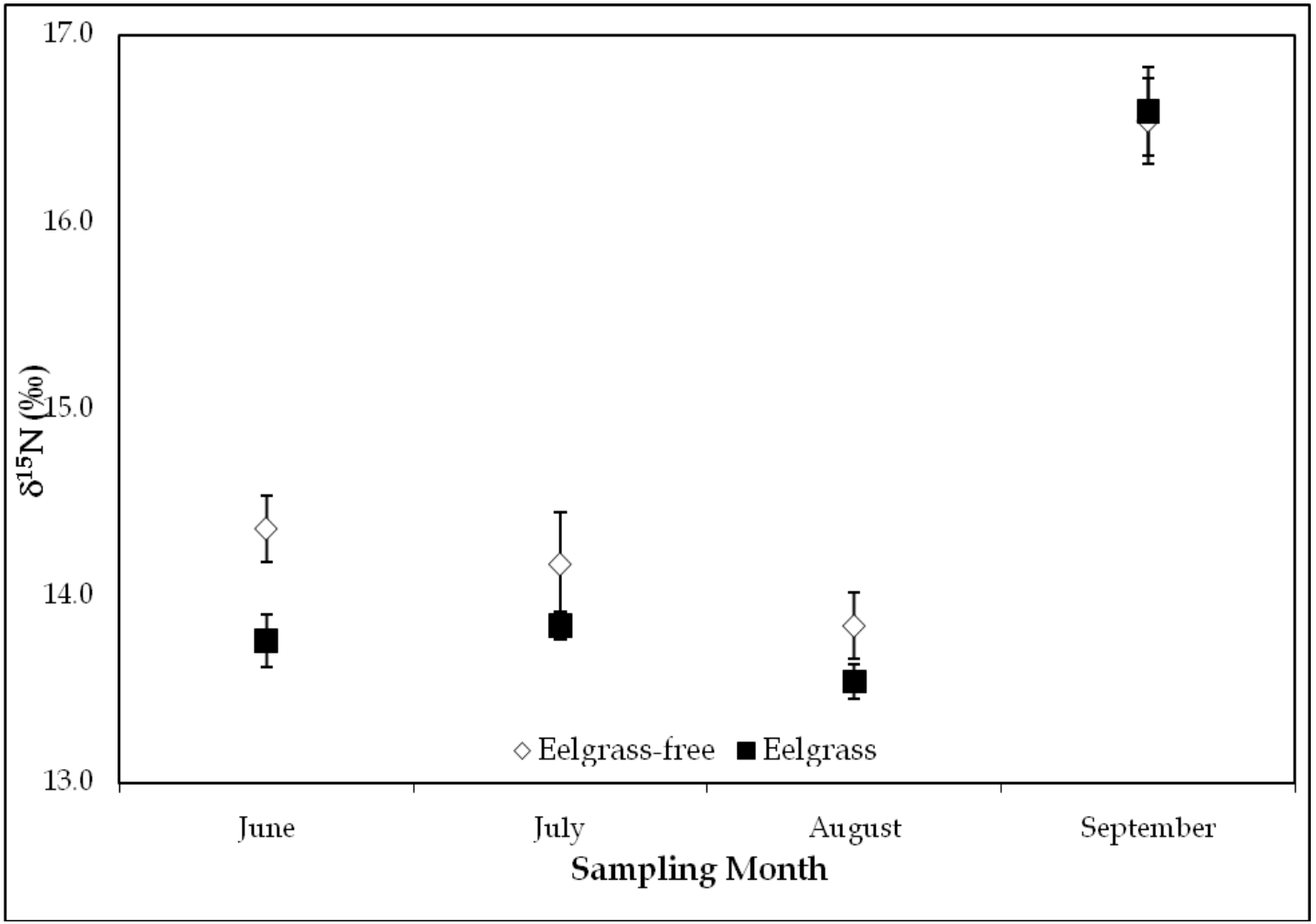


Figure 4.1c. Average $\delta^{34}S$ values (± standard error) of all fish in eelgrass plots vs. eelgrass-free sites by month.

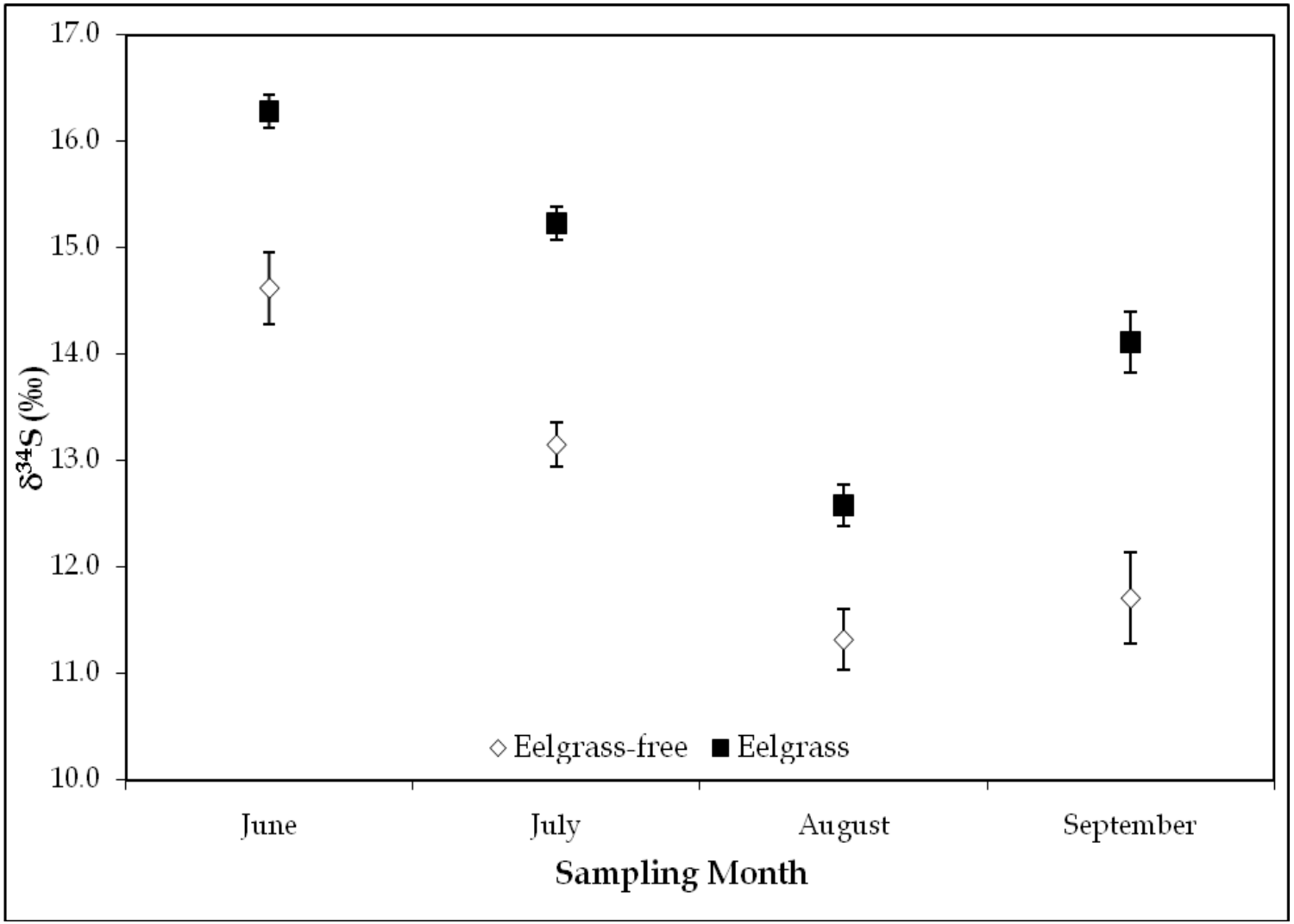


Figure 4.2a. Difference in average $\delta^{13}C$ and $\delta^{15}N$ values of fish in eelgrass plots vs. eelgrass-free sites by month. The symbol 'Δ' refers to the difference between consumer isotope values at the two sites (eelgrass minus eelgrass-free). Positive values indicate that fish from eelgrass sites have greater average isotope values.

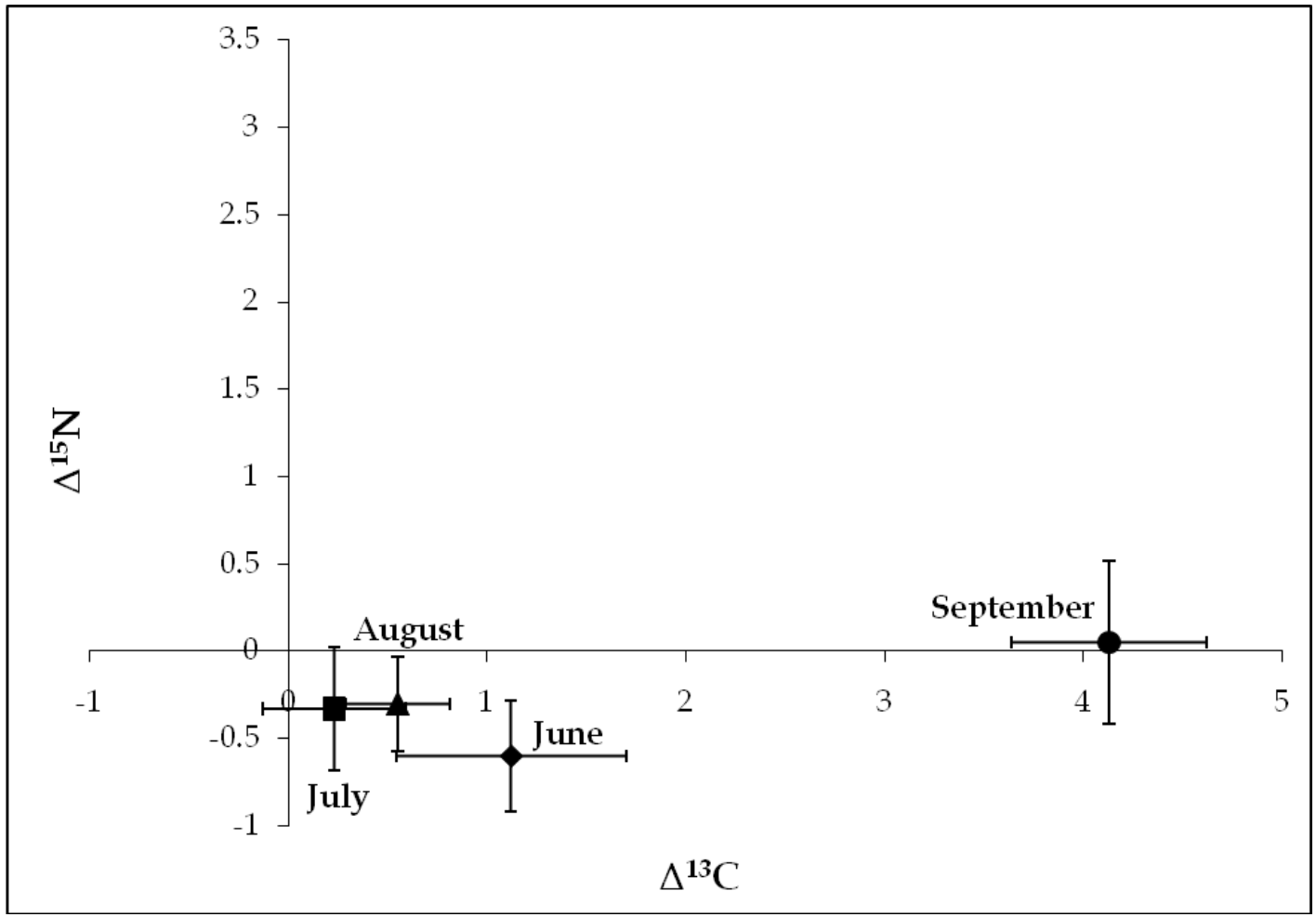


Figure 4.2b. Difference in average $\delta^{13}C$ and $\delta^{34}S$ values of fish in eelgrass plots vs. eelgrass-free sites by month. The symbol 'Δ' refers to the difference between consumer isotope values at the two sites (eelgrass minus eelgrass-free). Positive values indicate that fish in eelgrass sites have greater average isotope values.

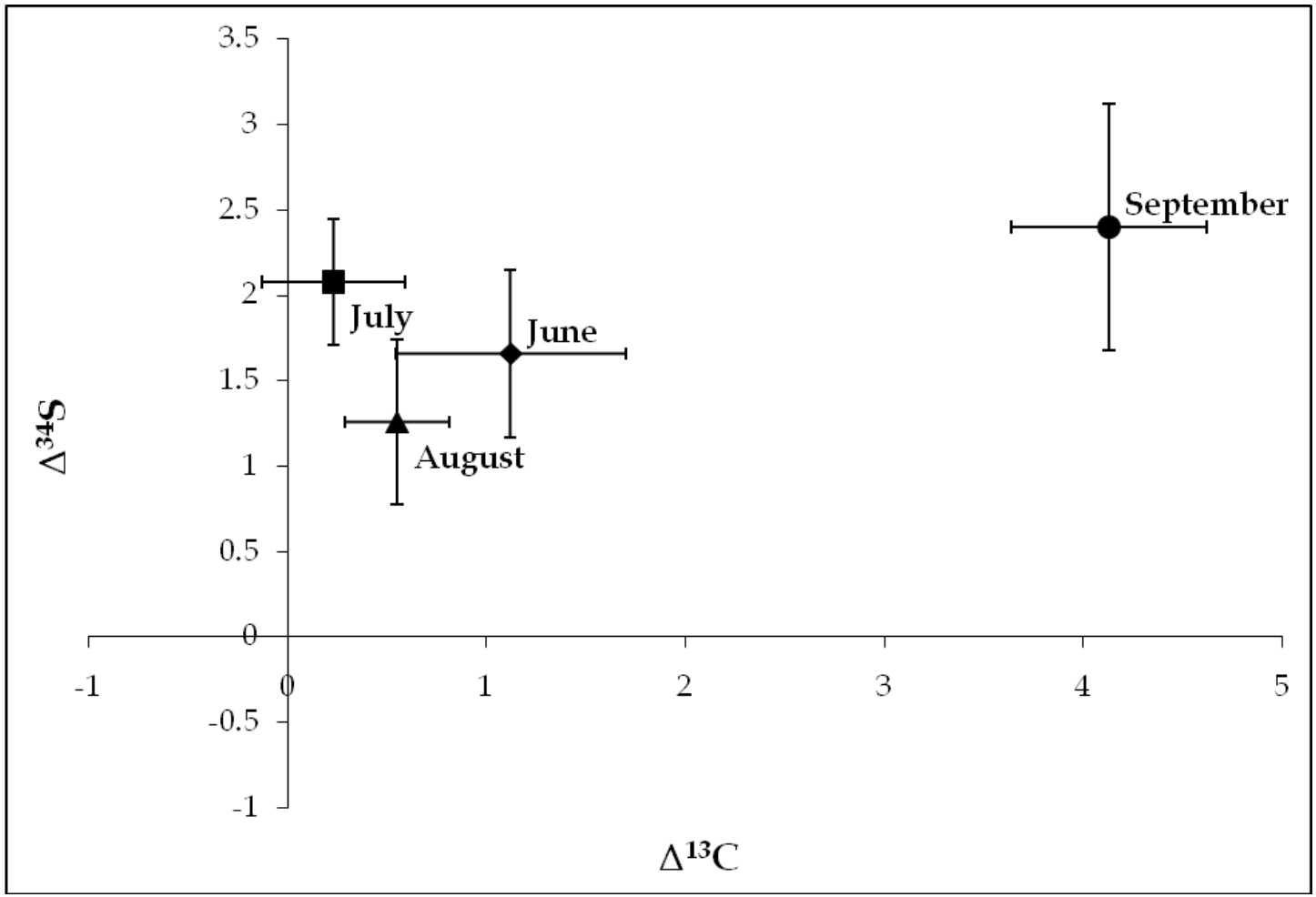


Figure 4.3. Average $\delta^{13}C$ vs. $\delta^{15}N$ values of spot tissue by month captured. Closed symbols represent fish from eelgrass meadows; open symbols represent fish from eelgrass-free sites. ◆ - June; ■ - July; ▲ - August; ● - September.

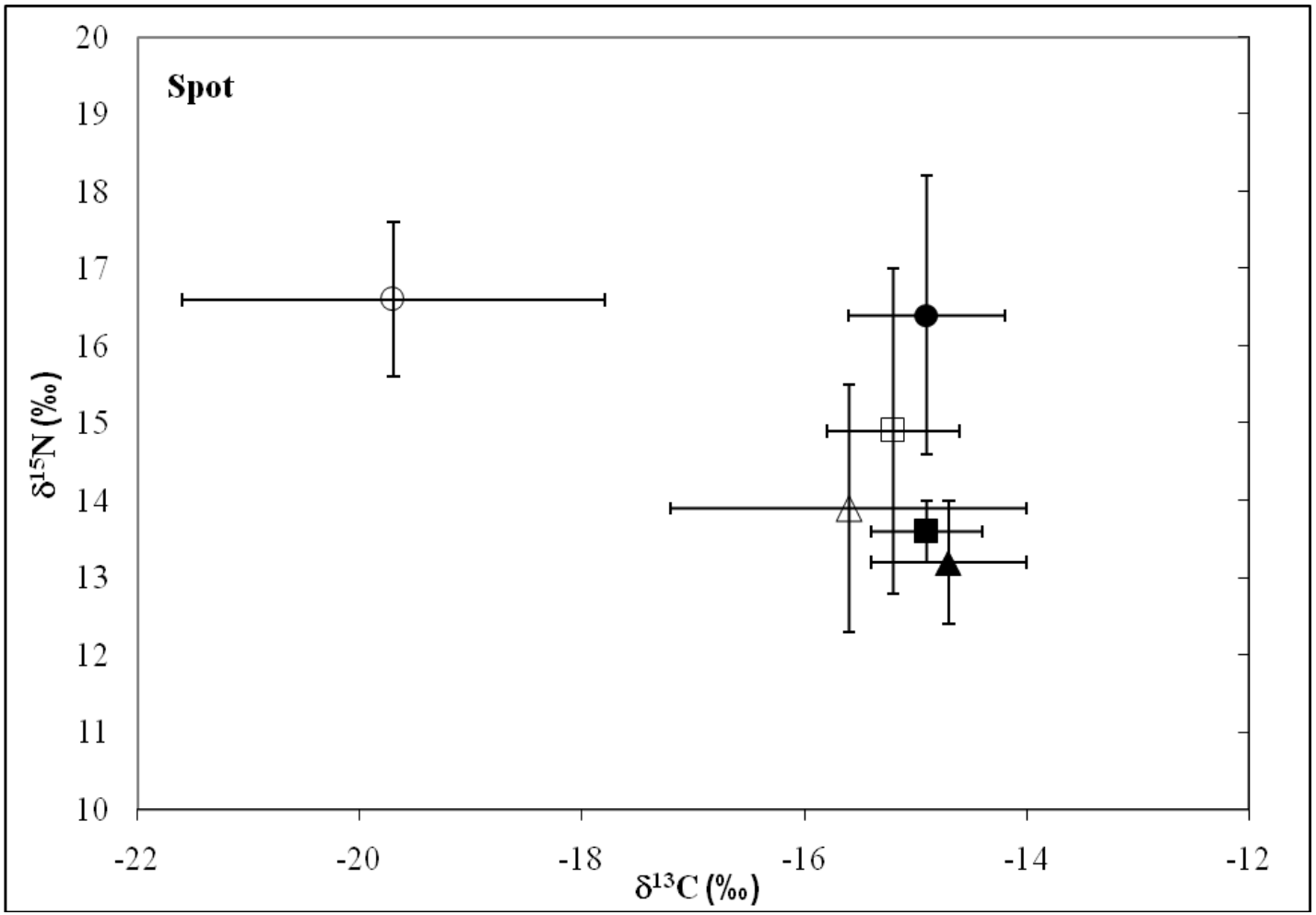


Figure 4.4. Average $\delta^{13}C$ vs. $\delta^{15}N$ values of summer flounder by month captured. Closed symbols represent fish from eelgrass meadows; open symbols represent fish from eelgrass-free sites. ◆ - June; ■ - July; ▲ - August; ● - September.

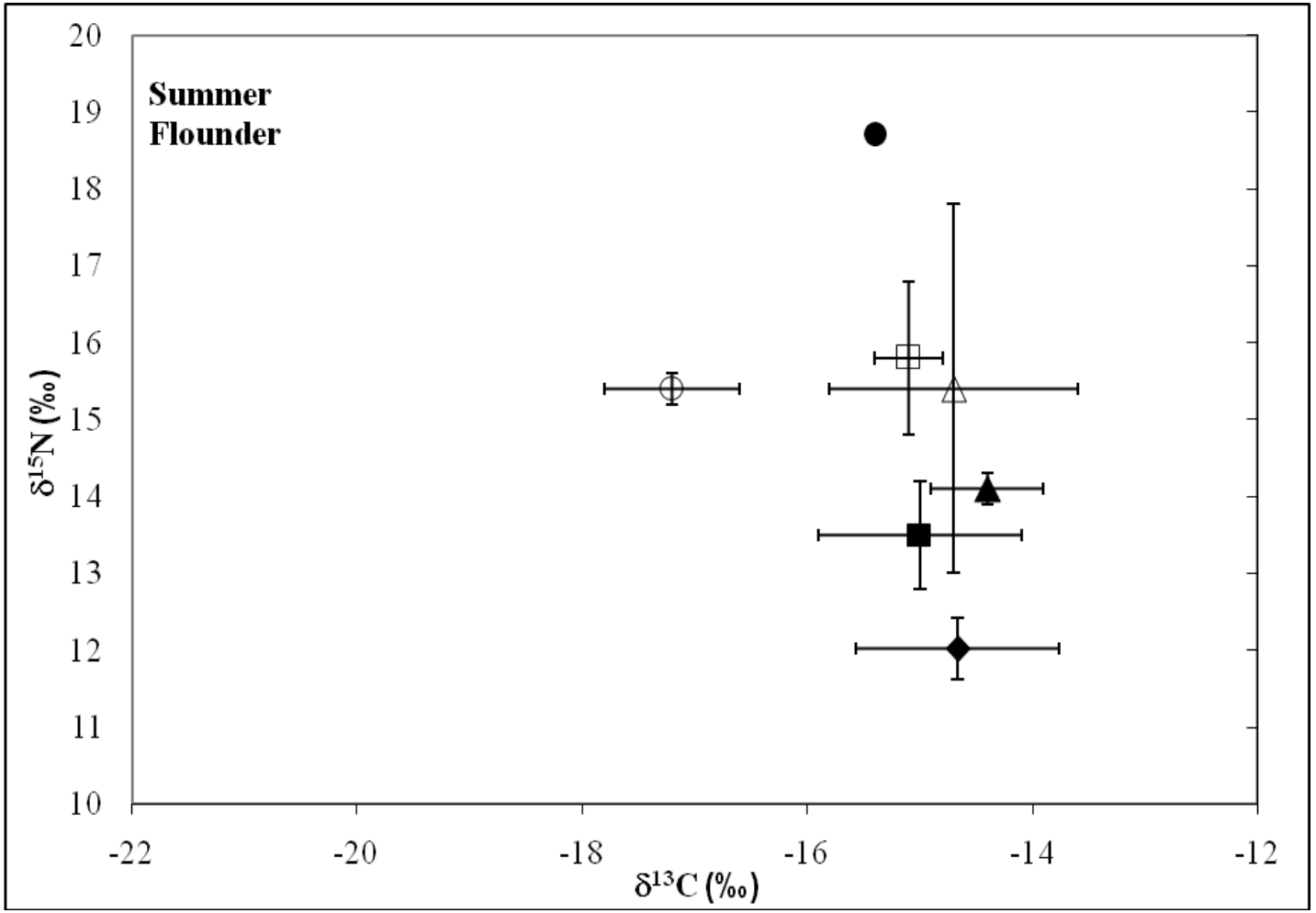


Figure 4.5. Average $\delta^{13}C$ vs. $\delta^{34}S$ values of spot by month captured. Closed symbols represent fish from eelgrass meadows; open symbols represent fish from eelgrass-free sites. ◆ - June; ■ - July; ▲ - August; ● - September.

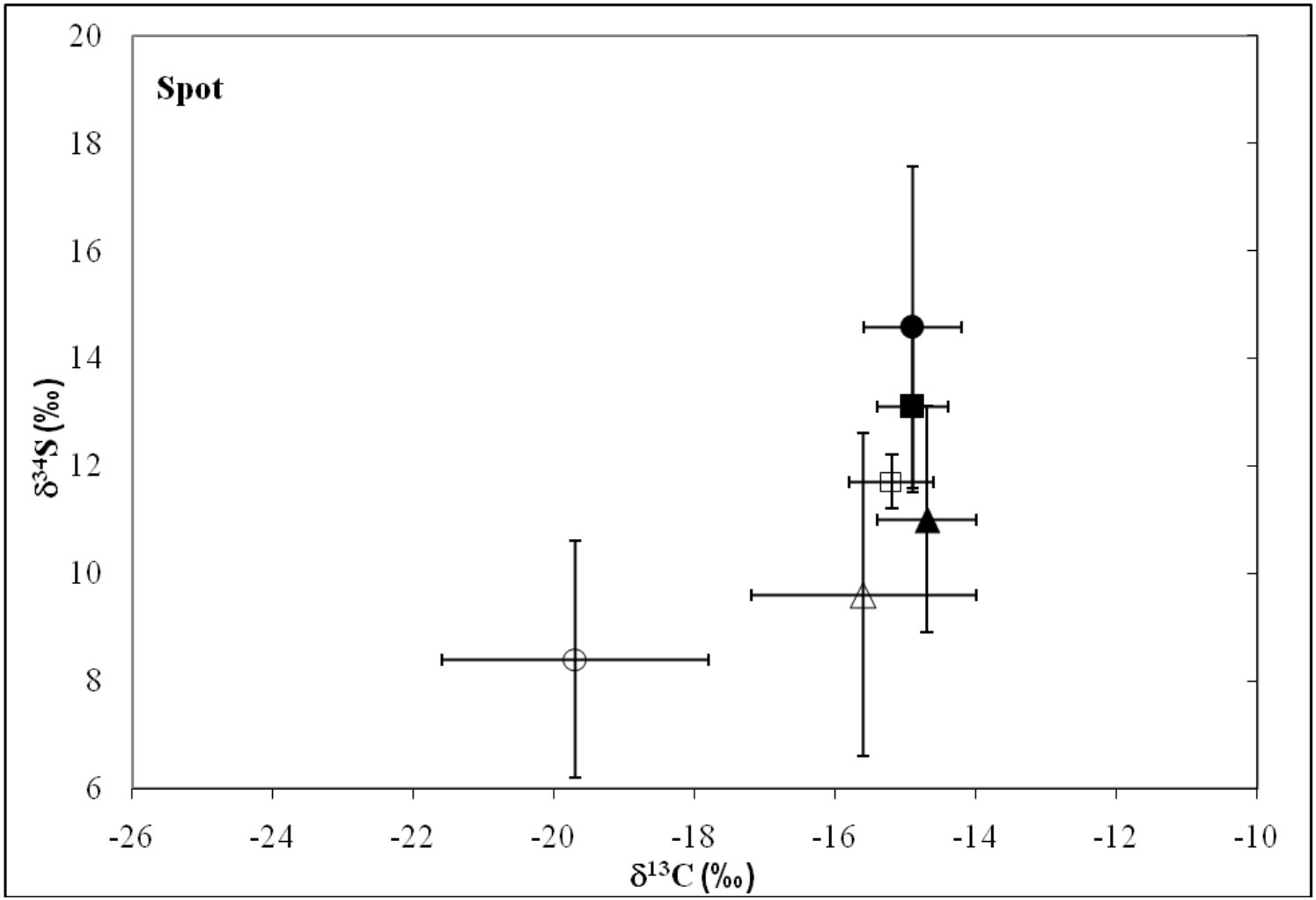


Figure 4.6. Average $\delta^{13}C$ vs. $\delta^{34}S$ values of summer flounder by date captured. Closed symbols represent fish from eelgrass meadows; open symbols represent fish from eelgrass-free sites. ◆ - June; ■ - July; ▲ - August; ● - September.

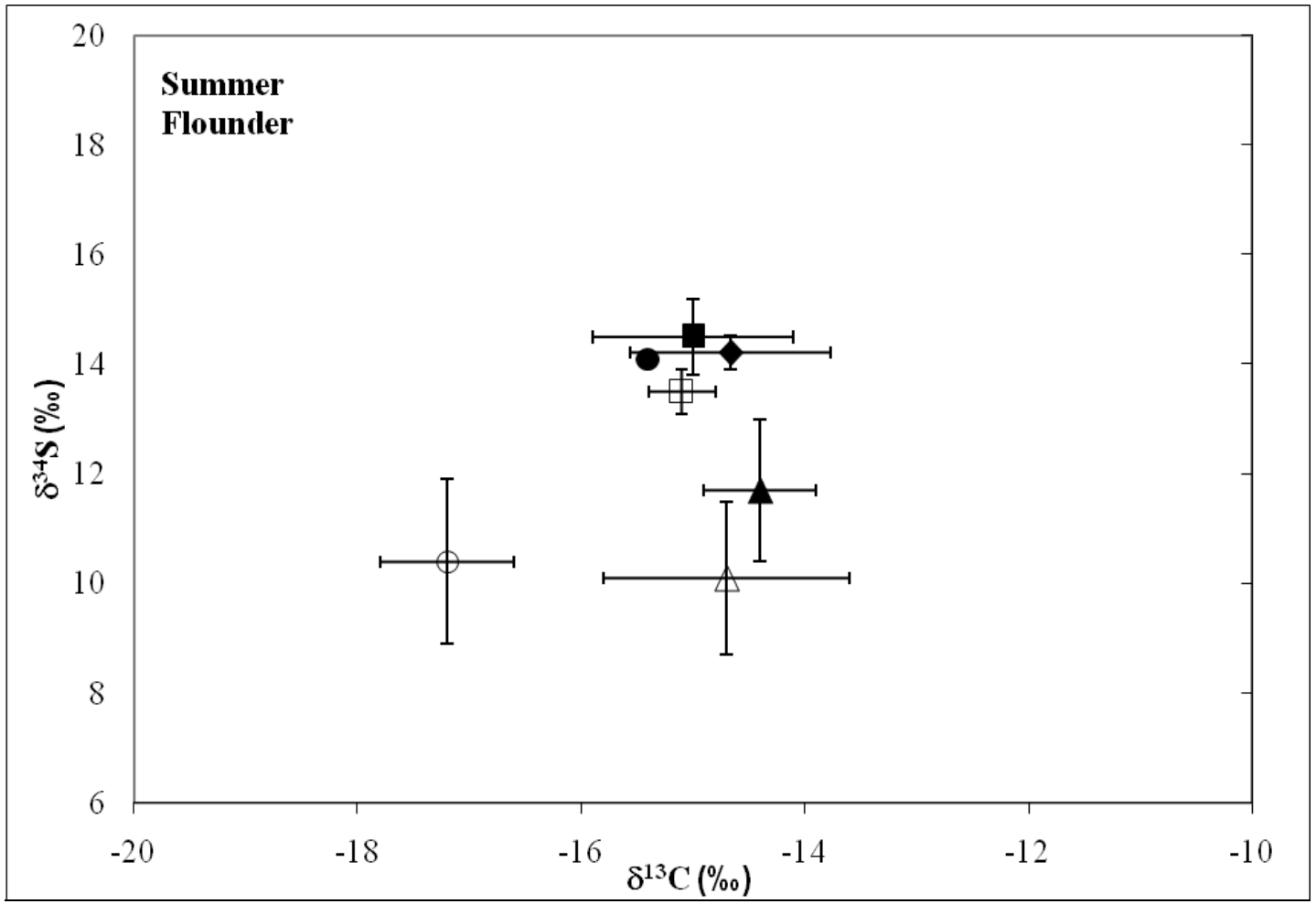


Figure 4.7. Average $\delta^{13}C$ vs. $\delta^{34}S$ values of silver perch by month captured. Closed symbols represent fish from eelgrass meadows; open symbols represent fish from eelgrass-free sites. ◆ - June; ■ - July; ▲ - August; ● - September.

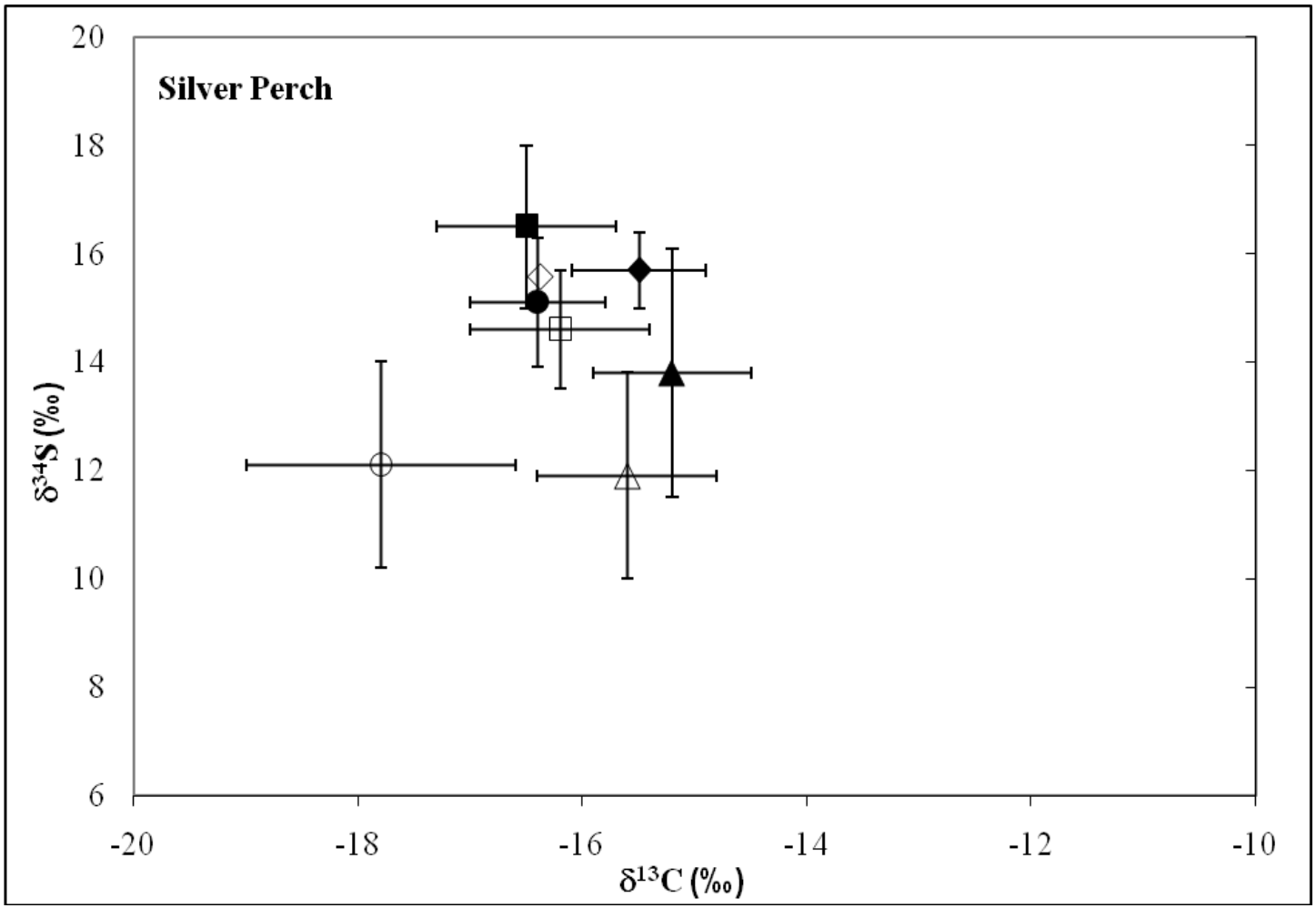


Figure 4.8. Diagram of the interaction of independent variables MONTH, COVER and SPECIES on $\delta^{13}C$ values of four species. Closed marks/dashed lines represent fish from restored eelgrass plots; open marks/solid lines represent fish from eelgrass-free plots. ● – Atlantic silversides; ▲ – northern pipefish; ■ – silver perch; ◆ – spot.

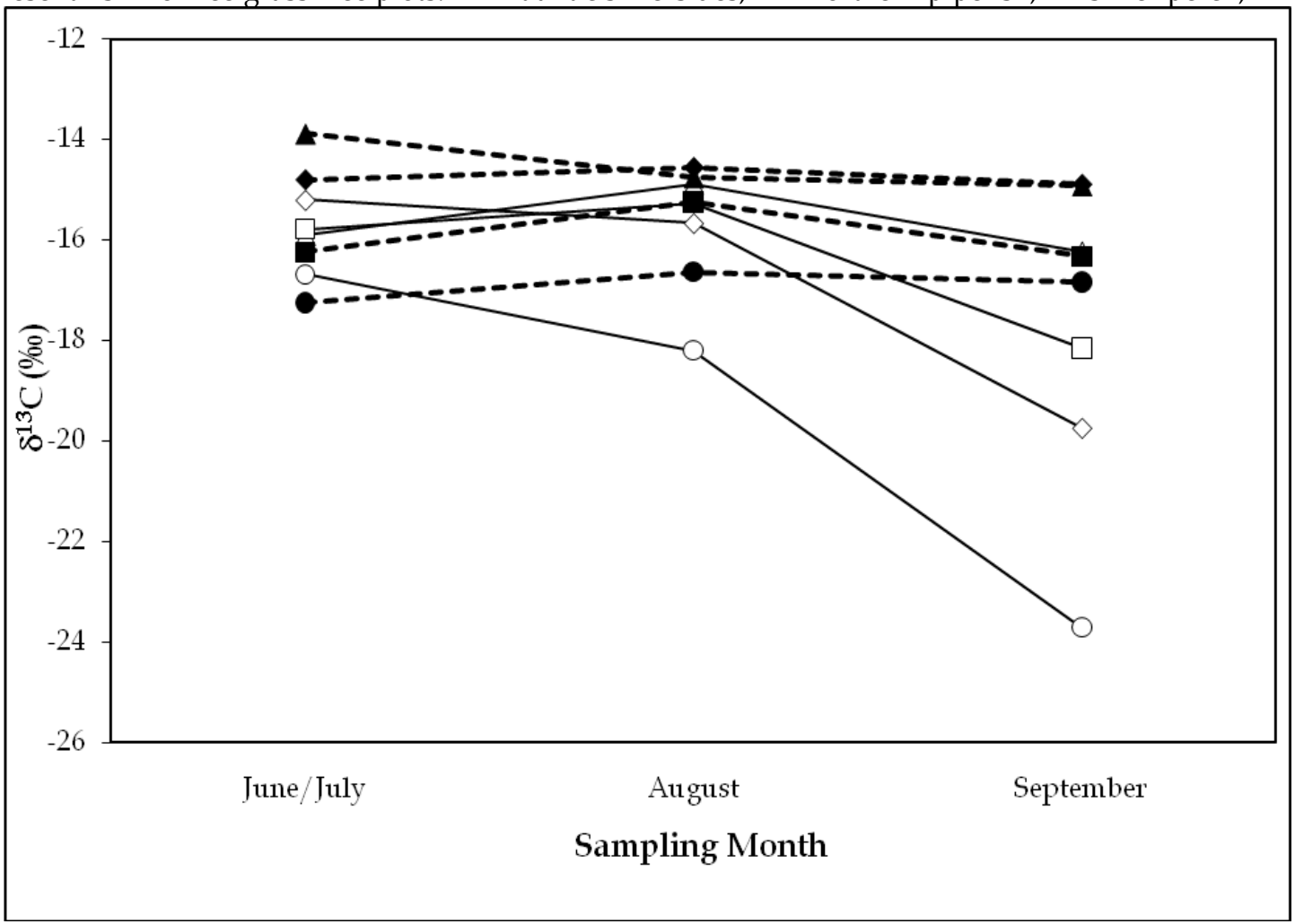


Figure 4.9. Diagram of the interaction of independent variables MONTH, COVER and SPECIES on $\delta^{34}S$ values of four species. Closed marks/dashed lines represent fish from restored eelgrass plots; open marks/solid lines represent fish from eelgrass-free plots. ● – Atlantic silversides; ▲ – northern pipefish; ■ – silver perch; ◆ – spot.

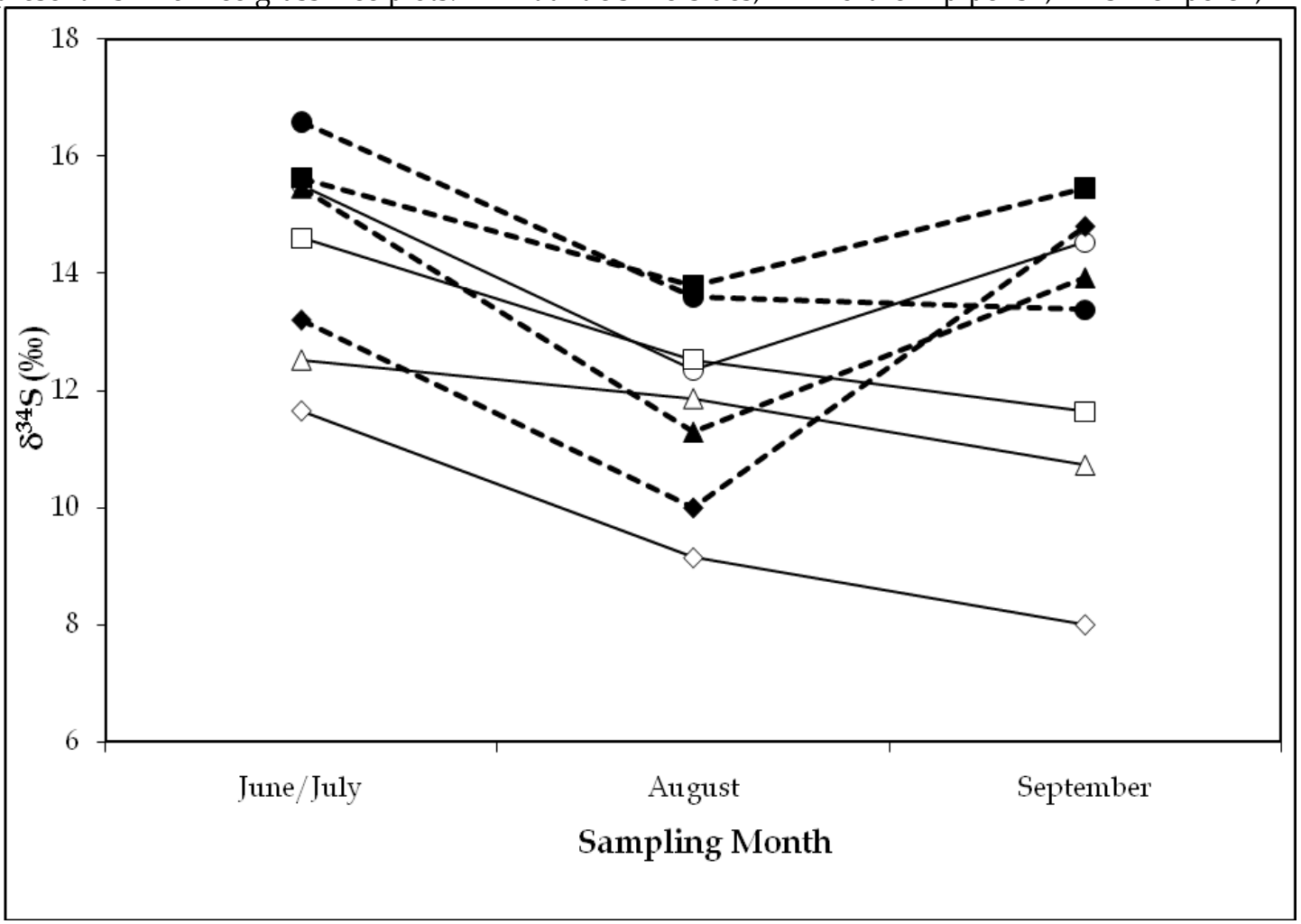


Figure 4.10. $\delta^{13}C$ vs. $\delta^{15}N$ of Atlantic silversides collected 9/12/2005. Closed marks indicate fish from eelgrass meadows; open marks indicate fish from eelgrass-free sites.

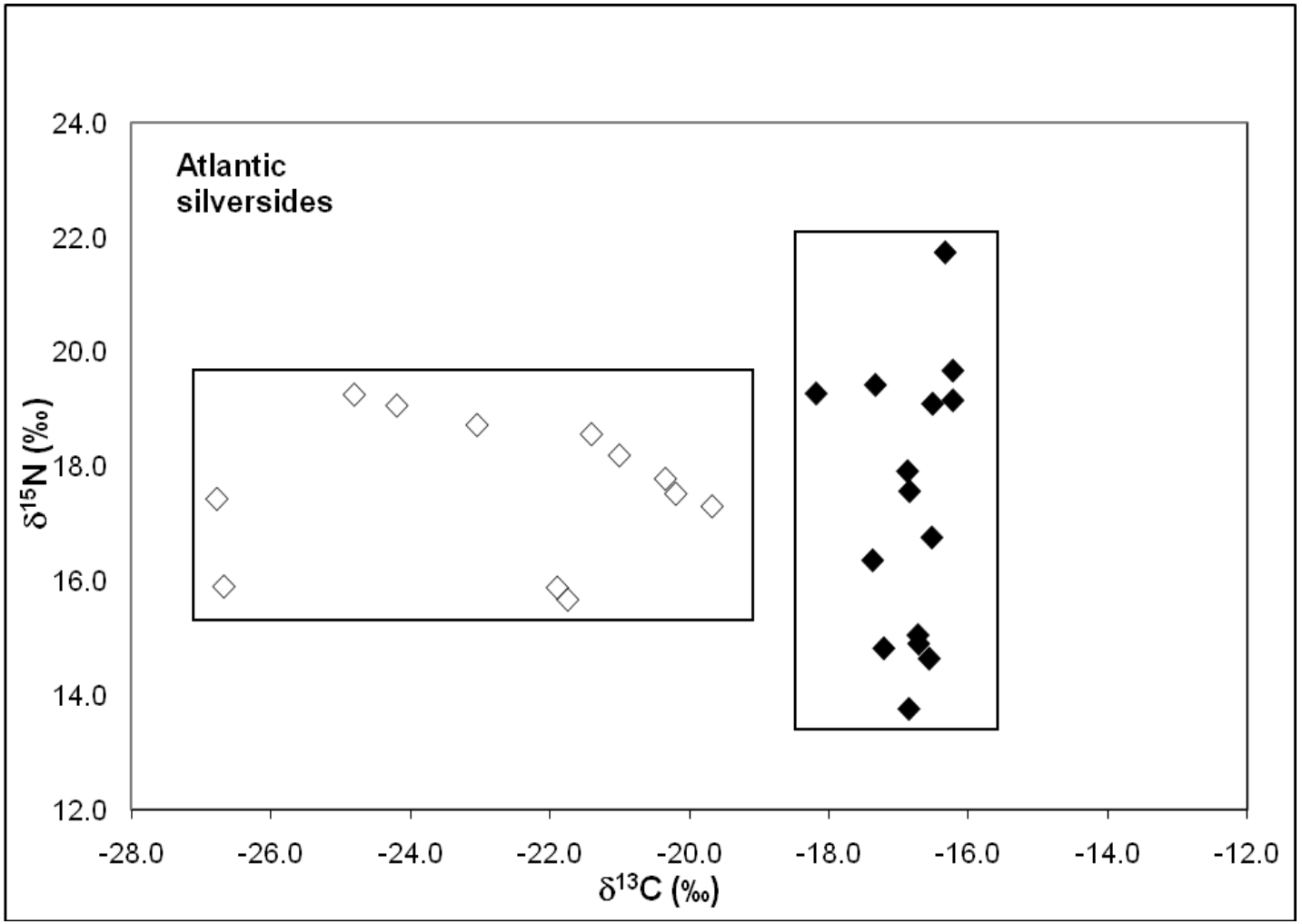


Figure 4.11. $\delta^{13}C$ vs. $\delta^{15}N$ of bay anchovy collected 9/12/2005. Closed marks indicate fish from eelgrass meadows; open marks indicate fish from eelgrass-free sites.

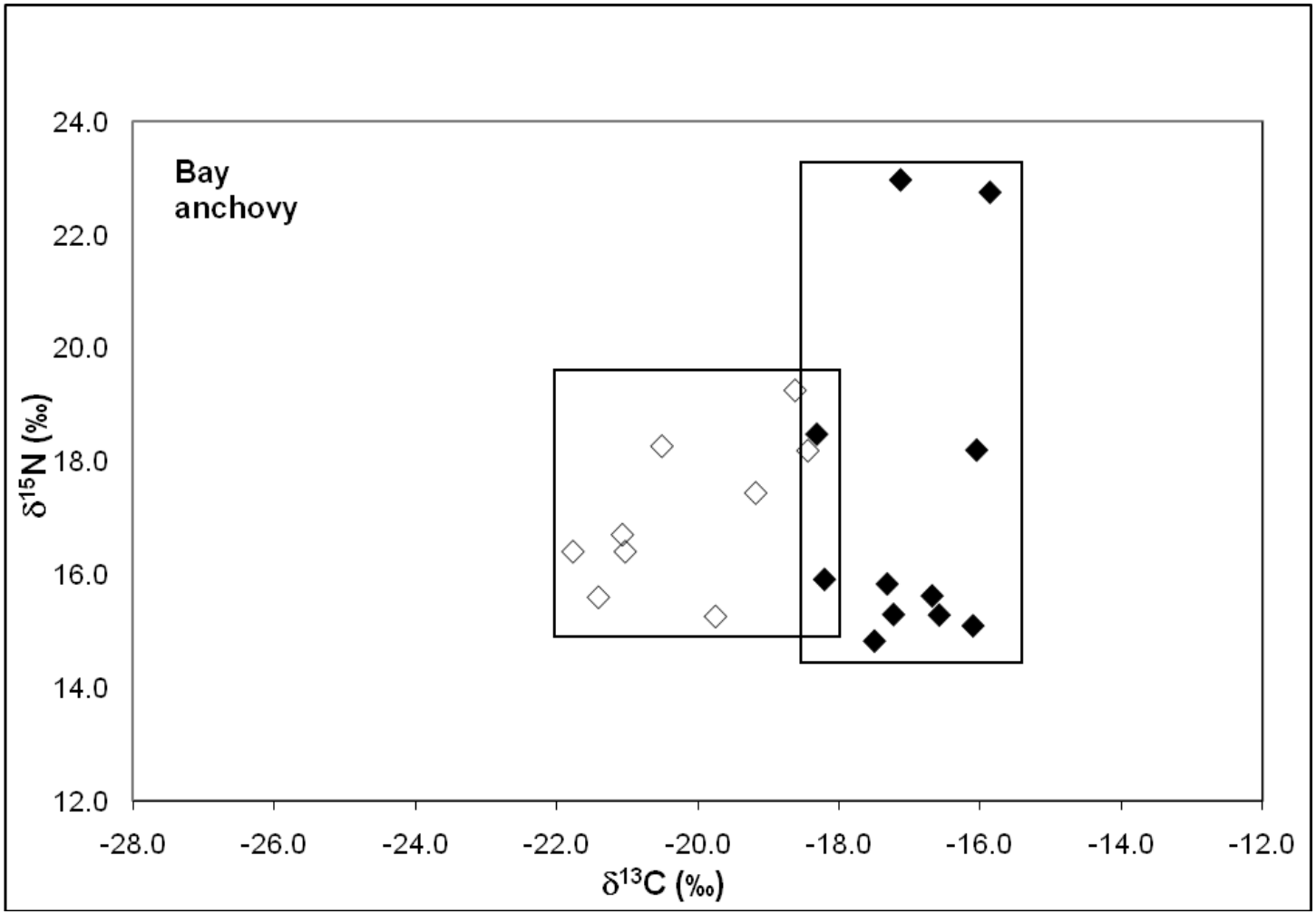


Figure 4.12. $\delta^{13}C$ vs. $\delta^{15}N$ of silver perch collected 9/12/2005. Closed marks indicate fish from eelgrass meadows; open marks indicate fish from eelgrass-free sites.

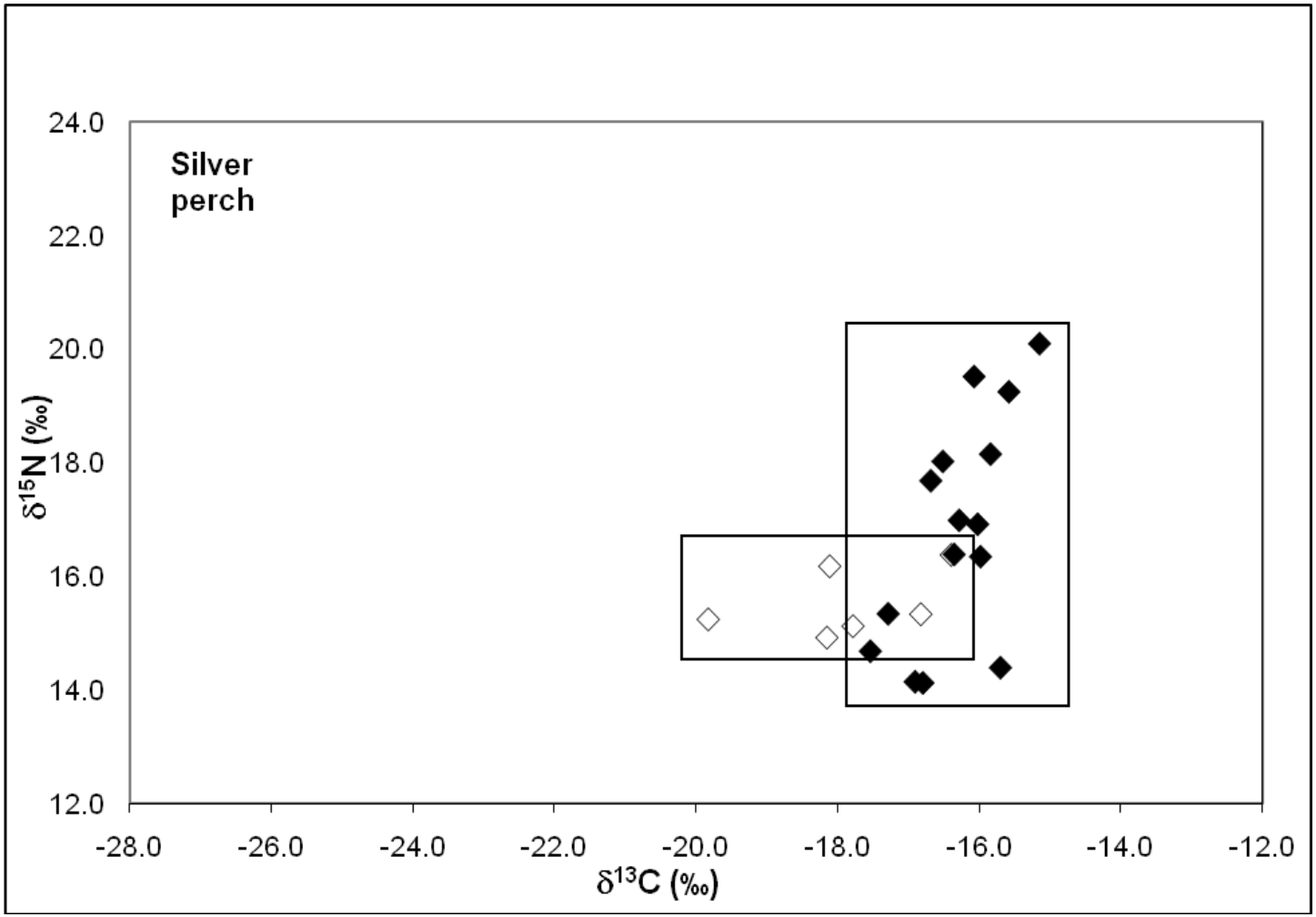


Figure 4.13. $\delta^{13}C$ vs. $\delta^{15}N$ of spot collected 9/12/2005. Closed marks indicate fish from eelgrass meadows; open marks indicate fish from eelgrass-free sites.

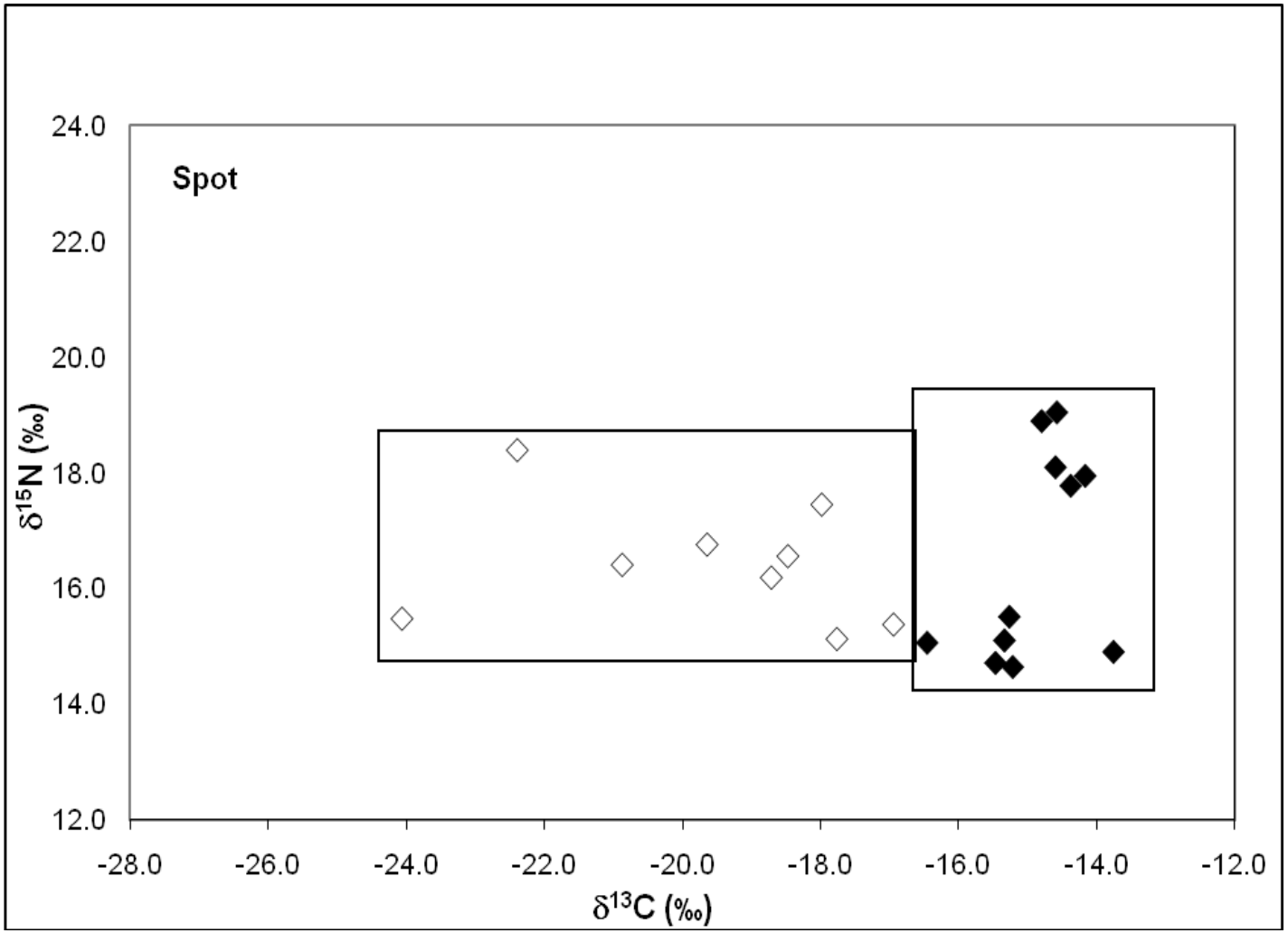


Figure 4.14. $\delta^{13}C$ vs. $\delta^{15}N$ of northern pipefish collected 9/12/2005. Closed marks indicate fish from eelgrass meadows; open marks indicate fish from eelgrass-free sites.

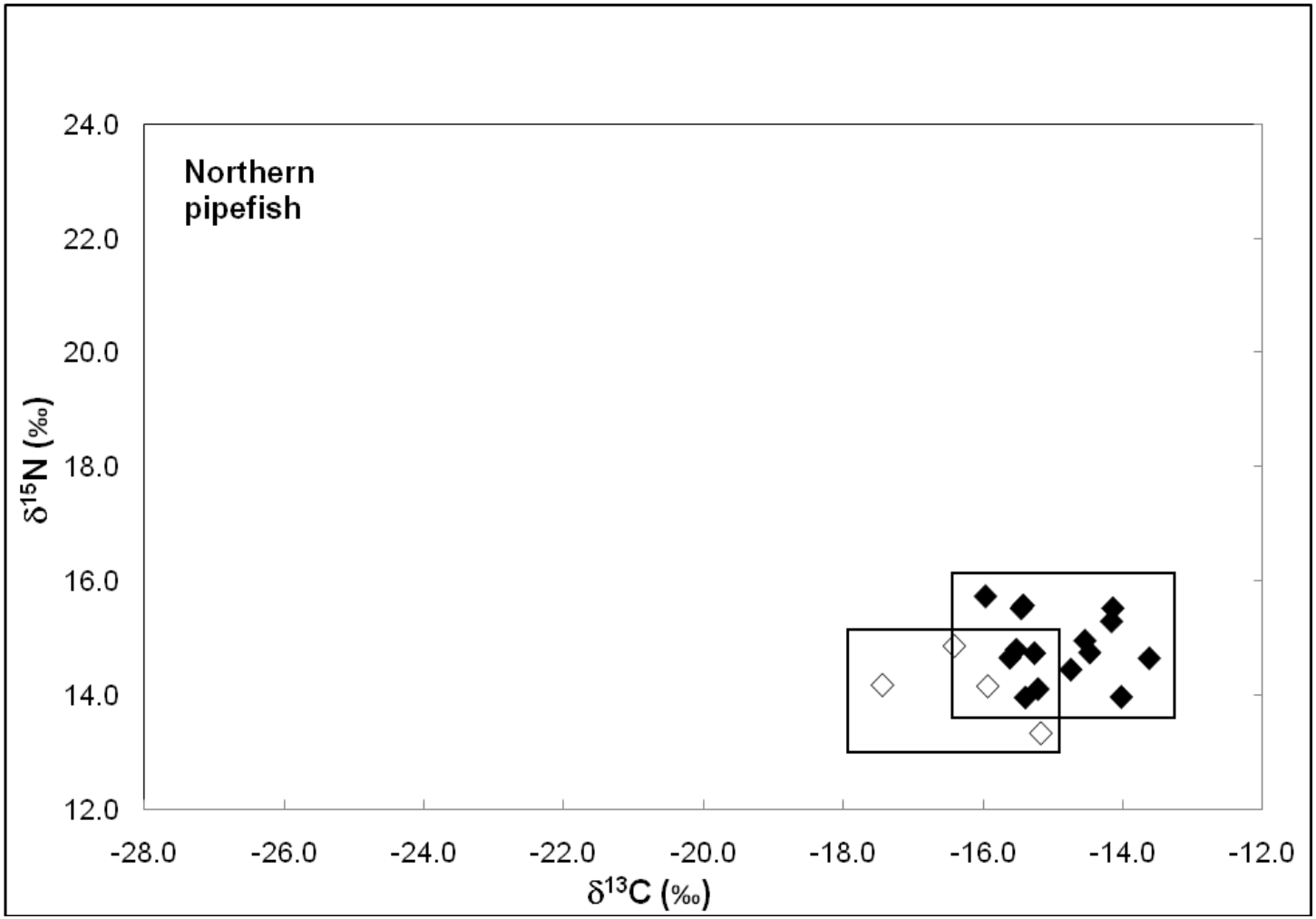


Figure 4.15. $\delta^{13}C$ vs. $\delta^{34}S$ of Atlantic silversides collected 9/12/2005. Closed marks indicate fish from eelgrass meadows; open marks indicate fish from eelgrass-free sites.

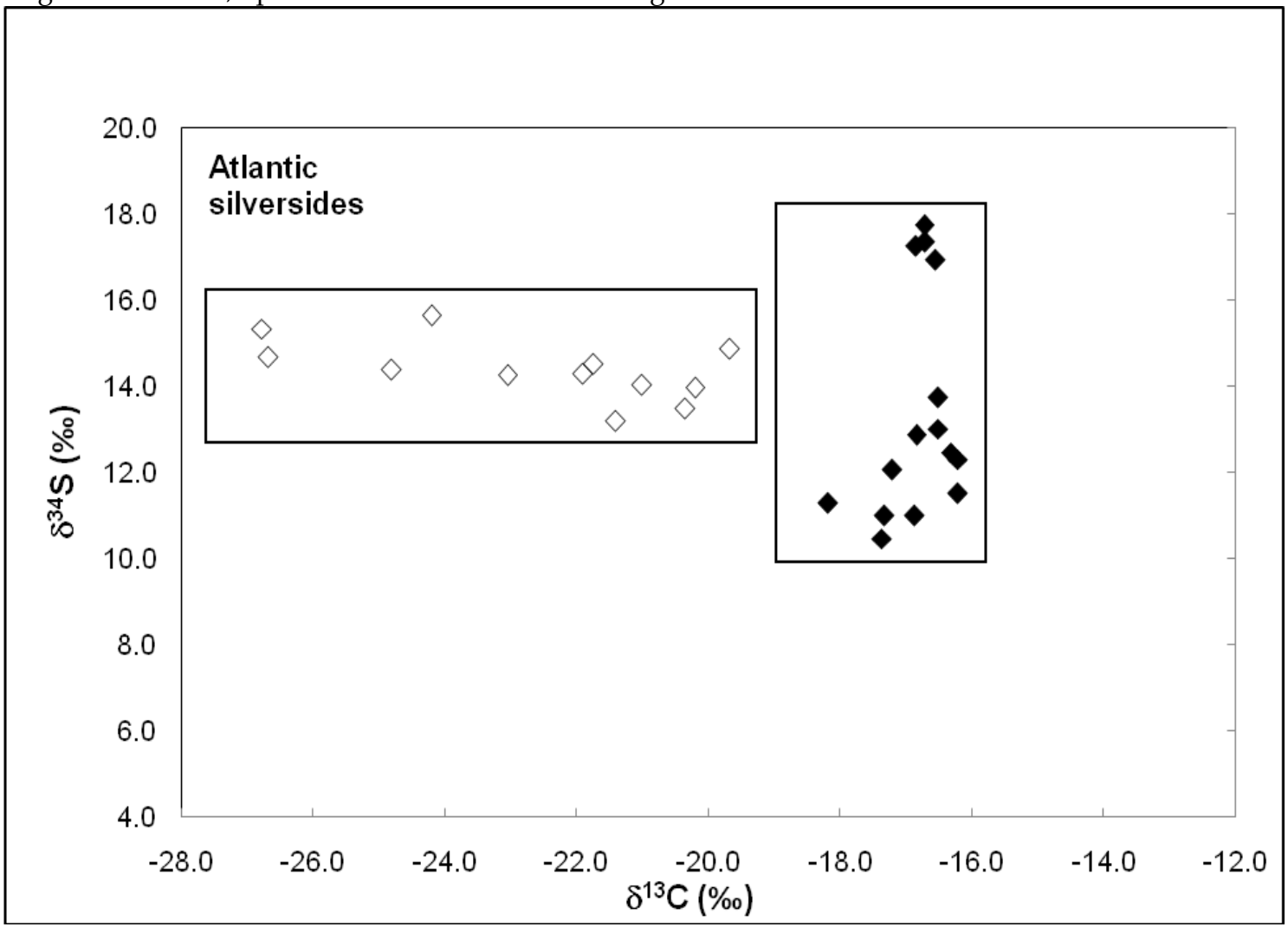


Figure 4.16. $\delta^{13}C$ vs. $\delta^{34}S$ of bay anchovy collected 9/12/2005. Closed marks indicate fish from eelgrass meadows; open marks indicate fish from eelgrass-free sites.

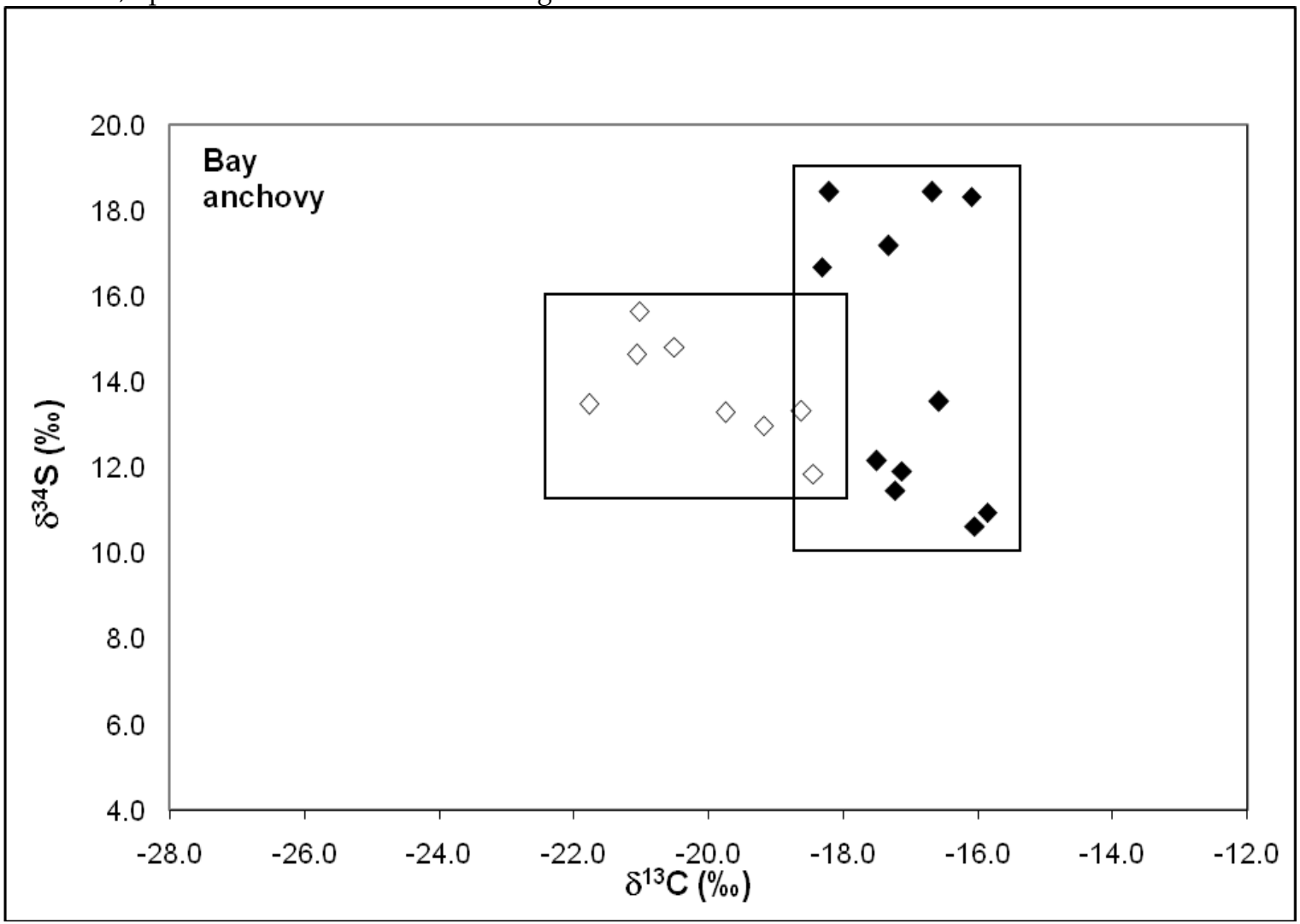


Figure 4.17. $\delta^{13}C$ vs. $\delta^{34}S$ of silver perch collected 9/12/2005. Closed marks indicate fish from eelgrass meadows; open marks indicate fish from eelgrass-free sites.

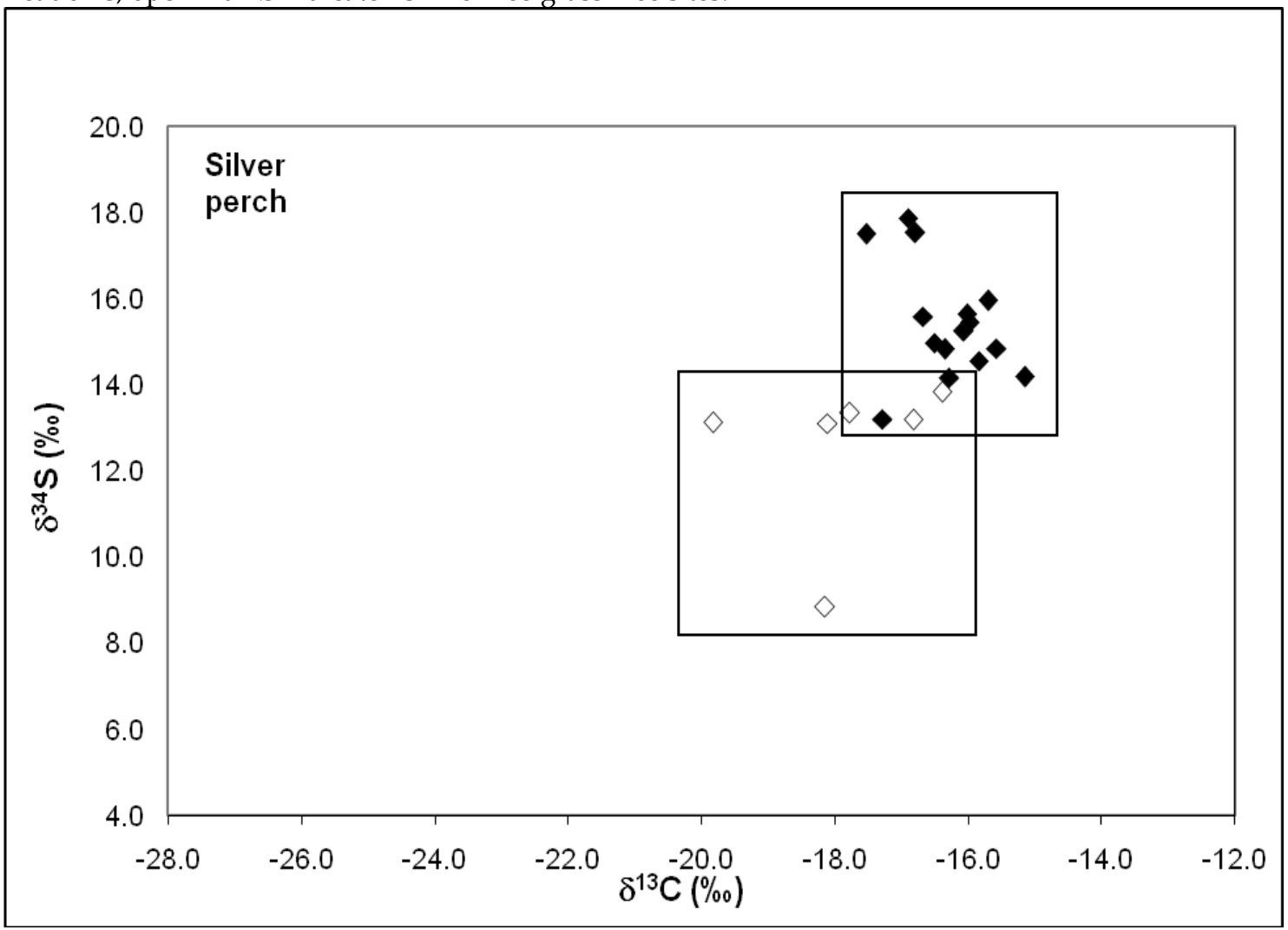


Figure 4.18. $\delta^{13}C$ vs. $\delta^{34}S$ of spot collected 9/12/2005. Closed marks indicate fish from eelgrass meadows; open marks indicate fish from eelgrass-free sites.

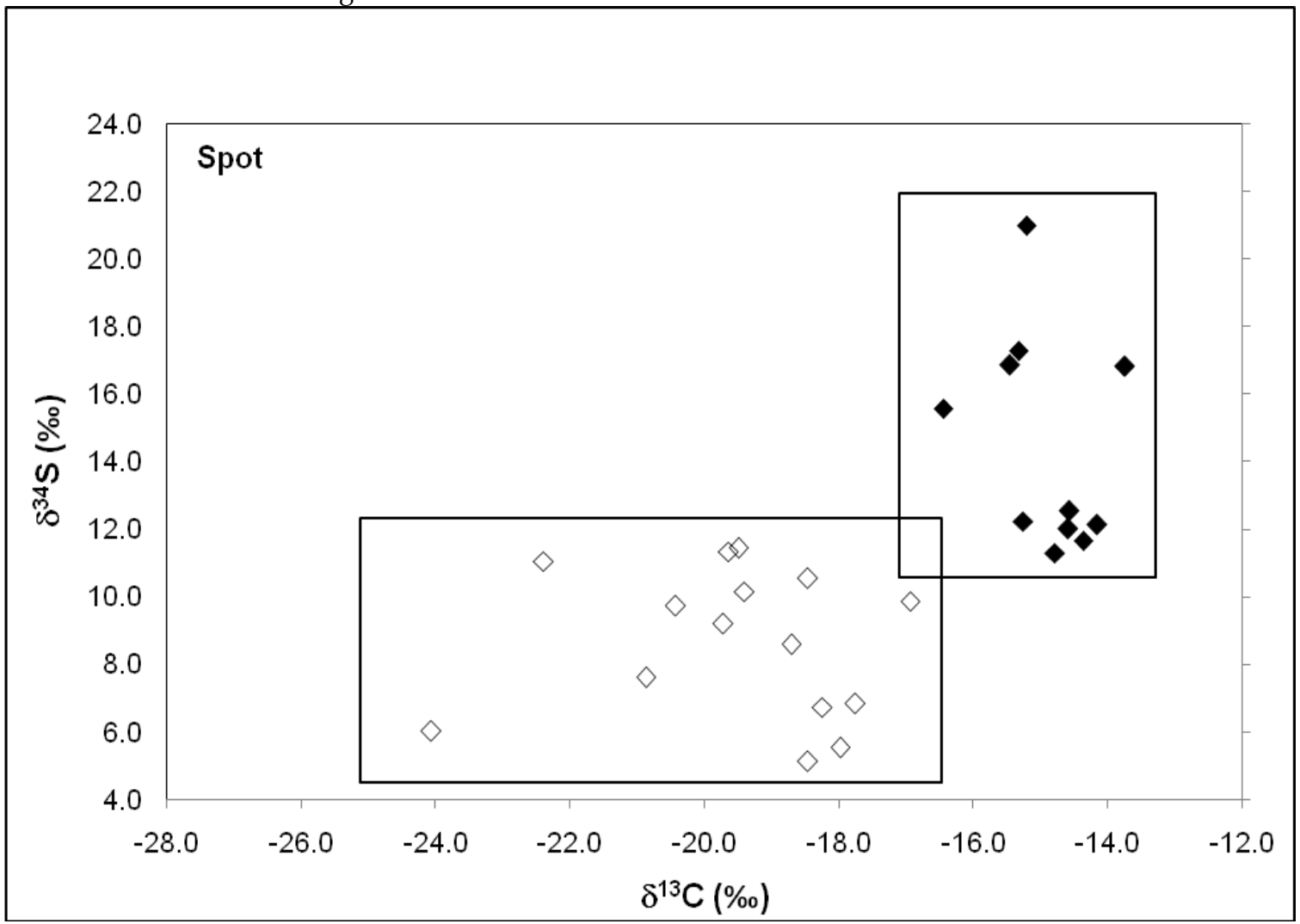
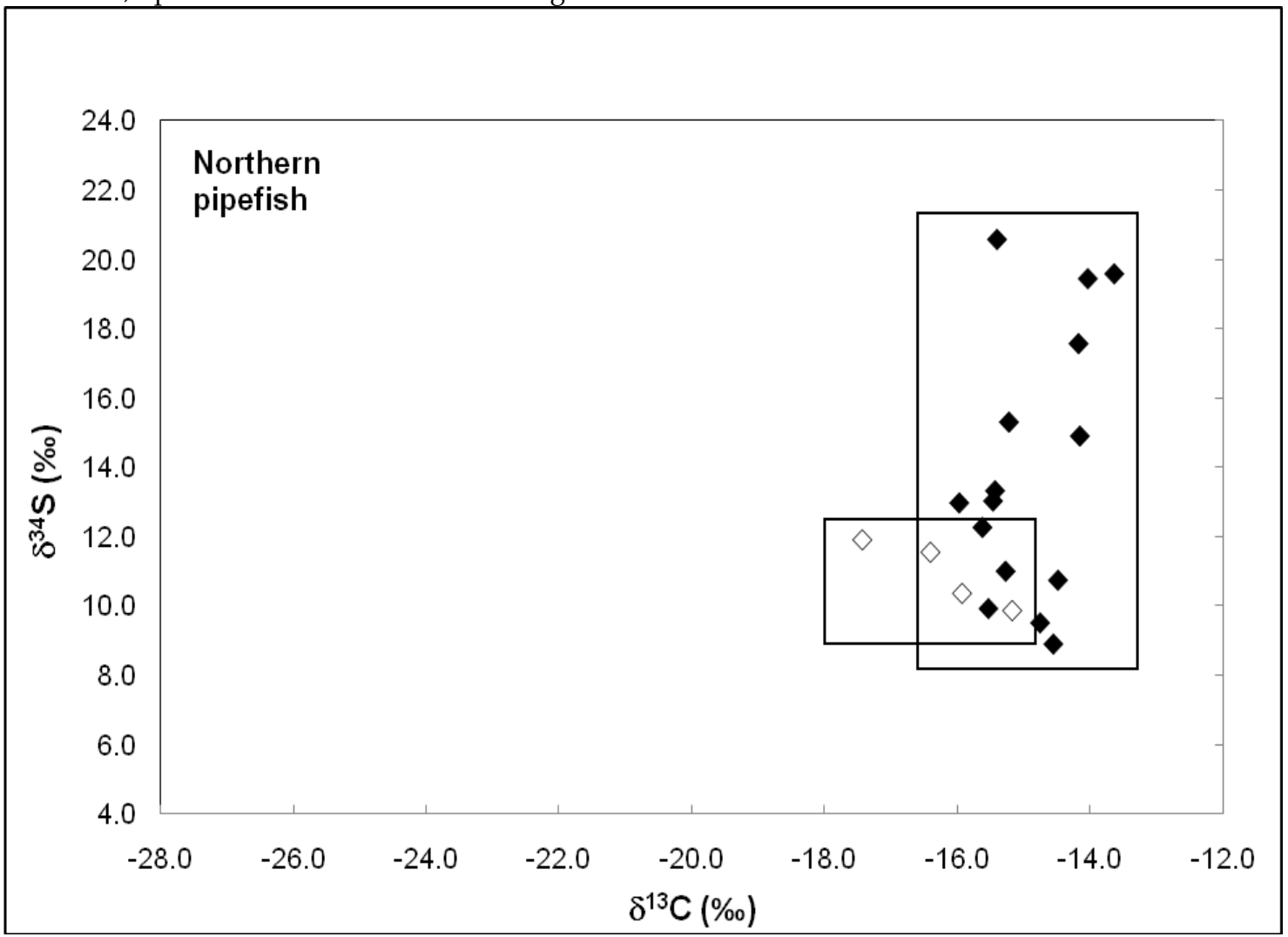


Figure 4.19. $\delta^{13}C$ vs. $\delta^{34}S$ of northern pipefish collected 9/12/2005. Closed marks indicate fish from eelgrass meadows; open marks indicate fish from eelgrass-free sites.



V. Chapter 5: Fatty acid profiles and relative abundances to evaluate primary production utilization by consumers in South Bay, VA

5.1. Introduction

Marine macrophytes such as seagrass, macroalgae, coastal marsh plants, and mangroves contribute a large proportion of the autotrophic biomass in shallow, near-shore systems around the world (Duarte and Cebrián, 1996; Thayer *et al.*, 1984). Seagrass systems in particular are noted for high productivity rates and complex faunal communities (Fry and Parker, 1979; Kikuchi, 1980). Seagrass meadows provide many ecosystems functions, notably providing structure, protection, and access to abundant food sources for various organisms.

The nutritional importance of seagrass tissues has been a topic for discussion for some time. In temperate systems in particular, the nutritional importance of seagrass tissues is still hotly debated; it appears that the extent of seagrass utilization by consumers is somewhat site-specific, though it is generally agreed that the dietary importance of seagrass primary production is less than in tropical seagrass systems (Kikuchi, 1980). Fewer fish in temperate marine systems are known to consume live seagrasses directly compared to tropical systems (Ogden, 1980; Thayer *et al.*, 1984). Pinfish (*Lagodon rhomboides*), which are common along the mid-Atlantic coast of the United States, have been found with fragments of eelgrass blades in their stomachs (Luczkovich and Stellwag, 1993).

In addition, studies in Australia have found that some fish species use seagrass primary production as an important nutritional source (i.e. Connolly, 2003). However, much of the evidence in the literature suggests that the majority of the seagrass organic matter is transferred to the food web in the form of detritus and partially decayed and dissolved organic matter (Deegan *et al*., 1990; De Leeuw *et al.*, 1995; Klumpp *et al.*, 1989; Mann, 1988; Connolly *et al*., 2005).

Live seagrass tissues are not particularly good nutritional sources for consumers as a large proportion of the components of seagrass tissues are highly refractory and few temperate-dwelling species have the enzymes necessary to fully break them down. Similarly, eelgrass (*Zostera marina*) detritus is highly refractory; eelgrass tissues generally only become a useful food source when partially decayed into a mass of organic matter and associated microbiota (Kikuchi, 1980; Zieman *et al*., 1984; Peterson and Howarth, 1987). Eelgrass primary production then transfers into the food web via the detrital pathway. First, bacteria and fungi decompose dead blades (Mann, 1988). Micro- and meiofauna then colonize the dead blades while feeding on the bacteria, fungi, and detrital and dissolved organic matter. At the same time, dissolved compounds released from the tissues during decomposition become available to phytoplankton and zooplankton. Finally, the mass of decomposing material and colonizing microbiota is available to detritivores.

The relative contribution of seagrass-derived detrital material as a bacterial carbon source has been studied in several different systems and by many methods (e.g. Thresher *et al.*, 1992; Boschker *et al.*, 2000; Holmer *et al.*, 2001, 2004; Bouillon *et al.*, 2004; reviewed by Bouillon and Boschker, 2006). The importance of seagrass organic matter as a carbon source to bacteria appears to be variable and site-specific, though previous studies have provided evidence that relationship is stronger in oligotrophic and non-impacted seagrass beds (Jones *et al.*, 2003; Holmer *et al.*, 2001, 2004) such as those found in South Bay, VA.

Eelgrass meadows are common along much of the eastern coastal United States and were widespread throughout the Chesapeake Bay and nearby coastal bays of the Delmarva Peninsula until the early 1930's when populations experienced rapid and extensive declines in biomass (Short *et al.*, 1988; Orth *et al.*, 2006). While eelgrass populations along the northern coast of the Delmarva Peninsula such as Sinepuxent Bay have rebounded somewhat, no natural eelgrass recovery was observed in southern Delmarva coastal bays until the discovery of small natural patches in South Bay in the 1990's (Orth *et al.*, 2006). Initial small-scale efforts using adult plants were followed by larger-scale restoration via seed distribution in a shallow subtidal section of South Bay in 2001 and 2002. Eelgrass recovery in South Bay has proven very successful; aerial photography from 2008 showed that the individual plots have coalesced to a nearly continuous single meadow (Orth *et al.*, 2010).

A main rationale of the present study was to analyze if eelgrass organic matter contributes a measurable fraction of primary production to the local food web, at least at the molecular level. This study endeavored to investigate two potential pathways that eelgrass is utilized by South Bay consumers; first was via direct consumption of live bulk tissues, and second was through the assimilation of microbially-modified eelgrass organic matter. I hypothesize that a measurable amount of eelgrass primary production enters the South Bay food web, either by a single pathway or a combination of the two. To that end, the fatty acid profiles of South Bay macrophytes (eelgrass and macroalgae) were compared with those of consumers (invertebrates and fish) in search of evidence of the assimilation of eelgrass material, keeping in mind the potential for bacterial influence.

An earlier part of this study utilized bulk tissue carbon, nitrogen, and sulfur stable isotope values of consumers and primary producers to investigate the nutritional importance of bulk eelgrass tissues to consumers in the South Bay restored eelgrass meadows. However, the results were inconclusive. Decomposition of source materials complicates the use of stable isotopes to determine the ultimate source of primary production to food webs; isotopic values of detritus may not reflect those of the original live parent material (Ember *et al.*, 1987; Boschker, *et al.*, 1999). Carbon, nitrogen and sulfur isotope ratios of plant detritus can change during decomposition due to variable decay rates of different components of the original material, or due to microbial activity

(Macko and Estep, 1984; Keough *et al.*, 1998; Peterson, 1999). Microbial metabolism results in large amounts of CO_2 loss by respiration as well as nitrogen assimilation, which are both also likely associated with isotopic change. Thus, the isotopic signal from eelgrass in South Bay consumers may be masked by a bacterial signal. It is expected that the ambiguities from the stable isotope study will be resolved by comparing the fatty acid chemistry of consumers and local primary producers to identify sources of primary production utilized by local consumers.

5.1.1. Fatty acid background

Fatty acids comprise one group of essential compounds for animals. The most common biologically active fatty acids range in length from two (acetic acid) to twenty-four (lignoceric acid) carbon atoms. Fatty acids from plants and animals are mainly straight-chained and even numbered, while branched and odd-chain fatty acids are typical of bacterial sources (Boschker *et al.*, 1999; Christie, 2003; Bouillon and Boschker, 2006). Animal fatty acids commonly vary from fourteen to twenty-two carbon atoms per chain, terminating in a carboxyl group at one end. Unsaturated fatty acids in this group have one or more double bonds (mono- or poly-unsaturated, respectively) in specific positions relative to the carboxyl group. In fatty acid nomenclature, the number following the 'ω' (omega) indicates the number of carbon atoms in the fatty acid chain between the methyl group and the first unsaturated bond.

Many saturated fatty acids can be synthesized anew from an acetate precursor. However, marine animals do not have the necessary enzymes necessary for *de novo* synthesis of a number of the unsaturated fatty acids required for normal, healthy growth (Olsen, 1999; Dalsgaard *et al*., 2003). These fatty acids are termed 'essential fatty acids' and must be obtained entirely through dietary sources.

Generally, all of the fatty acids with double bonds at the ω3 and ω6 positions of fatty acids present in marine systems are initially synthesized *de novo* by marine plants or bacteria (Dalsgaard *et al*., 2003). Longer-chain ω3 and ω6 fatty acids can be metabolically synthesized from shorter precursors. Heterotrophic marine organisms do not have enzymes needed to desaturate the carbon bonds at the ω6 and ω3 positions of fatty acids (Olsen, 1999); animal enzymes are only able to desaturate carbon linkages between an existing double bond and the carboxyl group. Plant enzymes, however, can insert a double bond in the terminal region of an unsaturated fatty acid. The terminus of biosynthesis for ω3 fatty acids for the majority of complex plant species is linolenic acid (18:3ω3) (Christie, 2003). Linolenic acid is the precursor to a group of polyunsaturated ω3 fatty acids that are formed by desaturation and chain elongation (Sargent *et al*., 1999). Similarly, linoleic acid (18:2ω6) is the dietary precursor to a family of polyunsaturated fatty acids with the ω6 terminal structure.

5.1.2. Lipid biomarkers in marine systems

Lipid biomarkers have been used with increasing frequency over the past few years to determine the ultimate sources of carbon assimilated by consumers. Lipids and fatty acids are ubiquitous biological compounds that are rich in carbon and easily assimilated and metabolized by organisms. Fatty acids contribute not only to energy and nutrient supplies to heterotrophic organisms, but also are requisite components of cell membranes (Dalsgaard *et al.*, 2003; Alfaro *et al*., 2006). In terms of biomarker analyses, these compounds are extracted relatively easily from natural samples and can distinguish between microbial groups and higher plants and animals (Boschker and Middleburg, 2002). While fatty acids cannot resolve primary production sources to the level of individual species, the presence and combination of certain fatty acids can be indicative of a particular class of organisms, for instance diatoms or Rhodophytes (red macroalgae) (Reuss and Poulsen, 2002; Dalsgaard *et al.*, 2003).

Previous studies have utilized fatty acid analyses to positively distinguish numerous marine primary producers (e.g. Khotimchenko *et al.*, 2002; Graeve *et al.*, 2002; Khotimchenko, 2003), including those found in eelgrass systems. To date, the fatty acids of marsh plants such as *Spartina alterniflora* (Canuel *et al*., 1997), eelgrass epiphytes (Kharlamenko *et al*., 2001), macroalgae (Vaskovsky *et al*., 1996; Sanina *et al*., 2004; Alfaro *et al*., 2006), mangroves (Bouillon *et al*., 2004; Alfaro *et al*., 2006), phytoplankton (Jaschinski *et al*., 2008), and bacteria (Boschker

and Middleburg, 2002; Alfaro *et al*., 2006) have been compared to those of seagrass including eelgrass. A few of the primary producers previously analyzed in the literature are common to South Bay and so are potential nutritional sources to local consumers. Based on the results from the aforementioned studies and others it is expected that the fatty acid profiles of primary producers in South Bay are distinct and therefore fatty acid analysis can be used as a tool to differentiate primary production sources utilized by consumers.

Fatty acid profiles of many seagrass species in other systems are available in the literature. Khotimchenko (1993) found that eelgrass in the Sea of Japan was rich in polyunsaturated C_{18} fatty acids, particularly linoleic and linolenic acids, and also contained small amounts of long-chain (C_{20} to C_{25}) saturated fatty acids, which is a combination common only to seagrass species in marine systems. Kharlamenko *et al.* (2001) utilized fatty acid analysis to study the food web in an eelgrass system off the coast of Siberia. The authors concluded that detrital eelgrass organic matter was an important component of the local food web. Conversely, Jaschinksi *et al.* (2008) found that eelgrass primary production contributed a negligible proportion of carbon to the food web in a *Zostera marina* meadow in the Baltic Sea; rather, epiphytes, diatoms and red algae were determined to be the main carbon sources at the site.

The fatty acids present in primary producer and consumer tissues were first identified in the current study; then consumer tissues were analyzed for

individual biomarker fatty acids or combinations of fatty acids particular to certain primary producer classes including seagrass and bacteria. Two particular fatty acids, 18:3ω3 (linolenic acid) and 18:2ω6 (linoleic acid), have been previously identified as biomarkers for live seagrass tissues in marine systems (Khotimchenko, 1993; Kharlamenko *et al*., 2001) and have been used to indicate utilization of eelgrass primary production by consumers. Fish do not possess the enzymes necessary to produce linoleic or linolenic acids from endogenously produced monounsaturated fatty acids (Olsen and Ringø, 1992) and must therefore obtain each of these essential fatty acids through dietary sources. The presence of linolenic and linoleic acids in consumer tissues will indicate the direct consumption of bulk eelgrass primary production. Alternatively, bacterial-specific fatty acids will indicate consumer utilization of organic sources that contain bacteria, likely detritus and decaying organic matter. Such fatty acids include 18:1ω7 which is typical of anaerobic bacteria, saturated odd-chain fatty acids, and saturated branched chain fatty acids, which are produced by sedimentary bacteria.

5.2. Methods

The analyses and results reported here were part of a larger project conducted in South Bay, VA. Field collection methods are described in a previous paper. Total fish abundance in both the restored eelgrass and eelgrass-free sites was greatest during July; thus samples for this part of the study were

selected from those captured in July such that the sample pool represented the greatest possible range of species. The exception was a group of spot (*Leiostomus xanthurus*) captured in September of 2005, which exhibited strongly different bulk stable isotope values compared to individuals collected during previous months. These individuals will be compared with spot from July to determine whether the fish fatty acid profiles can shed light on the origin or origins of the observed bulk stable isotope differences. The major primary producers present in South Bay were also subject to fatty acid analysis.

5.2.1. Lipid extraction and FAME preparation

Samples were lipid extracted and derivatized to fatty acid methyl esters (FAME) using the method of Ballentine (1996, modified from Barnes *et al.*, 1979; 1997). Glassware used for fatty acid sample preparation was washed thoroughly then ashed in a 550-degree Celsius muffle furnace for a minimum of 4 hours prior to use. All solvents (chromatography grade) were distilled prior to use for extractions in order to ensure purity. Glassware was cleaned and rinsed several times with the first-cut removed from distillation of dichloromethane (DCM). Extreme care was used not to touch the extraction thimble so as to prevent sample contamination by lipids from fingerprints.

Homogenized tissue samples were lipid-extracted in DCM for approximately 16 hours in a glass Soxhlet extraction tower to allow for continuous extractions of lipids. Triglycerides were saponified to individual fatty

acid salts by heating under reflux with 10 mL of 1 N KOH-methanol (31.9 grams 87.9 % assay KOH/500 ml distilled methanol). Non-saponifiable materials (straight-chain lipids) were removed from the remaining fatty acids with hexane. Saponifiable fatty acids were acid neutralized (1N HCl) drop by drop until neutral or slightly acidic. Neutral FA were extracted with hexane. Total fatty acids were concentrated by rotoevaporation (if necessary) and vacuum desiccation to dryness then refrigerated until derivatization.

Fatty acids are relatively non-volatile molecules, which require derivatization to the corresponding FAME prior to analysis. Approximately 2 mL of 15% boron tri-fluoride in excess methanol (BF_3 CH_2OH) were added to vials containing the dried fraction, which were then sealed and heated to 60°C for 8 minutes. Samples were cooled to room temperature, transferred to a separatory funnel, and washed with a saturated KCl solution. The fraction containing KCL was removed and discarded while the hexane fraction was transferred to a new vial. Water was removed by addition Na_2SO_4 (anhydrous) to excess. Samples were transferred by pipette to a clean, ashed vial sealed with a Teflon-lined cap. Vials were stored, refrigerated, until gas chromatographic analysis (from Ballentine, 1997).

The fatty acid methyl esters were analyzed using a Hewlett Packard 5890 Series II gas chromatograph fitted with a flame ionization detector (FID). A 60 meter J&W DB-5 column was used for analysis with helium as the carrier gas.

The injector temperature was maintained at 200°C. The general temperature program used for the separation of fatty acids for gas chromatographic analysis was as follows: hold at 50°C for 1 minute, ramp at 15°C/minute to 120°C, ramp at 2°C/minute to 210°C, hold for 5 minutes, ramp at 2°C/minute to 250°C, hold for 15 minutes. A Hewlett Packard 3390A integrator was used to record the elution of fatty acid peaks through the FID (Figure 5.7).

FAME were identified by comparison of peak retention times with the commercial standards SUPELCO C16 – C18 FA in water standard mix (#17973; all components at concentration of 0.4% w/v), SUPELCO Bacterial Acid FAMES mix in methyl caproate (#47080; 10mg/ml total concentration) as well as in-house standard mixes. Response factors of in-house standards were used for fatty acid quantification. Three to five injections were performed for each sample, depending on peak reproducibility. Fatty acid amounts are given as percent fractions of the total fatty acid composition.

5.2.2. Fatty acid nomenclature

The fatty acid shorthand nomenclature used here is of the 'ω' (omega) form. For this shorthand, given the fatty acid 18:2ω3 for instance, the '18' refers to the carbon chain length, '2' is the total number of double bonds in the chain, and the 'ω3' refers to the position of first double bond nearest the carboxyl group. To clarify, a designation of 'ω3' for a given fatty acid is equivalent to a designation of 'n-3' which is another commonly used shorthand for fatty acids. The

polyunsaturated fatty acids (PUFA) observed in this study are methylene interrupted – i.e. separated by a CH_2 group – as is typical for the more common biologically important fatty acids with multiple double bonds (Napolitano, 1999).

In this text, '*i*+*a*' is the shortened form of the sum of 'iso' and 'anteiso' fatty acids, which are designations that refer to the location of the branching methyl group. The branching methyl group of an 'iso'-branched fatty acid splits from the main chain at the second to last carbon opposite the carboxyl group, while the methyl group of an 'anteiso'-branched fatty acid splits two carbons from the end of the chain (Christie, 2003). For example, *i*15:0 represents a saturated fatty acid with 14 carbons in the main chain, with a single methyl group branching from the main chain at the second to last carbon opposite the carboxyl group.

5.3. Results

Fatty acid profiles were compiled for four species of primary producers, seven invertebrates, and twelve fish species collected in South Bay restored eelgrass sites. Fatty acid profiles were also determined for two primary producer species (plus a decaying fraction of one species), two invertebrates, and nine fish species in nearby eelgrass-free sites. The epiphytic red alga collected in the eelgrass sites was also collected from the eelgrass-free sites but was not included due to sample contamination during fatty acid extraction. Eelgrass was not collected from the eelgrass-free sites as these sites were selected and defined by

the visual lack of eelgrass cover. Although many of the same invertebrate species were collected from the eelgrass-free sites as from the restored eelgrass sites, too little tissue remained following bulk isotope analysis for fatty acid analysis; multiple individuals of the smallest invertebrates such as amphipods were combined as one sample in order to obtain enough tissue for stable isotope analysis. The fish species analyzed represented a range of habitat (i.e. benthic, pelagic, and schooling) and feeding preference (i.e. piscivores, planktivores, detritivores, and omnivores). Pigfish (*Lagodon rhomboides*), lined seahorse (*Hippocampus erectus*) and spiny boxfish (*Chilomycterus schoepfii*) were not collected from the eelgrass-free sites in July 2005 and therefore were not included in the fatty acid analyses for those sites.

Fatty acid compositions noted in the text and in the attached tables are given as percentages of the total fatty acids. Trace levels that represented less than 0.5 percent of the total fatty acids present were not included. Potential primary producers and their associated biomarker fatty acids are compiled in Table 5.1. Percent compositions of individual source biomarker fatty acids in consumer tissues are portrayed in Figures 5.1 through 5.4 for consumers in restored eelgrass meadows and Figures 5.5 and 5.6 for consumers in eelgrass-free sites. In restored eelgrass meadows, the percent composition of eelgrass, bacteria, zooplankton, and diatom fatty acid markers are depicted for fish and invertebrates, while in eelgrass-free sites the bacterial and zooplankton

biomarkers are depicted. Fatty acid compositions for primary producers are similar to those found in other studies (Khotomchenko, 1993; Canuel *et al.*, 1997; Khotimchenko *et al.*, 2002; Sanina *et al.*, 2004).

5.3.1 Fatty acids in primary producers

The fatty acid compositions of South Bay primary producers are given in Table 5.2. The dominant fatty acids of eelgrass, in order of decreasing abundance, are 18:3ω3, 16:0, 18:2ω6, 18:1ω9, and an unidentified 16-carbon chain PUFA [*ui* 16-C (b) in Table 5.2], which combined for nearly 80 percent of the total fatty acid content. The designation '*ui*' preceding a fatty acid (e.g. *ui* 17-C) indicates that the fatty acid in question was not conclusively identified; the number indicates the number of carbon atoms in the fatty acid chain. All unidentified fatty acids were mono- or polyunsaturated. Detrital fragments of eelgrass were dominated by 16:0, 14:0, 16:1ω7, and 18:0 which again accounted for nearly 80 percent of the total fatty acids. The major fatty acids in sea lettuce were 16:0, 18:1ω7, 16:1ω9, 18:0, and a second unidentified 16-carbon chain PUFA [*ui* 16-C (a) in Table 5.2; not the same *ui* 16-C PUFA found in eelgrass] within the eelgrass meadows, and 16:0, 18:0, 18:1ω7, 16:1ω9, and 18:2ω6 in the eelgrass-free sites. The fatty acid elution times for the two unidentified 16-C PUFA above indicated that *ui* 16-C (a) in sea lettuce was more highly unsaturated than *ui* 16-C (b) in eelgrass.

Total saturated fatty acid content of live eelgrass was at minimum 28 percent and up to 34 percent less than other primary producers tested (Table 5.3).

Detrital fragments of eelgrass were composed of almost 80 percent saturated fatty acids, on the other hand. All of the macroalgae sampled contained from 62 to 69 percent saturated fatty acids (SFA), though the monounsaturated fatty acid (MUFA) composition varied. Approximately 20 percent of the total fatty acids in live sea lettuce and an epiphytic red alga were MUFA, while only 6 percent of the fatty acids of decayed sea lettuce and *Gracilaria spp.* were monounsaturated. Eelgrass was the only macrophyte sampled that contained 18:3ω3, although all other primary producers except *Gracilaria spp*. contained measurable amounts of 18:2ω6s at both sites. Though all primary producers in eelgrass meadows contained long-chain saturated fatty acids (LCFA, 22 carbon atoms in the chain or more), only the decayed sea lettuce contained LCFA in the eelgrass-free sites.

5.3.2. Fatty acids of invertebrates

Relative amounts of invertebrate fatty acids are presented in Table 5.4. Invertebrates from both sites are included. For all invertebrates collected, 16:0 was the dominant SFA and 18:1ω9 was the dominant MUFA, with the exception of ghost shrimp (*Hippolyte spp*.) and an amphipod (*Gammarus sp*.) in eelgrass meadows, for which 16:1ω9 was the dominant MUFA.

The dominant PUFA varied between species. In eelgrass meadows, *ui* 16-C (a) was the dominant fatty acid for the gammarid amphipod, skeleton shrimp (*Paracaprella sp*.), and the snail (*Mitrella sp*.); 20:5ω3 (eicosapentaenoic acid or EPA) was the main PUFA for the unidentified amphipod and grass shrimp;

20:5ω6 was the most abundant PUFA in blue crab; and finally linoleic acid (18:2ω6) comprised nearly one quarter of the total fatty acids in ghost shrimp. In eelgrass-free sites, EPA was the most abundant PUFA for blue crab, while docosahexaenoic acid (DHA, 22:6ω3) was the dominant PUFA of grass shrimp.

Two invertebrates collected from the restored eelgrass meadows, blue crab and skeleton shrimp, contained measurable amounts of the eelgrass biomarkers 18:2ω6 and 18:3ω3 and LCFA (Table 5.7, Figure 5.1). No invertebrates in eelgrass-free sites contained 18:3ω3, while 18:2ω6 comprised less than two percent of the total fatty acids for both species. All invertebrates tested contained each of the three bacterial biomarkers – odd-chain and branched-chain fatty acids and 18:1ω7 (Figures 5.2 and 5.5) – except for the gammarid amphipod, which did not contain branched-chain fatty acids, and the snail, *Mitrella sp.*, which did not have a significant amount of 18:1ω7; each of these two invertebrates were collected in the restored eelgrass plots. Four of the seven invertebrates from restored eelgrass plots contained the diatom markers 16:1ω7 and 20:5ω3 (Figure 5.4), though the invertebrates in eelgrass-free sites contained only 20:5ω3. In the restored eelgrass meadows the only invertebrate that contained the zooplankton markers 20:1 (2.9 %) and 22:6ω3 (2.1 %) was the unidentified amphipod.

5.3.3. Fish fatty acid profiles

Fatty acid profiles and relative proportions of fish are presented in Tables 5.5 (restored eelgrass plots) and 5.6 (eelgrass-free sites). For all fish collected, 16:0

was the dominant SFA and 18:1ω9 was the dominant MUFA, with the exception of bay anchovy in the restored eelgrass for which 16:1ω9 was the dominant MUFA. As was the case with invertebrates, the dominant PUFA was less consistent between species. In the restored eelgrass sites, the main consumer PUFA were as follows: 20:5ω3 was the most abundant PUFA in Atlantic menhaden, bay anchovy, and tautog; 22:6ω3 was the main PUFA in Atlantic silversides, silver perch, spot collected in July, and summer flounder; 18:2ω6 was most abundant in spiny boxfish; 20:5ω6 was the dominant PUFA for lined seahorse and pipefish; the percentages of 18:2ω6 and 22:6ω3 were nearly equal in pigfish and pinfish; and finally, 20:5ω3 and 22:6ω3 were measured in equal proportions for spot collected in September. The dominant PUFA in eelgrass-free sites was 22:6ω3 for all fish except for northern pipefish, for which 20:5ω3 comprised the greatest fraction.

All organisms tested in all sites contained one or more odd-chained fatty acids (15:0, 17:0, *ui* 17-C, and 19:0) as well as the anaerobic bacteria-specific fatty acid 18:1ω7 (except for *Mitrella sp*.). In eelgrass meadows, six of the seven invertebrates and ten of the thirteen fish collected had branched-chain fatty acids (*i*+*a*15:0 and *i*+*a*17:0) in their tissues; all seven invertebrate species and five of the thirteen fish species had LCFA (≥ 22 carbon atoms per chain). In eelgrass-free sites, on the other hand, while both of the invertebrates and seven of the ten fish species had branched-chain fatty acids, only grass shrimp and silver perch

contained LCFA. Finally, while all organisms contained the eelgrass biomarker 18:2ω6 (linoleic acid), the other eelgrass biomarker 18:3ω3 (linolenic acid) was detected in blue crab, skeleton shrimp, and pigfish in the restored eelgrass plots (Figure 5.1).

5.4. Discussion

5.4.1. Differences between primary production sources

The suite of fatty acids detected in eelgrass was far different from the other primary producers from South Bay (Table 5.2). Nearly half of the total fatty acids were polyunsaturated in eelgrass, compared with the decaying *Ulva lactuca* in eelgrass sites which had the next highest concentration at 14.8 % (Table 5.3). Eelgrass was the only primary producer tested that contained either 18:3ω3 and/or *ui* 18-C, though all others except for *Gracilaria spp.* contained small amounts of 18:2ω6. Decayed *Ulva lactuca* contained the greatest proportion of LCFA, with greater than 17 %, though LCFA were present in all primary producers.

5.4.2. Individual consumer species

Palaemonetes spp. (mainly *Palaemonetes pugio* and *P. vulgaris*), also known as grass shrimp, are among the most abundant benthic macroinvertebrates within estuaries along the Atlantic coast of the U.S. As with many other marine species, grass shrimp are generalist feeders that may feed on live organisms as well as detritus. Therefore, grass shrimp represent an important link in detrital pathway

for both the breakdown of detritus and the transfer of detrital matter to higher trophic levels. In this study, grass shrimp contained by far the largest proportion of the anaerobic bacterial marker 18:1ω7 of species captured in eelgrass meadows, indicating that sedimentary bacteria, possibly colonizing decaying organic matter, are a significant contributor to the nutrition of grass shrimp.

Ghost shrimp (*Hippolyte spp.*), another invertebrate known to associate with eelgrass meadows on the east coast of the Unite States (Shield, 1978), had the most distinctive fatty acid profile of all consumers. Overall, ghost shrimp had the highest proportion of PUFA of all consumers and the highest proportion of MUFA of all invertebrates. Specifically, this species had the lowest concentration of 16:0 (6.9 % of total fatty acid content) and the highest concentrations of 19:0 (4.6 % of total fatty acids), 16:1ω9 (15.8 % of total fatty acids) and 18:2ω6 (23.7 % of total fatty acids) for all consumers. Ghost shrimp was the only consumer with levels of 19:0 greater than 1.5% of total fatty acid content.

Individual ghost shrimp were collected from within the eelgrass canopy. These invertebrates have been observed attached to eelgrass blades as a solid substrate; food items are then picked from the blades. The high levels of MUFA may indicate a diet composed mainly of epiphytes and fragments of detrital macroalgae such as *Ulva lactuca*, which was also the only primary producer found to contain appreciable levels of 19:0 in the eelgrass sites.

The fatty acid profiles of the two amphipods tested were very different. Of the two, the gammarid amphipod contained far more of the polyunsaturated 16 carbon chain fatty acids (8 % versus 1.6 % of total FA) and LCFA (2.5 % versus 0.5 % of total FA), while the unidentified amphipod had significant amounts of branched chain fatty acids though the gammarid had none. The unidentified amphipod contained the zooplankton markers 20:1 and 22:6ω3 while only 22:6ω3 was detected in the gammarid amphipod. There were also indications of diatom input to the diet of the unidentified amphipod. First, the diatom marker ratio was 2.3. Second, there were small amounts of 18:1ω7, which can be either a diatom marker or a bacterial marker depending on the presence of other specific biomarkers. Comparatively, the diatom marker ratio for the gammarid amphipod was nearly 1. Though there was a small trace of 16:1ω7, it is unlikely that diatoms contribute a significant amount to the diet of the gammarid amphipod.

Skeleton shrimp (*Paracaprella sp.*) were only collected in large enough quantities for fatty acid analysis from the eelgrass sites. These small invertebrates were collected among the eelgrass blades and eelgrass epiphytes rather than from along the sediment surface. *Paracaprella sp*. was one of only three species that contained both live eelgrass biomarkers, 18:2ω6 and 18:3ω3, and was also found to contain high levels of LCFA. At the same time, this species contained the lowest levels of branched-chain fatty acids and the anaerobic bacterial marker

18:1ω7 of all invertebrates. Since branched-chain fatty acids and 18:1ω7 are typical of bacteria living on or in sediments, the skeleton shrimp collected from the eelgrass meadows appear to obtain nutrition mainly from sources well above the sediment surface. This conclusion is reasonable since individuals collected for fatty acid analysis from the eelgrass meadow were collected from within the eelgrass canopy amongst the blades and epiphytes.

Greater than 6 percent of the total fatty acids of the snail (*Mitrella sp.*) were LCFA. The snail also contained the highest amount of odd-chain fatty acids of all invertebrates at more than 8 % of the total FA. These fatty acids, which included *i*+a15:0, 15:0, 17:0, and 19:0, are typical of bacterial sources. The presence of LCFA and several bacterial biomarker fatty acids is understandable as the snails were collected from a mass of detrital plant material tangled in eelgrass blades in the water column. A combination of several detrital primary producers, including live and decaying *Ulva lactuca* appears to be an important mixture of food sources for these snails; decaying *Ulva lactuca* from the eelgrass meadow has the potential to provide most of the above fatty acids, except for 15:0, which is present in the live tissues. *Ulva lactuca* is a floating macroalgae that is easily caught on material that it passes, including eelgrass stands or other macrophytes; also, live and decaying material is likely to be found in the same location. *Gracilaria spp.* and eelgrass are also potential minor nutritional components.

The snail also had trace amounts of the diatom fatty acid marker 16:1ω7. As noted in Table 6.1, another measure of the importance of diatoms to a consumer diet is the marker ratio 20:5ω3/22:6ω3 > 2; the ratio for the snail was 4.4, which again suggests that diatoms are a food source. Still, although the diatom marker ratio was high and indicated that diatoms were an important dietary source, the actual levels of 20:5ω3 and 22:6ω3 were relatively low compared to other consumers, which suggests that diatoms in fact are not a very important nutritional source for *Mitrella sp*. in the restored eelgrass meadows.

Spot in the eelgrass plots contained the highest proportion of odd-chain fatty acids of all sampled organisms and the highest proportion of branched chain fatty acids of all sampled fish in those sites (true for both months). This is a reasonable result, as spot are omnivorous and generally feed on smaller bottom-dwelling worms and crustaceans as well as detritus (Chao and Musick, 1977; O'Neil and Weinstein, 1987; van Montfrans *et al*., 2005) and these fatty acids are associated with sedimentary bacterial sources. Juveniles in particular are known to be strongly associated with bottom waters (O'Neil and Weinstein, 1987). The proportion of linoleic acid in spot was conversely the lowest of all fish species in eelgrass meadows, suggesting that live eelgrass provides little if any nutrition to spot at this site.

Pigfish were the only fish species collected that contained greater than trace amounts of linolenic acid. Gut content analysis showed that prey items for

pigfish were more varied in eelgrass meadows compared with eelgrass-free sites based on weight, although nearly 25 percent of the material was unidentifiable (van Montfrans, 2005). Of the remaining material, worms, decapods, and copepods were the most important dietary components in decreasing order (van Montfrans *et al.*, 2005). Pigfish contained 20:1 and 22:6ω3 (Figure 5.3), which in combination serve as a biomarker for zooplankton such as calanoid copepods. The ingested unidentified matter and the detritivores are equally possible sources of the linolenic acid.

Despite the evidence from this study that a few local invertebrate species assimilated small amounts of linolenic and linoleic acids into their tissues, linolenic acid was not detected in the local omnivorous fish (with the exception of pigfish from the eelgrass meadows) at more than trace levels. There are two potential explanations for this apparent contradiction. First, it has been postulated that linolenic acid is not an essential fatty acid for marine fish; rather, marine fish require ω3 fatty acids with carbon chains of 20 carbons or longer such as 20:5ω3 and 22:6ω3 (Takeuchi, 1997). The second potential explanation is that linolenic acid is in fact transferred through the food web, however all but trace amounts undergo chain elongation and desaturation in the consumers to longer chain polyunsaturated fatty acids rather than persisting in the original form (Kanazawa *et al*., 1979; Olsen and Ringø, 1992; Buzzi *et al.*, 1997). Although previous studies have shown that marine fish have capacity to produce longer-

chain polyunsaturated fatty acids from both linoleic and linolenic acids (Olsen *et al.*, 1990), there is evidence that at least for a few species the metabolic conversion of linolenic acid is prevalent over that of linoleic acid (Olsen and Ringø, 1992). It has been suggested that the overall ω3 and ω6 fatty acid content in dietary sources can either promote or inhibit the activity of enzymes responsible for *in vivo* desaturation and elongation of ω3 and ω6 fatty acids in fish (Xu and Kestemont, 2002). Specifically, high levels of these fatty acids in dietary sources will tend to inhibit *in vivo* conversion to longer-chain fatty acids.

Due to the large number of species collected for this study, testing for essential fatty acid efficiency for each of the species would have be cost and time prohibitive; therefore the cause of the scarcity of linolenic acid in consumers is not known. Still, it is important to note that previous studies either have not detected (Recks and Seaborn, 2008) or have found very low levels of (Iverson *et al.*, 2002) linolenic acid in several marine fish, including a few of the species collected for this study, despite finding similar levels of linoleic acid as this study in fish tissues. Therefore a dearth of linolenic acid is not unheard of in marine fish species.

5.4.3. Site differences

The fatty acid composition of the blue crab from the restored eelgrass plots differed greatly from the blue crab from the eelgrass-free sites. Nearly 60 percent of the total fatty acids of the blue crab from the restored eelgrass plots

were saturated, while only 46 percent were saturated in eelgrass-free sites. Three times as much of the total fatty acids in the tissues of the blue crab from the eelgrass-free sites were monounsaturated, while the proportion of polyunsaturated fatty acids was slightly more than half as much as the crab from the restored eelgrass. In addition, the tissues of the blue crab in the restored eelgrass contained all of the eelgrass biomarkers; linoleic and linolenic acids as well as LCFA. The blue crab from the eelgrass-free sites did not contain linolenic acid or LCFA.

The presence of eelgrass biomarkers indicates that a proportion of live eelgrass production was assimilated by the blue crab in the restored eelgrass meadows. One of the unidentified 16-C PUFA was detected in small amounts in the blue crab; the same fatty acid was also detected in live eelgrass and not in other primary producers, thus further linking eelgrass to the blue crab at this site. At the same time, the proportion of branched-chain fatty acids (*i*+*a*15:0 and *i*+*a*17:0) in the blue crab was far greater in eelgrass (7.6 %) versus the eelgrass-free area (1.3 %). The presence of sedimentary-specific bacterial biomarkers suggests that while the crab may be ingesting some live eelgrass, decaying detrital plant material is also important.

There were several differences in the fatty acid profiles of northern pipefish between the two site types. In eelgrass-free sites, pipefish contained 20:1 ui and 22:6ω3, which when noted together are indicators of zooplankton,

including calanoid copepods; 20:1 ui was absent in pipefish from the restored eelgrass sites. The pipefish from the eelgrass-free sites was one of only two fish that contained several biomarkers specific to diatoms. The first indicator was that the ratio of 20:5ω3 to 22:6ω3 was greater than 1 at 1.7; the second indicator was the presence of 16:1ω7. Based on the above biomarkers, it is likely that pipefish in eelgrass-free sites utilize diatoms as a dietary source. It is reasonable to expect that pipefish utilize microalgae such as diatoms since pipefish have a very small mouth which limits the size of potential food items.

Silver perch also showed significantly different fatty acid profiles between sites. The greatest differences were between the SFA content (45 % in eelgrass, 62 % in eelgrass-free) and the MUFA (approximately 38 % in eelgrass and approximately 18 % in eelgrass-free sites) content of silver perch. The levels of PUFA were similar, differing by less than two percent between sites. The silver perch in eelgrass contained very high levels of oleic acid (20.9 %), which is indicative of a highly carnivorous diet and was much greater than the amount of oleic acid in the silver perch from the eelgrass-free sites (9.5 %). Evidence of carnivory was expected based on gut-content analysis which concluded that decapods, particularly shrimp, were the most important prey items (van Montfrans *et al.*, 2005). Conversely, the amount of 18:0 was far greater for perch in eelgrass-free sites (20 %) compared to restored eelgrass sites (7 %). A larger single silver perch was sampled from the eelgrass-free sites in addition to the

juveniles. The sum of the SFA, MUFA, and PUFA of this larger individual more closely resembles that of juvenile silver perch from the restored eelgrass meadow (Tables 5.5 and 5.6). The larger size of this individual limits potential predators, allowing the fish to range more freely in search of food than juveniles collected from the same area; therefore the diet is likely to differ from juvenile silver perch collected at the same site.

The species noted above describe the most distinctive differences observed for individual species between the two sites. Other, smaller differences were also noted for other species. For instance, the percentage of the anaerobic bacterial marker 18:1ω7 found in grass shrimp was smaller for those captured in eelgrass-free sites, which suggests that grass shrimp in restored eelgrass sites obtain a greater proportion of nutritional sources within the eelgrass canopy, above the sediment surface. Also for grass shrimp, the levels of 20:5ω3 and 22:6ω3 were nearly equal with a slight predominance of 22:6ω3 in restored eelgrass plots, while in eelgrass-free sites, the percentage of 22:6ω3 was three times higher. Second, the ratio of 20:5ω3 to 22:6ω3 for bay anchovy was approximately 1.4 in eelgrass meadows, while in eelgrass-free meadows the ratio was reversed so that there was greater than double the amount of 22:6ω3. Third, spot from eelgrass-free sites had a higher proportion of 18:1ω9, which generally indicates a higher degree of omnivory. Finally, Atlantic silversides in eelgrass

meadows had nine times greater percentage of 18:2ω6 than silversides in eelgrass-free sites.

Many of these results listed above suggest that a wider variety of nutritional sources is available to and utilized by consumers in the restored eelgrass meadows compared to nearby eelgrass-free sites. In addition, there was further evidence that consumers in South Bay do not tend to move between eelgrass meadows and eelgrass-free sites. While several invertebrate and fish species in the eelgrass meadows contained both bacterial and eelgrass-specific fatty acid biomarkers, no seagrass-specific biomarkers were identified in consumers from eelgrass-free sites (Table 5.7 and Figures 5.1 and 5.2). These results support earlier evidence of habitat fidelity gleaned from stable isotope analysis of consumers in South Bay.

5.4.4. Presence of fatty acid biomarkers in consumers

Seagrass meadows are known to be some of the most productive systems in the marine environment (Duarte, 1989; Kharlamenko *et al.*, 2001), generally producing far more organic carbon than required by the ecosystem (Duarte and Cebrián, 1996). Such high productivity results in the formation of large amounts of excess organic matter. Some of this matter is likely to be transported out of the system with the movements of the tides. However, it is also likely that a portion of the organic matter produced within the meadows is retained and recycled within the system. Much of the organic matter currently produced in marine

settings is recycled in either the water column or at the sediment surface by bacteria as opposed to being buried into the sediments (Peterson, 1999). The decaying and detrital material represents a significant resource of recyclable carbon that is available to microbes for fixation and eventual transfer through the grazer food web (Keough *et al.*, 1998).

In eelgrass meadows, invertebrates had higher proportions of odd-chain fatty acids, with the exception of spot which had the highest proportion of all organisms at those sites. The pelagic fish *Menidia menidia* and *Anchoa mitchilli* and the schooling fish *Brevoortia tyrannus* had some of the lowest percentages of odd-chain FAs, and branched-chain fatty acids comprised 0.6 % or less of total fatty acids. This is not surprising as bacteria tend to grow in greater quantities at the sediment surface or within the upper centimeters of sediment, while these species tend to feed on prey items in the water column. On average, benthic invertebrates had higher proportions of branched-chain, odd-chain, and long-chain fatty acids in their tissues than fish in the restored eelgrass plots, but lower proportions of 18:1ω7, 20:5ω3 and 22:6ω3. The presence of larger amounts of bacterial fatty acids in benthic invertebrate species is generally expected since most of these species feed on detritus and invariably ingest the bacteria that colonizes and decomposes detrital matter.

Bacteria are the only natural source of branched-chain saturated fatty acids in the marine environment. These fatty acids are less common in higher

organisms and appear to be produced almost exclusively by bacterial sources (Johns *et al*., 1977). Therefore, if such fatty acids are found in consumers it follows that they originate from a bacterial source, likely a combination of decaying detritus and the colonizing microbiota. Odd-chain saturated FA, which are again attributable to bacteria, were present in every consumer that was collected (Table 5.7 and Figure 5.2), while the anaerobic bacteria-specific FA 18:1ω7 was noted in all consumers except the snail, *Mitrella sp.*, in the restored eelgrass plots. The consistent occurrence of numerous types of bacterial fatty acids regardless of species or habitat types validates the original assumption of this study that bacteria play an important role in the food web in South Bay by mediating the recycling of detrital material to local consumers.

Several consumers from both site types had a combination of fatty acids normally attributed to zooplankton. Specifically, the combination of 22:6ω3 (docosahexaenoic acid), and high levels of the total of all monounsaturated 20 and 22 carbon chain fatty acids is attributed to zooplankton such as calanoid copepods (Table 5.1). All consumers collected at all sites had the fatty acid 22:6ω3. Each of the two invertebrates and six of the fish from the eelgrass-free sites also contained small amounts of 20:1. Similarly, one invertebrate and seven fish collected from the restored eelgrass plots contained low levels of 20:1. Fatty acid analyses indicate that zooplankton were a dietary source for numerous consumers regardless of habitat in South Bay.

Diatoms are not believed to be a major carbon source to consumers in the restored eelgrass meadows. All of the consumers sampled contained the diatom marker 20:5ω3 (eicosapentaenoic acid, or EPA) and generally in large proportions; however, first, EPA is also a general algal biomarker and second, the other fatty acids indicative of diatom inputs were not present, were found at low levels, or the specific ratios were too low. For instance, 16:1ω7 was present at very low levels (< 1 percent of the total FAs) in four of the five invertebrates (Table 5.7 and Figure 5.4) and was absent from all fish. Second, the FA ratio 16:1ω7/16:0 > 1, which is also indicative of diatom inputs, was far less than one for all organisms that contained 16:1ω7. Together, these factors suggest little direct diatom influence on the food web in the restored eelgrass meadows.

The importance of benthic microalgae to consumers in this system remains unclear from the current data. Fatty acid biomarkers commonly attributed to benthic microalgae include 16:2ω12, 16:4ω1, 18:4ω3 (dinoflagellates), and 18:5ω3 (Table 5.1). Three polyunsaturated fatty acids that were potential benthic microalgal biomarkers but were not conclusively identified by chromatographic analysis were detected in consumer tissues; *ui* 16-C (a), *ui* 16-C (c), and *ui* 18-C. First, *ui* 16-C (a) was detected in several consumer species; skeleton shrimp (16.4 % of total FA), a snail (10.7 %), ghost shrimp (6.1 %), and gammaridean amphipod (6.1 %). It is possible that *ui* 16-C is the benthic biomarker 16:4ω1. It is understandable a benthic microalgal marker should be

detected in the species listed above, as many are benthic feeders; however, as the marker identification is not certain, it cannot be designated as a distinctive biomarker fatty acid. Another complicating factor is that detrital eelgrass, live and decaying *Ulva lactuca,* and *Gracilaria spp.* also contained *ui* 16-C (a) (Table 5.2). The three macroalgal species had relatively high levels of this fatty acid in both eelgrass sites as well as eelgrass-free sites. The second unidentified PUFA was also a 16 carbon chain fatty acid [*ui* 16-C (c)]; the gas chromatrograph elution time indicated that *ui* 16-C (c) contained fewer double bonds (i.e. was less unsaturated) than *ui* 16-C (a). Further analyses would be needed to determine whether *ui* 16-C (c) was the benthic microalgal biomarker 16:2ω12. Two invertebrate and two fish species collected from the eelgrass meadows contained *ui* 16-C (c), though in all cases the fatty acid comprised less than 2 percent of the total tissue fatty acids. As was the case with *ui* 16-C (a), low levels of *ui* 16-C (c) were also detected in macrophyte primary producers (live eelgrass and decayed sea lettuce). The final potential benthic microalgal biomarker was *ui* 18-C, which was more highly unsaturated that 18:3ω3, again based on fatty acid elution time. It is possible that *ui* 18-C represents either 18:4ω3 or 18:5ω3. *ui* 18-C comprised 3.1 % of the total fatty acids in ghost shrimp in eelgrass sites. No other consumer exhibited *ui* 18-C, though it was present (< 2% of total FA) in live and detrital eelgrass. Considering the ambiguities noted above, I have not found conclusive evidence for significant benthic microalgal input into the local food web.

5.5. Conclusions and future work

Fatty acid biomarkers in the marine environment are widely used as qualitative indicators of food web dynamics in natural systems. Comparisons of fatty acid compositions have been used to differentiate species (Iverson *et al.*, 2002), as well as to distinguish individuals within a species which feed at different habitats (Iverson *et al.*, 1997, 2002). However, it is very difficult to obtain definitive, quantifiable measures of energy transfer between trophic levels based on fatty acid compositions alone, outside of a controlled laboratory environment. Fatty acid analysis is best applied to corroborate food web relationships that have been indicated through unrelated analyses.

Future work may include compound-specific isotope analysis (CSIA) of fatty acids, which may resolve ambiguities related to this study. CSIA will give rise to a more quantitative estimate of seagrass inputs to the local food webs. This study assumes that eelgrass is the only source of linolenic acid that is available to consumers in the selected sites. However, terrestrial plants and some marsh plants are also known to contain this combination of fatty acids. For instance, *Spartina alterniflora* is known to contain linolenic and linoleic acids as well as a large proportion of long-chain saturated FAs (Jeffries, 1972; Meziane *et al.*, 1997). *Spartina alterniflora* is common in the nearby marsh systems. However, no visible fragments of cordgrass or other terrestrial plant species were collected within the restored meadows. Also 18:3ω3 was not detected in any higher trophic

level fish in eelgrass-free sites, which were located more closely to marsh stands. Still, it is possible that these plants contribute to the organic matter in South Bay.

For the species in which eelgrass biomarkers are present, the $\delta^{13}C$ value of that fatty acid from both the eelgrass and the consumer tissue can be compared to reveal whether eelgrass is a source of those particular fatty acids to the consumer. In the particular case of the blue crab, skeleton shrimp, and pigfish from seagrass meadows which had significant amounts of the combination of eelgrass biomarker FAs, CSIA analysis could more conclusively identify the original source of these fatty acids. If direct incorporation of dietary FAs is the dominant biosynthetic pathway it is expected that the dietary fatty acids and the animal tissue FAs will be isotopically similar. Conversely, if a metabolic process such as desaturation and elongation is dominant, the isotopic signatures of the animal FAs will more closely resemble the bulk isotopes of the dietary sources (Stott *et al*., 1997). Finally, in this system, it was assumed for this study that eelgrass was the only substantial source of linolenic acid available to the food web. However, terrestrial angiosperms and several marsh plants are also known to contain significant proportions of linolenic acid. CSIA analyses will likely be able to differentiate between these sources, considering that the isotope signature of the carbon sources available to these plants and also the bulk carbon isotope values of these terrestrial plants differ from those of eelgrass at this site.

As it appears that the bacteria that colonize detrital material are important mediators for the transfer of plant FA to the local food chain, it then follows that the ultimate carbon sources utilized by bacteria for FA production should be more fully investigated. Sedimentary bacteria are likely the best choice to study as they can be more easily collected in sufficient quantities for CSIA analysis. In particular, it can be determined whether there is a direct incorporation of eelgrass FA or whether the material ingested is used for *de novo* synthesis of FA (e.g. MacAvoy *et al.*, 2003). Also, if the FA are synthesized from preexisting carbon sources in the diet, such as proteins and carbohydrates, their respective carbon isotopic values will resemble those of the bulk sources (Stott *et al.*, 1997).

CSIA analysis can help to determine whether bacteria preferentially select for material originating from eelgrass or not. If direct uptake, followed by fatty acid desaturation and chain elongation, is the main pathway for bacterial uptake of eelgrass fatty acids, then the carbon isotope value of bacterial fatty acids should reflect the $\delta^{13}C$ of their respective precursor fatty acids in seagrass. Conversely, if bacteria do not selectively choose eelgrass-derived carbon, it would be expected that the $\delta^{13}C$ of bacterial fatty acids reflect the $\delta^{13}C$ of the total organic carbon in the sediment.

Previous studies have investigated the carbon sources utilized by bacteria in marine sediments. In a review of several coastal systems, Bouillon and Boschker (2006) found that in systems containing from one to ten percent total

organic carbon by weight, the $\delta^{13}C$ of bacterial biomarkers (specifically, the fatty acids *i*+*a*15:0) generally follow that of the $\delta^{13}C$ of sediment TOC. Little or no selectivity was found even in systems with mixed inputs to the TOC from algal and macrophyte sources. However, for sediments with TOC contents lower than one percent, bacteria tended to select for an isotopically enriched source. This could indicate the preferential use of organic material such as macrophyte root exudates which are generally simple organic molecules that are readily available to and usable by sedimentary bacteria (Moriarty *et al*., 1986). Eelgrass and other seagrass species are believed to exude small amounts of simple organic molecules through their leaves (Penhale and Smith, 1977; Robertson *et al*., 1982) and root and rhizome systems (Moriarty *et al*., 1986; Welsh *et al*., 1997). Root exudates are a potentially important carbon source to bacteria that inhabit the sediments surrounding seagrass roots.

This study did not investigate whether root exudates compounds were used by sedimentary bacteria. Also, as sedimentary percent TOC was not determined during this study, it is unclear whether the TOC in the sediments within the eelgrass meadows or in nearby locations lies within the one to ten percent range. A similar study would be useful in South Bay in order to further investigate the pathway for eelgrass incorporation into the food web.

5.6. References

Alfaro, A.C., Thomas, F., Sergent, L., and Duxbury, M. 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuarine Coastal and Shelf Science 70: 271-286.

Ballentine, D.C., Macko, S.A., Turekian, V.C., Gilhooly, W.P., and Martincigh, B. 1996. Compound specific isotope analysis of fatty acids and polycyclic aromatic hydrocarbons in aerosols: implications for biomass burning. Organic Geochemistry 25 (1-2): 97-104.

Ballentine, D. C. 1997. Tracing combustion-derived atmospheric pollutants using compound specific isotope analysis. PhD. Dissertation, Department of Chemistry, University of Virginia. 172 pages.

Boschker, H.T.S., de Brouwer, J.F.C., and Cappenberg, T.E. 1999. The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: Stable carbon isotope analysis of bacterial biomarkers. Limnology and Oceanography 44 (2): 309-319.

Boschker, H.T.S., Wielemaker, A., Schaub, B.E.M., and Holmer, M. 2000. Limited coupling of macrophyte production and bacterial carbon cycling in the sediments of *Zostera spp.* meadows. Marine Ecology Progress Series 203: 181-189.

Boschker, H.T.S., and Middleburg, J.J. 2002. Minireview: Stable isotopes and biomarkers in microbial ecology. FEMS Microbiology Ecology 40: 85-95.

Bouillon, S., Moens, T., and Dehairs, F. 2004. Carbon sources supporting benthic mineralization in mangrove and adjacent seagrass sediments (Gazi Bay, Kenya). Biogeosciences 1: 71-78.

Bouillon, S., and Boschker, H.T.S. 2006. Bacterial carbon sources in coastal sediments: a cross-system analysis based on stable isotope data of biomarkers. Biogeosciences 3: 175-185.

Buzzi, M., Henderson, R.J., and Sargent, J.R. 1997. The biosynthesis of docosahexaenoic acid [22:6(n-3)] from linolenic acid in primary hepatocytes isolated from wild northern pike. Journal of Fish Biology 51 (6): 1197-1208.

Canuel, E.A., Freeman, K.H., and Wakeham, S.G. 1997. Isotopic compositions of lipid biomarker compounds in estuarine plants and surface sediments. Limnology and Oceanography 42 (7): 1570-1583.

Chao, L.N., and J.A. Musick. 1977. Life history, feeding habits, and functional morphology of juvenile sciaenid fishes in the York River estuary. U.S. Natl. Mar. Fish. Serv. Fish. Bull. 75: 657-702.

Christie, W. 2003. Lipid analysis: isolation, separation, and structural analysis of lipids. Bridgewater (England): The Oily Press. 416 pages.

Connolly, R.M. 2003. Differences in trophodynamics of commercially important fish between artificial waterways and natural coastal wetlands. Estuarine, Coastal and Shelf Science 58: 929-936.

Connolly, R.M., Gorman, D., and Guest, M.A. 2005. Movement of carbon among estuarine habitats and its assimilation by invertebrates. Oecologia 144: 684-691.

Dalsgaard, J., St. John, M., Kattner, G., Muller-Navarra, D., and Hagen, W. 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46: 225-340.

Deegan, L.A., Peterson, B.J., Portier, R. 1990. Stable isotopes and cellulase activity as evidence for detritus as a food source for juvenile Gulf menhaden. Estuaries 13: 14-19.

De Leeuw, J.W., Rijpstra, W.I.C., and Nienhuis, P.H. 1995. Free and bound fatty acids and hydroxy fatty acids in the living and decomposing eelgrass *Zostera marina*. Organic Geochemistry 23 (8): 721-728.

Duarte, C.M. 1989. Temporal biomass variability and production biomass relationship of seagrass communities. Marine Ecology Progress Series 51: 269-276.

Duarte, C.M. and Cebrián, J. 1996. The fate of marine autotrophic production. Limnology and Oceanography 41 (8): 1758-1766.

Ember, L.M., Williams, D.F., and Morris, J.T. 1987. Processes that influence carbon isotope variations in salt marsh sediments. Marine Ecology Progress Series 36: 33–42.

Fry, B., and Parker, P.L. 1979. Animal diet in Texas seagrass meadows: $\delta^{13}C$ evidence for the importance of benthic plants. Estuarine and Coastal Marine Science 8: 499-509.

Graeve, M., Kattner, G., Wiencke, C., and Karsten, U. 2002. Fatty acid composition of Arctic and Antarctic macroalgae: indicator of phylogenetic and trophic relationships. Marine Ecology Progress Series 231: 67-74.

Holmer, M., Andersen, F.Ø., Nielsen, S.L., and Boschker, H.T.S. 2001. The importance of mineralization based on sulfate reduction for nutrient regeneration in tropical seagrass sediments. Aquatic Botany 71 (1): 1-17.

Holmer, M., Duarte, C. M., Boschker, H. T. S., and Barrón, C. 2004. Carbon cycling and bacterial carbon sources in pristine and impacted Mediterranean seagrass sediments. Aquatic Microbiology and Ecology 36: 227-237.

Iverson, S.J., Frost, K.J., and Lang, S.L.C. 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. Marine Ecology Progress Series 241: 161-181.

Iverson, S.J., Frost, K.J., and Lowry, L.F. 1997. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Marine Ecology Progress Series 151: 255-271.

Jaschinski, S., Brepohl, D.C., and Sommer, U. 2008. Carbon sources and trophic structure in an eelgrass *Zostera marina* bed, based on stable isotope and fatty acid analysis. Marine Ecology Progress Series 358: 103-114.

Jeffries, H.P. 1972. Fatty-acid ecology of a tidal marsh. Limnology and Oceanography 17 (3): 433-440.

Jones, W.B., Cifuentes, L.A., and Kaldy, J.E. 2003. Stable carbon isotope evidence for coupling between sedimentary bacteria and seagrasses in a sub-tropical lagoon. Marine Ecology Progress Series 255: 15-25.

Kanazawa, A., Teshima, S.-I., and Ono, K. 1979. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comparative Biochemistry and Physiology 63B: 295-298.

Keough, J.R., Hagley, C.A., Ruzycki, E., and Sierszen, M. 1998. $\delta^{13}C$ composition of primary producers and the role of detritus in a freshwater coastal ecosystem. Limnology and Oceanography 43 (4): 734-740.

Kharlamenko, V.I., Kiyashko, S.I., Imbs, A.B., and Vyshkvartzev, D.I. 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. Marine Ecology Progress Series 220: 103–117.

Khotimchenko, S.V. 1993. Fatty acids and polar lipids of seagrasses from the Sea of Japan. Phytochemistry 33 (2): 369-372.

Khotimchenko, S.V. 2003. The fatty acid composition of glycolipids of marine macrophytes. Russian Journal of Marine Biology 29 (2): 126-128.

Khotimchenko, S.V., Vaskovsky, V.E., and Titlyanova, T.V. 2002. Fatty acids of marine algae from the Pacific Coast of North California. Botanica Marina 45: 17-22.

Kikuchi, T. 1980. Faunal relationships in temperate seagrass beds. Pages 153-172 in: R.C. Phillips and C.P. McRoy (eds.), *Handbook of Seagrass Biology: An Ecosystem Perspective*. New York: Garland STPM Press. 353 pages.

Klumpp, D.W., Howards, R.K., and Pollard, D.A. 1989. Trophodynamics and nutritional ecology of seagrass communities. Pages 394-457 in: A.W.D. Larkum, A.J. McComb and S.A. Shepherd (eds.), *Biology of Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region.* Amsterdam: Elsevier Science Publishing. 841 pages.

Luczkovich, J.J., and Stellwag, E.J. 1993. Isolation of cellulolytic microbes from the intestinal tract of the pinfish, *Lagodon rhomboides*: size-related changes in diet and microbial abundance. Marine Biology 116: 381-388.

MacAvoy, S.E., Macko, S.A, and Carney, R.S. 2003. Links between chemosynthetic production and mobile predators on the Louisiana continental slope: stable carbon isotopes of specific fatty acids. Chemical Geology 201: 229-237.

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

Mann, K.H. 1988. Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnology and Oceanography 33 (4, part 2): 910-930.

Meziane, T., Bodineau, L., Retiere, C., and Thoumelin, G. 1997. The use of lipid markers to define sources of organic matter to sediment and food web of the intertidal salt-marsh-flat ecosystem of Mont-Saint-Michel Bay, France. Journal of Sea Research 38: 47-58.

Moriarty, D.J.W., Iverson, R.L., and Pollard, P.C. 1986. Exudation of organic carbon by the seagrass *Halodule wrightii* Aschers and its effect on bacterial growth in the sediment. Journal of Experimental Marine Biology and Ecology 96 (2): 115-126.

Napolitano, G.E. 1999. Fatty acids as trophic and chemical markers in freshwater ecosystems. Pages 21-44 in: Arts, M.T. and Wainman, B.C. (eds.), *Lipids in Freshwater Ecosystems.* New York: Springer-Verlag Inc. 319 pages.

Ogden, J.C. 1980. Faunal relationships in Caribbean seagrass beds. Pages 173-198 in: R.C. Phillips and C.P. McRoy (eds.), *Handbook of Seagrass Biology: An Ecosystem Perspective*. New York: Garland STPM Press. 353 pages.

Olsen, R.E., Henderson, R.J., and McAndrew, B.J. 1990. The conversion of linoleic acid and linolenic acid to longer chain polyunsaturated fatty acids by *Tilapia* (*Oreochromis*) *nilotica in vivo*. Fish Physiology and Biochemistry 8 (3): 261-270.

Olsen, R.E., and Ringø, E. 1992. Lipids of the Arctic charr, *Salvelinus alpines* (L.) II. Influence of dietary fatty acids on the elongation and desaturation of linoleic and linolenic acid. Fish Physiology and Biochemistry 9 (5/6): 393-399.

Olsen, Y. 1999. Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture? Pages 161-202 in: M.T. Arts and B.C. Wainwright (eds.), *Lipids and Freshwater Ecosystems*. New York: Springer-Verlag New York, Inc. 319 pages.

O'Neil, S.P. and Weinstein, M.P. 1987. Feeding habits of spot, *Leistomus xanthurus*, in polyhaline versus meso-oligohaline tidal creeks and shoals. Fishery Bulletin 85 (4): 785-796.

Orth, R.J., Luckenbach, M.L., Marion, S.R., Moore, K.A., and Wilcox, D.J. 2006. Seagrass recovery in the Delmarva Coastal Bays, USA. Aquatic Botany 84: 26-36.

Orth, R.J., Marion, S.R., Moore, K.A., Wilcox, D.J. 2010. Eelgrass (*Zostera marina* L.) in the Chesapeake Bay region of mid-Atlantic Coast of the USA: Challenges in Conservation and Restoration. Estuaries and Coasts 33: 139-50.

Penhale, P.A. and Smith Jr., W.O. 1977. Excretion of dissolved organic carbon by eelgrass (*Zostera marina*) and its epiphytes. Limnology and Oceanography 22 (3): 400-407.

Peterson, B.J., and Howarth, R.W. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnology and Oceanography 32 (6): 1195-12.13.

Peterson, B.J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. Acta Oecologia 20 (4): 479-487.

Recks, M.A., and Seaborn, G.T. 2008. Variation in fatty acid composition among nine forage species from a southeastern US estuarine and nearshore coastal ecosystem. Fish Physiology and Biochemistry 34: 275-287.

Reuss, N., and Poulsen, L.K. 2002. Evaluation of fatty acids as biomarkers for a natural plankton community. A field study of a spring bloom and a post-bloom period off West Greenland. Marine Biology 141: 423-434.

Robertson, M.L., Mills, A.L., and Zieman, J.C. 1982. Microbial synthesis of detritus-like particulates from dissolved organic carbon released by tropical seagrasses. Marine Ecology Progress Series 7: 279-285.

Sanina, N.M., Goncharova, S.N., and Kostetsky, E.Y. 2004. Fatty acid composition of individual polar lipid classes from marine macrophytes. Phytochemistry 65: 721-730.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., and Estevez, A. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177: 191-199.

Shi, W., Sun, M-Y., Molina, M., and Hodson, R.E. 2001. Variability in the distribution of lipid biomarkers and their molecular isotopic composition in Altahama estuarine sediments: implications for the relative contribution of organic matter from various sources. Organic Geochemistry 32 (4): 453-467.

Shield, P.D. 1978. Larval development of the Caridean shrimp, *Hippolyte pleuracanthus* (Stimpson), reared in the laboratory. Estuaries 1 (1): 1-16.

Short, F.T., Ibelings, B.W., and den Hartog, C. 1988. Comparison of a current eelgrass disease to the wasting disease in the 1930s. Aquatic Botany 30: 295–304.

Stott, A.W., Davies, E., and Evershed, R.P. 1997. Monitoring the routing of dietary and biosynthesized lipids through compound-specific stable isotope ($\delta^{13}C$) measurements at natural abundance. Naturwissenschaften 84: 82-86.

Takeuchi, T. 1997. Essential fatty acid requirements of aquatic animals with emphasis on fish larvae and fingerlings. Reviews in Fisheries Science 5 (1): 1-25

Thayer, G.W., Bjorndal, K.A., Ogden, J.C., Williams, S.L., and Zieman, J.C. 1984. Role of larger herbivores in seagrass communities. Estuaries 7 (4a): 351-376.

Thresher, R.E., Nichols, P.D., Gunn, J.S., Bruce, B.D., and Furlani, D.M. 1992. Seagrass detritus as the basis of a coastal planktonic food chain. Limnology and Oceanography 37 (8): 1754-1758.

van Montfrans, J., Macko, S.A., Harbeson, S.A., and Combs, D. 2005. Trophic interactions of fish predators utilizing restored seagrass beds in Virginia's Coastal Bays. Final Report for FY03, Task 12.05. 7 pages.

Vaskovsky, V.E., Khotimchenko, S.V., Bangmei, X., and Li., W. 1996. Polar lipids and fatty acids of some marine macrophytes from the Yellow Sea. Phytochemistry 42 (5): 1347-1356.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., and Garland, C.D. 1998. Fatty acids and lipid compositions of 10 species of microalgae used in mariculture. Journal of Experimental Marine Biology and Ecology 128: 219-240.

Wannigama, G.P., Volkman, J.K., Gillan, F.T., Nichols, P.D., and Johns, R.B. 1981. A comparison of lipid components in the fresh and dead leaves and pneumatophores of the mangrove *Avicennia marina*. Phytochemistry 20(4): 659-666.

Welsh, D.T., Bourguès, S., de Wit, R., and Auby, S. 1997. Effect of plant photosynthesis, carbon sources and ammonium availability on nitrogen fixation rates in the rhizosphere of *Zostera noltii*. Aquatic Microbial Ecology 12: 285-290.

Xu, X., and Kestemont, P. 2002. Lipid metabolism and FA composition in tissues of Eurasian perch *Perca fluviatilis* as influenced by dietary fats. Lipids 37 (3): 297-304.

Zieman, J.C., Macko, S.A., and Mills, A.L. 1984. Role of seagrasses and mangroves in estuarine food webs: temporal and spatial changes in stable isotope composition and amino acid content during decomposition. Bulletin of Marine Science 35(3): 380-392.

Table 5.1. Marine primary producers and their associated fatty acid biomarkers. Adapted from Alfaro *et al*. (2006): a - Alfaro *et al*. (2006). b - Boschker and Middleburg (2002). c - Shi *et al.* (2001). d - Khotimchenko, S.V. (1993). e - Volkman *et al*. (1998). f - Wannigama *et al*. (1981). g - Dalsgaard *et al*. (2003). h - Kharlamenko *et al*. (2001).

Organisms	**Biomarkers**
Bacteria[a,b,c,h]	18:1ω7 (anaerobic bacteria), branched-chain (sedimentary bacteria) and odd-chain fatty acids, 15:0 (aerobic bacteria), *i*14:0
Brown algae[a]	18:1ω9 (in sediments)
Live seagrass[a,d,h]	18:2ω6 + 18:3ω3, Long-chain saturated fatty acids (LCFA, contain ≥ 22 carbons per chain)
Benthic microalgae[e] (excluding diatoms and dinoflagellates)	16:2ω12, 18:5ω3, 16:4ω1
Terrestrial plants[f]	LCFA (≥ 22 carbons per chain)
Dinoflagellates[g]	combined high levels: 22:6ω3, 18:4ω3, 18:5ω3
Red algae[a,g]	[20:5ω3/20:4ω6 > 10], High 20:4ω6, low C18 PUFA
Diatoms[a,h] (microalgae)	20:5ω3, [20:5ω3/22:6ω3 > 1], [16:1/16:0 > 1.6], [∑16/∑18 >2], 16:1ω7
Zooplankton[h]	Sum of all 20:1 and 22:1, 22:6ω3

Table 5.2. FA profiles of primary producers in restored eelgrass meadows and nearby eelgrass-free sites. FA amounts are given as percentages of total fatty acids ± SD when applicable. *ui* – unidentified unsaturated fatty acid. 'n' is the number of samples.

Fatty Acid	**Eelgrass Meadows**						**Eelgrass-free Sites**		
	Eelgrass *Zostera marina*	**Detrital eelgrass** *Zostera marina*	**Sealettuce** *Ulva lactuca*	**Decayed sealettuce** *Ulva lactuca*	**Epiphytic red alga** (*Ceramium rubrum*)	**Graceful redweed** *Gracilaria spp.*	**Sealettuce** *Ulva lactuca*	**Decayed sealettuce** *Ulva lactuca*	**Graceful redweed** *Gracilaria spp.*
n	3	2	2	1	1	1	1	1	1
11:0				1.9				0.5	
12:0	0.6 ± 0.2				0.5			0.6	
13:0								0.6	
14:1ω9			1.3	2.0					
14:0	1.5 ± 0.6	6.3	3.0	1.8	2.4	3.9	2.8	3.4	
i+a 15:0			2.4	1.1	2.0		1.0	0.8	
15:0	0.5 ± 0.0		1.2		2.3	2.1	1.0	1.5	
ui 16-C (a)		2.0	6.3	3.2		4.5	1.7	8.7	12.3
ui 16-C (b)	6.0 ± 1.5								
ui 16-C (c)	0.8 ± 0.3			1.2					
16:1ω9	0.6 ± 0.1	1.0	7.2	1.1	4.7	2.8	4.8	0.5	1.2
16:1ω7	1.5 ± 0.6	4.9		0.8				0.5	
16:0	24.4 ± 2.7	62.7	44.9	17.0	34.9	42.3	42.0	58.1	47.3
ui 17-C					1.7		1.2		
i+a 17:0					1.9		0.7		
17:0			1.6	2.0	6.5	2.3	1.2	0.8	2.0
ui 18-C	1.4 ± 0.1	0.6							
18:3ω3	26.4 ± 2.2	0.5							
18:2ω6	12.2 ± 0.7		1.6	1.0	0.5		4.0	1.1	
18:1ω9	8.6 ± 0.3	0.6	4.7		10.4	2.6	3.2	0.5	0.7
18:1ω7	0.9 ± 0.1	1.0	7.5	1.6	4.4	0.7	5.6	0.6	0.7
18:0	2.8 ± 0.1	3.8	6.3	17.2	13.6	9.4	11.7	6.8	15.8
19:0				3.4			1.3		2.1
20:5ω6					0.5				
20:5ω3	0.5 ± 0.1		3.2				2.9	1.0	
20:4ω6					4.5	6.7			3.5
ui 20-C					0.5				
20:2ω6	0.6 ± 0.1			1.6	0.6	2.1	1.2		1.6
20:1				1.1					
20:0	1.8 ± 0.1	2.7	1.2	4.0	0.7	2.8	0.5	1.6	1.4
ui 22-C				4.0					
ui 22-C				3.8					4.4
22:0	0.8 ± 0.1	2.1	1.4	14.5	0.6	4.8		2.5	
24:0	1.3 ± 0.4	1.5		3.1					

Table 5.3. Summary of fatty acid classes identified in South Bay primary producers. Values given represent the percentage of total fatty acid composition. **Σ** indicates the sum of all fatty acids of each type. [a] Saturated fatty acids include branched-chain fatty acids. [b] Long chain fatty acids are the sum of 22:0, 23:0, 24:0.

Eelgrass meadows	**Σ SFA**[a]	**Σ MUFA**	**Σ PUFA**	**Σ LCFA**[b]	**18:2ω6**	**18:3ω3**
Zostera marina (Live)	33.7	11.6	47.9	2.1	12.2	26.4
Z. marina (Detrital)	79.1	7.5	3.1	3.6	--	0.5
Ulva lactuca (Live)	62.0	20.7	11.1	1.4	1.6	--
U. lactuca (Decayed)	66.0	6.6	14.8	17.6	1.0	--
Epiphytic algae	65.4	21.2	6.6	0.6	0.5	--
Gracilaria spp.	67.6	6.1	13.3	4.8	--	--
Eelgrass-free sites	**Σ SFA**[a]	**Σ MUFA**	**Σ PUFA**	**Σ LCFA**[b]	**18:2ω6**	**18:3ω3**
Ulva lactuca (Live)	62.2	14.8	9.8	--	4.0	--
U. lactuca (Decayed)	77.1	2.1	10.8	2.5	1.1	--
Gracilaria spp.	68.6	2.6	21.8	--	--	--

Table 5.4. FA profiles of invertebrates. FA totals are given as percentages of total fatty acids ± SD when applicable. *ui* – unidentified unsaturated fatty acid. 'n' is the number of samples.

Fatty Acid	Eelgrass Meadows							Eelgrass-free sites	
	Amphipod *Gammarus sp.*	**Unidentified amphipod**	**Blue Crab** *Callinectes sapidus*	**Grass shrimp** *Palaemonetes spp.*	**Ghost shrimp** *Hippolyte spp.*	**Skeleton shrimp** *Paracaprella sp.*	**Snail** *Mitrella sp.*	**Blue crab** *Callinectes sapidus*	**Grass shrimp** *Palaemonetes spp.*
n	1	1	1	2	1	1	1	1	3
12:0	1.0	0.5	1.5		0.9				
14:1ω9	0.9		1.4						
14:0	2.3	2.7	1.6	2.7	2.2	2.8	1.9	0.8	3.7 ± 0.3
i+a 15:0		3.3	7.6		1.0	0.9	1.0		0.5 ± 0.0
15:0	2.5	1.4	0.5	1.9	0.8	1.3	1.9	0.8	1.5 ± 0.2
ui 16-C (a)	6.1	1.6			6.1	16.4	10.7	0.7	
ui 16-C (b)			0.6						
ui 16-C (c)	1.9					0.9			
16:1ω9	2.3	1.3	1.0	6.6	15.8	0.9		5.3	11.6 ± 1.0
16:1ω7	0.6		0.7			0.6	0.6		
16:0	37.4	28.1	35.2	32.8	6.9	27.0	39.8	24.8	29.9 ± 3.5
ui 17-C		1.2	2.1	0.9		1.4	2.9	0.8	0.5 ± 0.1
i+a 17:0		0.5		1.9	1.4			1.3	1.7 ± 0.3
17:0	2.4	3.1	1.0	2.2	1.2	4.0	2.9	3.3	2.1 ± 0.1
ui 18-C					3.1				
18:3ω3			2.4			0.9			
18:2ω6	2.4	0.9	3.7	0.9	23.7	0.9	1.1	1.4	1.1 ± 0.2
18:1ω9	1.8	3.5	2.4	13.5	8.6	5.1	0.5	20.6	13.4 ± 2.9
18:1ω7	1.6	2.9	5.6	11.0	6.2	1.1		11.7	7.5 ± 0.7
18:0	10.3	15.4	6.8	9.5	3.4	6.2	16.9	14.0	4.0 ± 1.5
19:0		0.6	1.4		4.6		0.5	0.6	0.6 ± 0.1
20:5ω6			5.0		0.8			1.3	0.8 ± 0.2
20:5ω3	4.3	4.9	2.5	2.9	2.0	3.2	2.2	2.7	1.5 ± 0.4
20:4ω6		0.8		0.5					
20:2ω6		5.3		0.9	1.6	2.9		2.3	1.2 ± 0.2
20:1		2.9						0.8	0.5 ± 0.0
20:0	1.1	0.6	0.7			0.7	1.3	0.5	0.5 ± 0.1
22:6ω3	4.2	2.1	4.2	2.6	2.9	2.6	0.5	2.1	4.5 ± 0.0
22:5ω3		0.7	0.6	0.7		1.9			
ui 22-C		2.1							
ui 22-C						3.6			
22:0	2.0		3.5		1.0	2.2	4.2		0.5 ± 0.0
23:0						4.2	0.5		
24:0	0.5	0.6		0.6			1.4		

Table 5.5. FA profiles of fish collected from restored eelgrass meadows. FA amounts are given as percentages of total fatty acids. *ui* – unidentified unsaturated fatty acid. 'n' is the number of samples.

Fatty Acid	**Atlantic menhaden** *Brevoortia tyrannus*	**Atlantic silverside** *Menidia menidia*	**Bay anchovy** *Anchoa mitchilli*	**Lined seahorse** *Hippocampus erectus*	**Northern pipefish** *Syngnathus fuscus*	**Pigfish** *Orthopristis chrysoptera*	**Pinfish** *Lagodon rhomboides*	**Silver perch** *Bairdiella chrysoura*	**Spiny boxfish** *Chilomycterus schoepfii*	**Spot** *Leiostomus xanthurus*	**Spot 9/2005** *Leiostomus xanthurus*	**Summer flounder** *Paralichthys dentatus*	**Tautog** *Tautoga onitis*
n	2	3	3	1	1	2	3	3	3	2	4	1	3
14:0	3.1	2.4 ± 0.3	1.3 ± 0.3	1.1	1.5	3.4	1.9 ± 0.3	2.2 ± 0.4	1.7 ± 0.4	1.1	0.9 ± 0.2	2.1	1.7 ± 0.1
i+*a*15:0		0.6 ± 0.1		0.5		1.5							
15:0	0.9	1.1 ± 0.2	0.7 ± 0.1	0.7	0.8		1.4 ± 0.1	0.6 ± 0.0	1.1 ± 0.1	3.0	2.6 ± 0.6	1.9	1.1 ± 0.2
ui 16-C (a)			0.6 ± 0.1							0.5	0.5 ± 0.1		
ui 16-C (c)										0.9	0.6 ± 0.1		
16:1ω9	6.7	4.8 ± 0.5	5.2 ± 0.3	2.6	4.7	6.1	5.6 ± 0.7	6.9 ± 0.6	5.1 ± 0.4	2.8	3.7 ± 0.6	5.1	5.4 ± 0.4
16:0	36.8	33.2 ± 4.3	39.1 ± 2.6	27.2	33.5	28.7	29.5 ± 2.5	34.5 ± 3.4	32.4 ± 2.0	31.1	33.2 ± 4.6	30.0	34.8 ± 4.3
ui 17-C	0.8					1.7	1.1 ± 0.0	1.2 ± 0.2		2.5	1.5 ± 0.1	0.5	0.9 ± 0.1
i+*a*17:0				0.9	0.7		0.5 ± 0.0		0.8 ± 0.1	1.8	1.6 ± 0.3	1.4	0.7 ± 0.0
17:0	1.7		1.4 ± 0.2	2	1.1	1.7	1.4 ± 0.1	0.9 ± 0.1	1.9 ± 0.1	3.7	4.4 ± 0.9	1.9	1.9 ± 0.2
18:3ω3						1.9							
18:2ω6	2.9	4.8 ± 0.3	4.8 ± 0.2	2.5	1.3	2.7	3.1 ± 0.2	0.9 ± 0.1	3.7 ± 0.5	0.5	0.7 ± 0.1	1.1	1.1 ± 0.1
18:1ω9	11.9	12.1 ± 1.7	4.2 ± 0.3	17.3	17.3	14.3	14.6 ± 1.7	20.9 ± 1.2	9.6 ± 0.3	7.4	7.0 ± 0.6	11.1	13.9 ± 1.6
18:1ω7	4.2	6.1 ± 0.6	4.3 ± 0.3	7.1	9.5	7.9	9.3 ± 0.3	8.5 ± 0.7	7.5 ± 1.1	4.4	5.5 ± 0.8	5.3	7.4 ± 0.5
18:0	6.3	11.8 ± 1.4	11.5 ± 0.5	16.7	12.9	9.3	10.1 ± 1.1	6.5 ± 0.1	17.3 ± 0.8	16.3	14.5 ± 1.3	9.2	7.6 ± 0.5
19:0						0.5			0.5 ± 0.1	0.7	0.9 ± 0.2		
20:5ω6		0.7 ± 0.1		3.3	2.2	1.6	2.8 ± 0.4	0.5 ± 0.2		0.9	0.8 ± 0.3	3.0	4.2 ± 0.2
20:5ω3	6.7	3.5 ± 0.1	5.3 ± 0.4	2.5	1.9	2.4	2.7 ± 0.3	2.1 ± 0.1	2.1 ± 0.2	3.3	2.3 ± 0.2	6.9	4.5 ± 0.1
20:4ω6	0.9	0.5 ± 0.0	1.5 ± 0.1	0.7		0.6	0.5 ± 0.1	0.9 ± 0.0	0.9 ± 0.1	0.8	0.5 ± 0.0	0.5	
20:2ω6	2.6	1.0 ± 0.0	0.5 ± 0.1	0.8	0.7	1.2	1.4 ± 0.4	1.4 ± 0.3	1.2 ± 0.3	1.1	1.7 ± 0.2	1.2	1.7 ± 0.1
20:1		0.6 ± 0.1		0.7		0.8	0.9 ± 0.1			0.9	0.9 ± 0.2		0.6 ± 0.1

20:0		0.5 ± 0.1					0.8 ± 0.1		0.8 ± 0.1	0.5	0.5 ± 0.1		0.5 ± 0.1
22:6ω3	4.5	5.1 ± 0.5	3.9 ± 0.3	2.8	1.9	2.9	3.2 ± 0.2	3.6 ± 0.0	1.9 ± 0.1	4.5	2.3 ± 0.1	9.4	2.0 ± 0.0
22:5ω3			1.1 ± 0.1	1.2		0.6	1.0 ± 0.2	0.8 ± 0.1	0.5 ± 0.1			3.0	0.9 ± 0.1
ui 22-C	2.5	1.1 ± 0.1						0.8 ± 0.1					
22:0			0.7 ± 0.0		0.5				0.5 ± 0.1		0.8 ± 0.1		0.5 ± 0.0
24:0					0.8				0.8 ± 0.0		0.7 ± 0.1		0.5 ± 0.0

Table 5.5. continued.

Table 5.6. FA profiles of fish collected from eelgrass-free sites. FA amounts are given as percentages of total fatty acids. *ui* – unidentified unsaturated fatty acid. 'n' is the number of samples.

Fatty Acid	**Atlantic menhaden** *Brevoortia tyrannus*	**Atlantic silverside** *Menidia menidia*	**Bay anchovy** *Anchoa mitchilli*	**Northern pipefish** *Syngnathus fuscus*	**Pinfish** *Lagodon rhomboides*	**Silver perch** *Bairdiella chrysoura*	**Large silver perch** *B. chrysoura*	**Spot** *Leiostomus xanthurus*	**Spot 9/2005** *L. xanthurus*	**Summer flounder** *Paralichthys dentatus*	**Tautog** *Tautoga onitis*
n	4	2	3	1	4	2	1	2	4	1	2
14:0	7.4 ± 0.4	2.2	2.2 ± 0.6	1.5	2.5 ± 0.4	0.8	2.2	1.1	0.8 ± 0.1	1.6	1.5
i+a 15:0					1.0 ± 0.1						
15:0	1.1 ± 0.3	1.1	1.0 ± 0.1	0.8	1.1 ± 0.0	1.0		1.6	2.1 ± 0.5	3.1	1.0
ui 16-C (a)			0.5 ± 0.0			2.9			0.6 ± 0.1		0.5
ui 16-C (c)		1.1					1.1		0.6 ± 0.1		
16:1ω9	7.1 ± 0.5	4.0	3.3 ± 0.3	4.9	6.9 ± 1.2	2.5	6.7	4.2	2.8 ± 0.2	2.0	4.2
16:1ω7				1.8					0.5 ± 0.0		
16:0	30.7 ± 1.6	30.7	36.0 ± 4.2	29.4	31.4 ± 2.4	34.9	38.6	31.7	32.4 ± 1.4	32.8	35.3
ui 17-C	0.5 ± 0.0		0.6 ± 0.1		1.4 ± 0.2	0.7	0.5	1.2	2.6 ± 0.3		
i+a 17:0		0.7	0.5 ± 0.0	0.5	0.8 ± 0.2			1.2	1.5 ± 0.3	1.6	0.6
17:0	1.1 ± 0.2	1.6	1.9 ± 0.2	1.1	1.7 ± 0.2	2.3	0.7	3.1	4.2 ± 0.7	2.4	1.7
18:2ω6	2.5 ± 0.1	0.5	3.7 ± 0.5	1.7	1.7 ± 0.2	0.5	0.5	1.2	2.4 ± 0.1	4.5	0.8
18:1ω9	9.2 ± 0.4	12.2	16.0 ± 0.5	17.9	14.5 ± 1.6	9.5	14.6	15.1	12.3 ± 0.5	10.8	19.0
18:1ω7	3.7 ± 0.1	4.1	4.3 ± 0.2	7.3	6.1 ± 0.5	5.4	8.2	4.3	5.0 ± 0.9	6.5	3.9
18:0	5.3 ± 0.6	12.3	9.1 ± 0.7	12.9	9.6 ± 0.6	20.6	5.8	11.3	10.7 ± 0.5	8.9	9.2
19:0					0.5 ± 0.0	0.5	0.7	0.5	0.9 ± 0.2		0.7
20:5ω6		1.1		2.2	0.7 ± 0.5	0.5		0.5		2.3	1.2
20:5ω3	5.7 ± 0.3	3.7	3.4 ± 0.4	4.9	0.9 ± 0.6	3.6	2.6	2.7	3.2 ± 0.2	3.2	3.7
20:4ω6	1.9 ± 0.1	0.6			0.5 ± 0.0	0.7	0.9	1.4		1.5	

20:2ω6	1.1 ± 0.1	1.0	0.7 ± 0.1	0.7	3.8 ± 0.2	1.4	1.5	2.2	1.4 ± 0.2	1.1	1.2
20:1	0.5 ± 0.0	0.6		0.9	0.8 ± 0.1				0.8 ± 0.1		0.7
20:0				0.8		0.5		0.5	0.5 ± 0.1		
22:6ω3	9.1 ± 0.3	6.8	7.1 ± 0.3	2.9	5.2 ± 0.3	4.3	5.4	7.2	5.1 ± 1.0	5.3	6.8
22:5ω3	1.8 ± 0.1	1.2		0.6			0.5			1.2	
ui 22-C	1.5 ± 0.1				0.7 ± 0.6			1.4	0.5 ± 0.0		
22:0						1.0					
24:0				1.0		0.6					

Table 5.6. continued.

Table 5.7. Summary of fatty acid classes identified in consumers including biomarkers indicating bacteria and eelgrass sources. **Σ** indicates the sum of all fatty acids of each type. [a] Saturated fatty acids do not include branched-chain fatty acids. [b] Long chain fatty acids are the sum of 22:0, 23:0, 24:0.

	Eelgrass meadows	**Σ SFA[a]**	**Σ MUFA**	**Σ PUFA**	**Σ Odd-chain FAs**	**Σ Branched FAs**	**Σ LCFA[b]**	**18:2ω6**	**18:3ω3**
Invertebrates	*Gammarus sp.*	59.5	7.2	18.9	4.9	--	2.5	2.4	--
	Unidentified amphipod	53.0	11.8	18.4	6.3	3.8	0.6	0.9	--
	Callinectes sapidus	52.2	13.2	19.0	5.0	7.6	3.5	3.7	2.4
	Palaemonetes spp.	49.7	32.0	8.5	5.0	1.9	0.6	0.9	--
	Hippolyte spp.	21.0	30.6	40.2	6.6	2.4	1.0	23.7	--
	Paracaprella sp.	48.4	9.1	33.3	6.7	0.9	6.4	0.9	0.9
	Mitrella sp.	71.3	4.0	14.5	8.2	1.0	6.1	1.1	--
Fish	*Brevoortia tyrannus*	48.8	23.6	20.1	3.4	--	--	2.9	--
	Menidia menidia	49.0	23.6	16.7	1.1	0.6	--	4.8	--
	Anchoa mitchilli	54.7	13.7	17.7	2.1	--	0.7	1.8	--
	Hippocampus erectus	47.7	27.7	13.8	2.7	1.4	--	2.5	--
	Syngnathus fuscus	51.1	31.5	8.0	1.9	0.7	1.3	1.3	--
	Orthopristis chrysoptera	45.1	30.8	13.9	3.9	1.5	--	2.7	1.9
	Lagodon rhomboides	45.1	31.5	14.7	3.9	0.5	--	3.1	--
	Bairdiella chrysoura	44.7	37.5	11.0	2.7	--	--	0.9	--
	Chilomycterus schoepfii	57.0	22.2	10.3	3.5	0.8	1.3	3.7	--
	Leiostomus xanthurus	56.4	18.0	12.5	9.9	1.8	--	0.5	--
	L. xanthurus, 9/2005	58.5	18.6	9.4	9.4	1.6	1.5	0.7	--
	Paralichthys dentatus	43.1	24.0	25.1	4.3	1.4	--	1.1	--
	Tautoga onitis	48.6	28.2	14.4	3.9	0.7	1.0	1.1	--
	Eelgrass-free sites	**Σ SFA[a]**	**Σ MUFA**	**Σ PUFA**	**Σ Odd-chain FAs**	**Σ Branched FAs**	**Σ LCFA[b]**	**18:2ω6**	**18:3ω3**
Invert.	*Callinectes sapidus*	44.8	39.2	10.5	5.5	1.3	--	1.4	--
	Palaemonetes spp.	42.8	33.5	9.1	4.7	2.2	0.5	1.1	--
Fish	*Brevoortia tyrannus*	45.6	21.0	23.6	2.7	--	--	2.5	--
	Menidia menidia	47.9	20.9	16.0	2.7	0.7	--	0.5	--
	Anchoa mitchilli	50.2	24.2	15.4	35.0	0.5	--	3.7	--
	Syngnathus fuscus	34.9	32.8	13.0	1.9	0.5	1.0	1.7	--
	Lagodon rhomboides	46.8	29.7	13.5	4.7	1.8	--	1.7	--
	Bairdiella chrysoura	62.2	18.1	13.9	4.5	--	1.6	0.5	--
	Large B. chrysoura	48.0	30.0	12.5	1.9	--	--	0.5	--
	Leiostomus xanthurus	49.8	24.8	16.6	6.4	1.2	--	1.2	--
	L. xanthurus, 9/2005	51.6	24.0	13.3	9.8	1.5	--	2.4	--
	Paralichthys dentatus	48.8	19.3	19.1	5.5	1.6	--	4.5	--
	Tautoga onitis	49.4	27.8	14.2	3.4	0.6	--	0.8	--

Figure 5.1. Percent fraction of eelgrass biomarker fatty acids in consumers collected from restored eelgrass meadows. Ga – *Gammarus sp.*; Ua – unidentified amphipod; Cas – *Callinectes sapidus*; Psp – *Palaemonetes spp.*; Hp – *Hippolyte spp.*; Pas – *Paracaprella sp.*; Ms – *Mitrella sp.*; Bt – *Brevoortia tyrannus*; Mm – *Menidia menidia*; Am – *Anchoa mitchilli*; He – *Hippocampus erectus*; Sf – *Syngnathus fuscus*; Oc – *Orthopristis chrysoptera*; Lr – *Lagodon rhomboides*; Bc – *Bairdiella chrysoura*; Chs – *Chilomycterus shoepfii*; Lx – *Leiostomus xanthurus*; Lex – *L. xanthurus* from 9/2005; Pd – *Paralichthys dentatus*; To – *Tautoga onitis*.

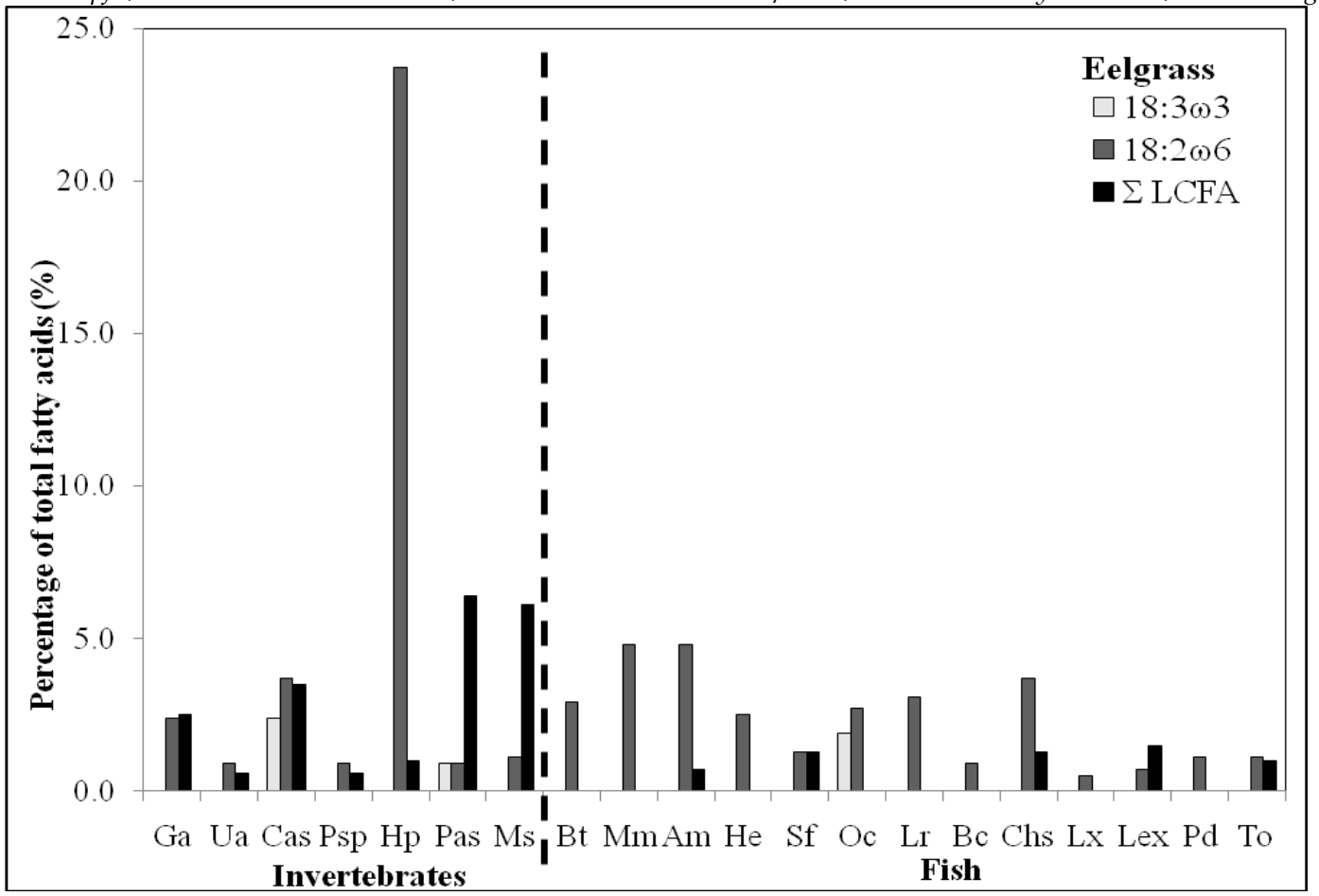


Figure 5.2. Percent fraction of bacterial biomarker fatty acids in consumers collected from restored eelgrass meadows. Ga – *Gammarus sp.*; Ua – unidentified amphipod; Cas – *Callinectes sapidus*; Psp – *Palaemonetes spp.*; Hp – *Hippolyte spp.*; Pas – *Paracaprella sp.*; Ms – *Mitrella sp.*; Bt – *Brevoortia tyrannus*; Mm – *Menidia menidia*; Am – *Anchoa mitchilli*; He – *Hippocampus erectus*; Sf – *Syngnathus fuscus*; Oc – *Orthopristis chrysoptera*; Lr – *Lagodon rhomboides*; Bc – *Bairdiella chrysoura*; Chs – *Chilomycterus shoepfii*; Lx – *Leiostomus xanthurus*; Lex – *L. xanthurus* from 9/2005; Pd – *Paralichthys dentatus*; To – *Tautoga onitis*.

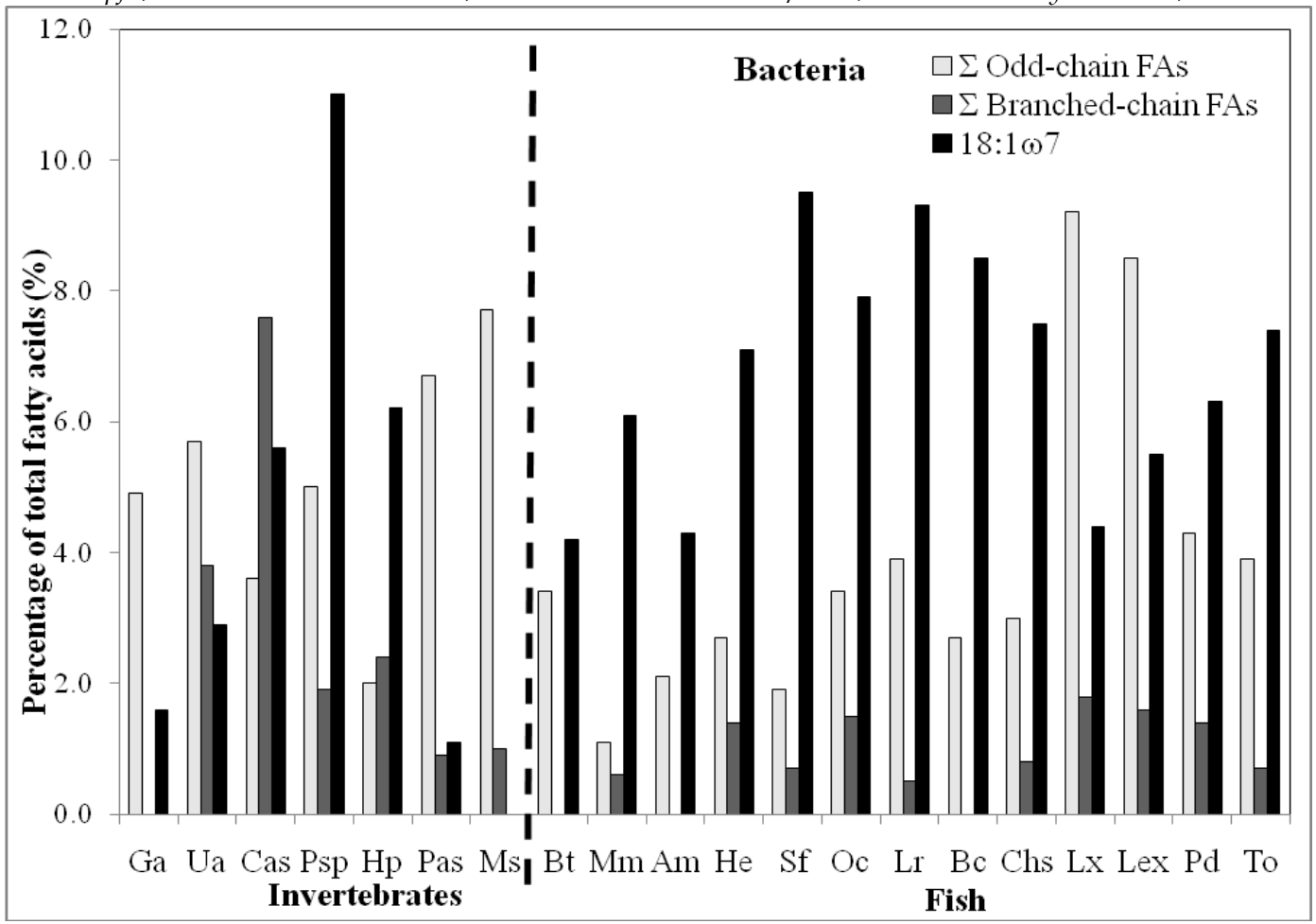


Figure 5.3. Percent fraction of zooplankton biomarker FA in consumers from restored eelgrass meadows. Ga – *Gammarus sp.*; Ua – unidentified amphipod; Cas – *Callinectes sapidus*; Psp – *Palaemonetes spp.*; Hp – *Hippolyte spp.*; Pas – *Paracaprella sp.*; Ms – *Mitrella sp.*; Bt – *Brevoortia tyrannus*; Mm – *Menidia menidia*; Am – *Anchoa mitchilli*; He – *Hippocampus erectus*; Sf – *Syngnathus fuscus*; Oc – *Orthopristis chrysoptera*; Lr – *Lagodon rhomboides*; Bc – *Bairdiella chrysoura*; Chs – *Chilomycterus shoepfii*; Lx – *Leiostomus xanthurus*; Lex – *L. xanthurus* from 9/2005; Pd – *Paralichthys dentatus*; To – *Tautoga onitis*.

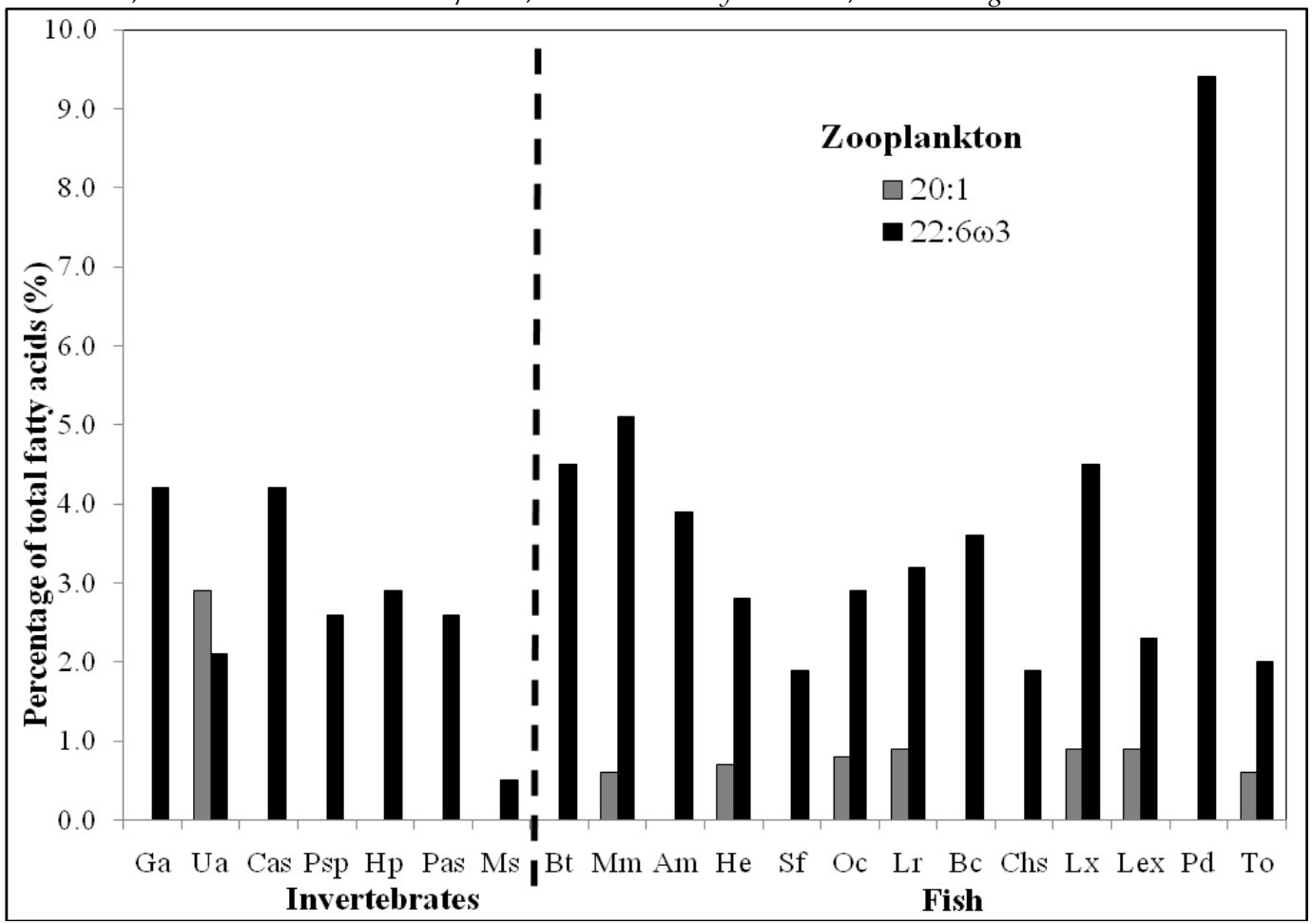


Figure 5.4. Percent fraction of diatom fatty acid markers in invertebrates in restored eelgrass plots. Ga – *Gammarus sp.*; Ua – unidentified amphipod; Cas – *Callinectes sapidus*; Psp – *Palaemonetes spp.*; Hp – *Hippolyte spp.*; Pas – *Paracaprella sp.*; Ms – *Mitrella sp.*

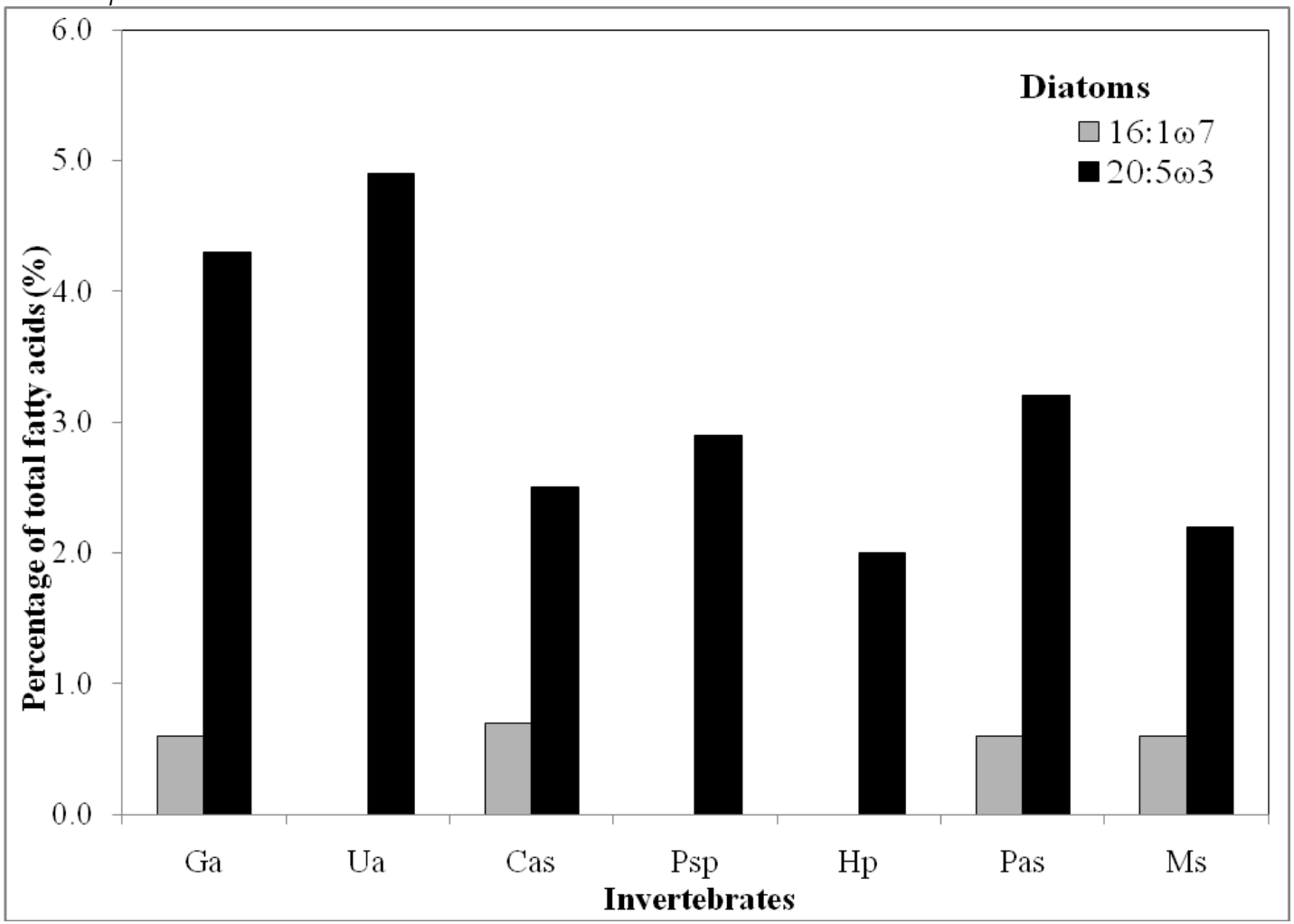


Figure 5.5. Percent fraction of bacterial biomarker fatty acids in consumers collected from South Bay eelgrass-free sites. Cas – *Callinectes sapidus*; Psp – *Palaemonetes spp.*; Bt – *Brevoortia tyrannus*; Mm – *Menidia menidia*; Am – *Anchoa mitchilli*; Sf – *Syngnathus* fuscus; Lr – *Lagodon rhomboides*; Bc – *Bairdiella chrysoura;* LBc – large *B. chrysoura*; Lx – *Leiostomus xanthurus*; Lex – *L. xanthurus* from 9/2005; Pd – *Paralichthys dentatus*; To – *Tautoga onitis*.

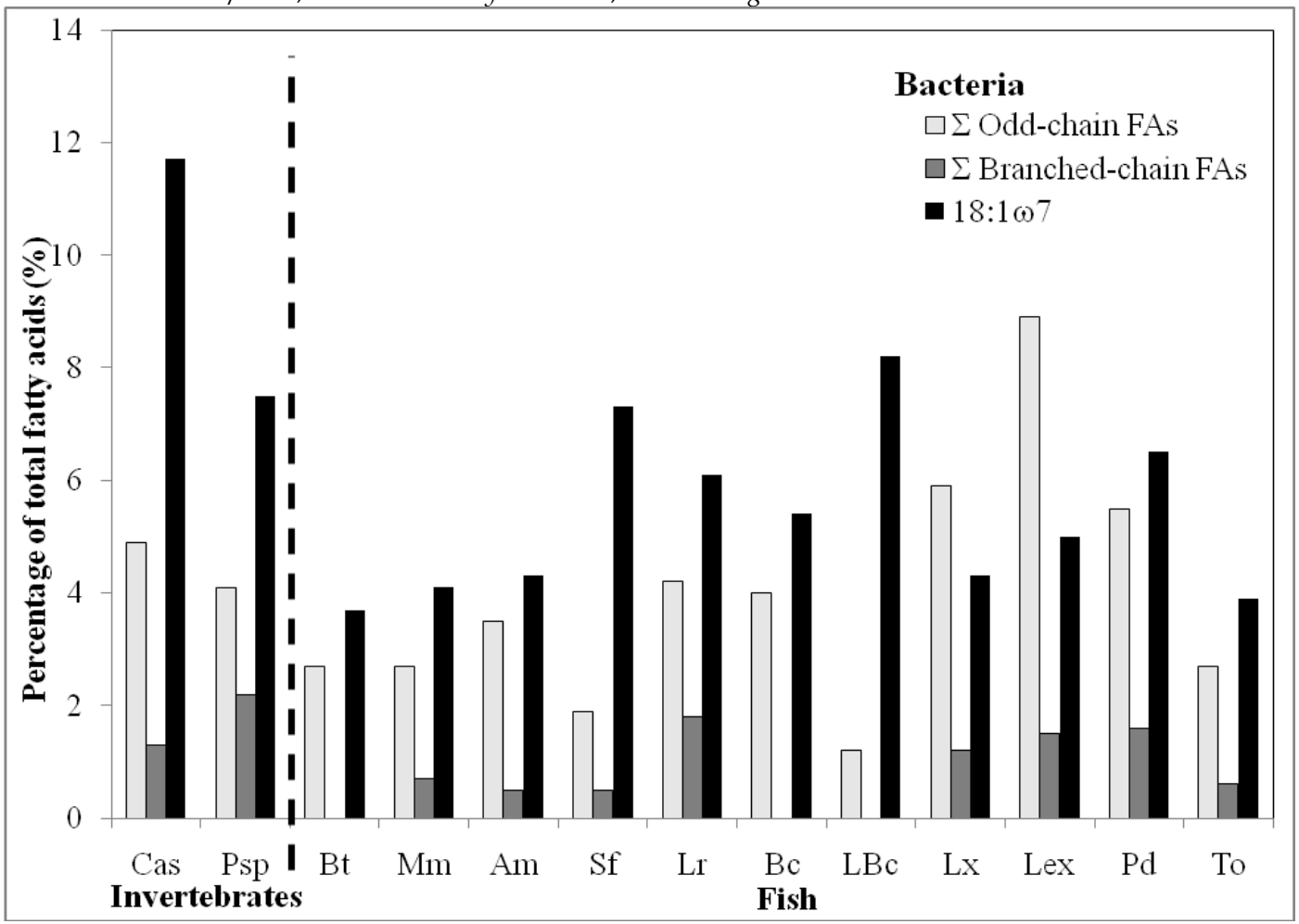


Figure 5.6. Percent fraction of zooplankton biomarkers in invertebrates and fish collected from eelgrass-free sites. Cas – *Callinectes sapidus*; Psp – *Palaemonetes spp.*; Bt – *Brevoortia tyrannus*; Mm – *Menidia menidia*; Am – *Anchoa mitchilli*; Sf – *Syngnathus fuscus*; Lr – *Lagodon rhomboides*; Bc – *Bairdiella chrysoura*; LBc – large *B. chrysoura*; Lx – *Leiostomus xanthurus*; Lex – *L. xanthurus* from 9/2005; Pd – *Paralichthys dentatus*; To – *Tautoga onitis*.

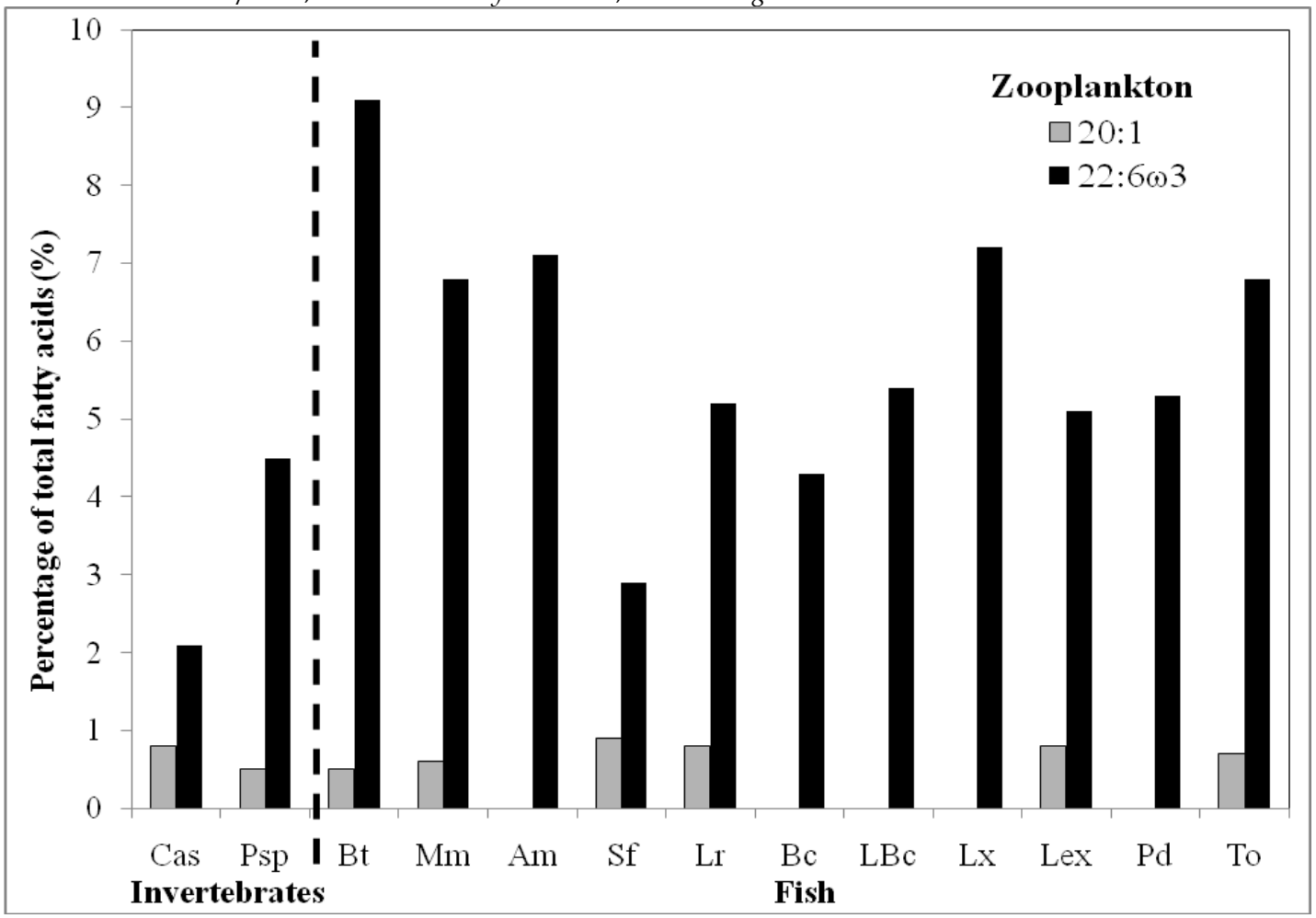
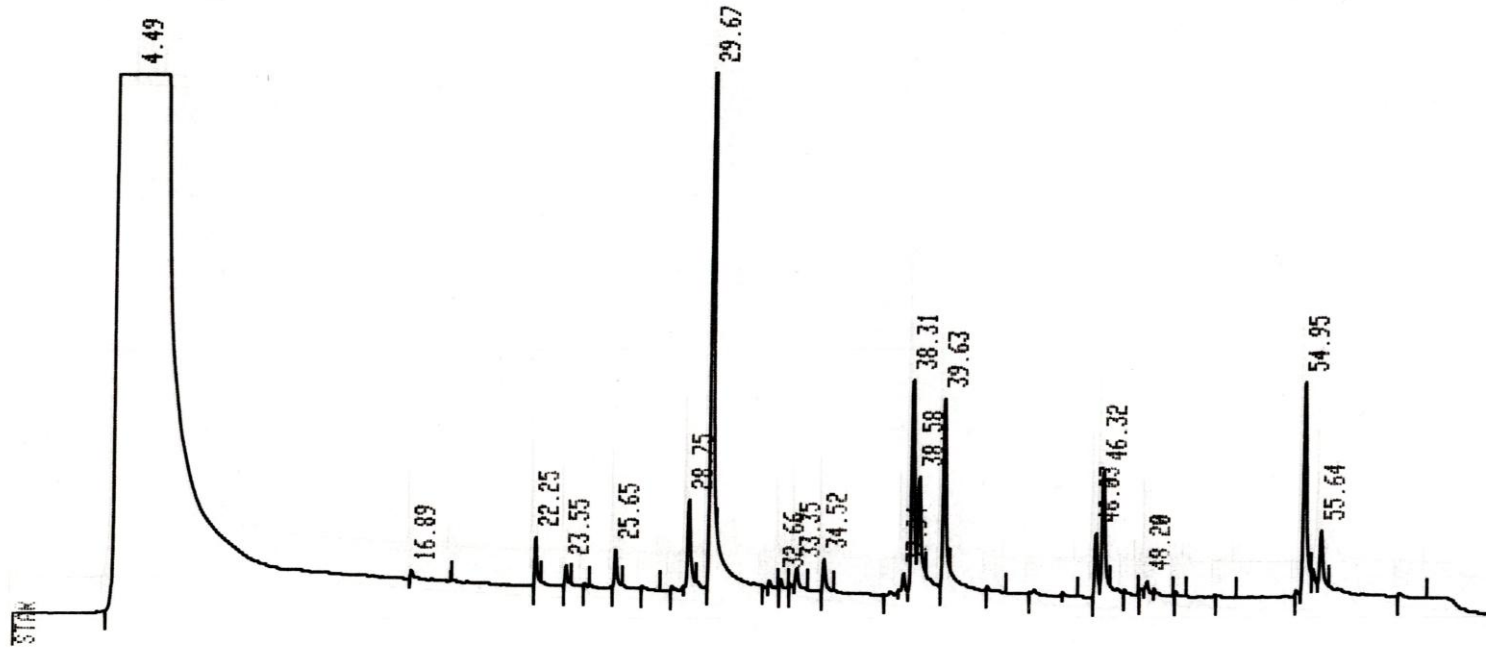


Figure 5.7. Fatty acid chromatogram for summer flounder (*Paralychthis dentatus*). Elution times are given, in minutes, above each peak. Solvent peak is first, left.



VI. Chapter 6: Summary and Conclusions

Seagrass meadows are valuable coastal resources that provide numerous ecosystem services. Seagrasses have been labeled 'coastal canaries' due to a high sensitivity to local water quality (Orth *et al.*, 2006a; Hughes *et al.*, 2009); relatively small variation in water temperature, light and nutrient availability, and dissolved oxygen levels, among other environmental variables strongly affect seagrass productivity (Fourqurean *et al.*, 1997; Short *et al.*, 2007; Moore and Jarvis, 2008; Fonseca and Uhrin, 2009). Seagrass, in turn, positively affects fish and decapod survival. Seagrass can influence the nutritional status of consumers through direct ingestion; indirectly, seagrass can increase fish survival and production by providing shelter and safety from predators and via increased food availability (Minello *et al.*, 2003; Harris *et al.*, 2004; Hughes *et al.*, 2009). As a consequence, the National Marine Fisheries Service designated eelgrass beds as an 'Essential Fish Habitat' effectively identifying these critical habitats to the general public. Yet in general, seagrass meadows are ignored by major media outlets and published news reports compared to coral reefs, mangroves, and tropical forests (Orth *et al.*, 2010), though the scientific community in general acknowledges that seagrass meadows provide numerous ecosystem services, including indicating the overall health of a coastal ecosystem.

Identifying and, when possible, quantifying the many sources of organic matter is essential to understanding nutrient dynamics in estuarine systems (Kwak and Zedler, 1997; Childers *et al.*, 2000). The food webs of coastal and estuarine systems are highly interconnected. Two major pathways of nutrient transfer and utilization, one based on live material and the other on dead and decomposing material, combine and allow for the recycling of nutrients within the system. Microbial decomposers free up nutrients bound in dead organic matter, thus making previously fixed nutrients available to plants. Though a few species are known to eat live eelgrass directly, most studies in temperate systems have found that eelgrass primary production contributes a far greater proportion of biomass to detrital organic matter compared with the minor input made of live material to local food webs (Klumpp *et al.*, 1989; Vähätalo and Søndergaard, 2002).

I utilized multiple stable isotope analysis and fatty acid chemistry to address the long-standing subject of energy transfer in eelgrass systems by tracing the transfer of eelgrass primary production to local organisms in a recently restored system. The major objectives of this study were: 1) To evaluate whether *Zostera marina* significantly influences the diet of consumers in restored meadows, 2) to determine whether consumers in the restored eelgrass meadows utilize different nutritional sources than consumers from nearby eelgrass-free sites, and 3) to compare the fatty acid suites of primary producers and fish and

invertebrates to determine organic matter sources utilized by consumers. The observations combined to give broad insight into the nutritional use and cycling of primary productivity in the food webs of this relatively young system.

6.1. Main findings – Dietary differences relating to habitat

There were no indications that any species feed solely on live eelgrass tissues. Many fish captured in this study are omnivorous, however, and so a mixture of food sources containing a small proportion of eelgrass is possible. The seagrass fatty acid biomarkers 18:3ω3 and 18:2ω6 were found in blue crab, skeleton shrimp, and pigfish in eelgrass sites, indicating that eelgrass provides a proportion of the total nutrition to these species. No consumers in the eelgrass-free sites contained measurable amounts of 18:3ω3 or 18:2ω6.

Several species had significantly different isotope compositions depending on the habitat in which each was collected, which corroborates a related study that compared the gut contents of these fish (van Montfrans *et al*., 2006). Differences were more pronounced in fish collected in 2005 than 2004, which was either due to changes in the sampling protocol between the two years or due to the unusually warm temperatures experienced throughout the Chesapeake Bay region during the summer of 2005 (Moore and Jarvis, 2008; Orth *et al*., 2010).

A bay-wide algal biomass crash during the mid-summer of 2005 allowed for further comparison of consumers collected at the two site types. Eelgrass

biomass also decreased during the same time period, but to a lesser degree. The unusually high water temperatures in the region are believed to have caused the macroalgae die-off. Macrophytes in the Chesapeake Bay suffered major losses during the summer of 2005; however, the degree of damage was less severe in the coastal bays, possibly due to periodic flushing from the Atlantic Ocean (ref). Site type and species type as well as the interaction of these two factors were tested as variables for differences in consumer isotope values. Isotope compositions of many species differed based on site type, though fish collected in September in particular were widely different between the two sites. In general, carbon and sulfur isotope values of fish in restored meadows were relatively higher, while nitrogen isotope values were relatively lower than fish in eelgrass-free sites. Sulfur isotope values were shown to be best for distinguishing fish by habitat.

For all statistically significant comparisons, the average carbon isotope values of fish species in the eelgrass meadows were higher than in eelgrass-free sites. The unweighted average carbon isotope value of all consumers was less negative in the restored eelgrass meadows, and did not mimic the large decrease observed in September for all consumers from the eelgrass-free sites. Approximately half of the significant comparisons for fish nitrogen isotope values showed an increase between restored eelgrass and eelgrass-free sites, while the other half showed a decrease. For all statistically significant

comparisons, the sulfur isotope values of consumers were higher in the restored eelgrass plots than in eelgrass-free sites. The trend of the change of consumer sulfur isotope values through the season was similar for the two sites, though the values differed by approximately 1.2 ‰ up to 2.5 ‰. Overall, the gap between the average carbon and sulfur isotope values of consumers at the two sites suggests that the main base primary production sources are different at the sites. The gap in isotope values also suggests that the nutritional sources in the restored eelgrass plots are more stable than those in the eelgrass-free sites, which is a reasonable supposition since the macroalgae collected from the eelgrass-free sites are ephemeral and are not permanently attached to a substrate. A shift toward decomposing macroalgal matter as the main source for primary consumers, and therefore the prevalence of the detrital pathway, would fit this scenario.

The distinct differences between the isotope values of consumers depending on the habitat from which they were collected indicates that these consumers tend to remain within one or the other habitat and tend not to move between the restored meadows and the eelgrass-free sites. Though the degree of difference that was observed was unexpected, such site fidelity is reasonable, considering that the majority of all consumers collected were vulnerable juveniles which are less likely to traverse the essentially bare sediment areas between habitat types.

6.2. Main findings – Microbial recycling in South Bay

The average nitrogen isotope values of fish increased dramatically in September 2005, by 2.5 ‰ in restored meadows and by nearly 3 ‰ in eelgrass-free sites. The observed shift is approximately equal to one trophic level of enrichment (DeNiro and Epstein, 1981; Peterson and Fry, 1987; Fry, 1988; Hoefs, 1997). It was postulated that the shift in nitrogen isotopes resulted from a mid-summer release of previously fixed nitrogen during the decomposition of much of the macroalgal biomass throughout the bay. The released nitrogen would have a nitrogen isotopic signature reflecting the higher values of the available nitrate; thus when assimilated by other primary producers, it could appear that an additional trophic level would have been added to the food web. McGlathery *et al*. (2001) had previously noted an extensive release of available nitrogen following the senescence and decomposition of much of the macroalgal biomass in nearby Hog Island Bay, which is another coastal bay off the Delmarva Peninsula.

Bacteria may mediate the transfer of seagrass organic matter to the food web via decomposition and eventual uptake through the detrital pathway. All consumer tissues contained one or more bacterial biomarkers; in most cases, several were present in one individual. Invertebrates generally had greater levels of bacterial biomarkers in their tissues as compared to fish. This is not surprising as most invertebrates tested were benthic species; many of these species feed on

the detritus and the colonizing microbiota on the sediment surface. Considering that bacterial biomarker fatty acids were found among numerous consumer species as well as across habitat types, it is not expected that bacteria specifically choose to colonize seagrass organic matter in this system. Rather, bacteria are likely generalists that will use any available carbon, including what is bound in seagrass detritus. In a somewhat nutrient-limited system such as this one, much of the nutrients assimilated by primary producers are likely to be recycled within the system.

It is clear that microbial decomposition and the general recycling of nutrients are important processes within South Bay. Unlike in tropical seagrass systems, there are few large consumers in temperate regions that feed on seagrass directly. Therefore, eelgrass mainly influences the local food web indirectly via decomposition and the detrital pathway.

6.3. Main findings – Restored versus natural eelgrass meadows

A small natural eelgrass meadow was discovered in the mid-1990's adjacent to Fisherman's Island, VA. The meadow is at the southernmost point of the Delmarva Peninsula, with the outlet of the Chesapeake Bay to the west, and the open Atlantic Ocean to the east. The eelgrass blades were heavily colonized by epiphytes, mainly calcareous algae, unlike the South Bay eelgrass, which had little epiphyte growth. Algae are able to assimilate nitrogen from the water column far more quickly than eelgrass. Therefore, it was expected that the

Fisherman's Island site was exposed to high nutrient loading from the eutrophic Chesapeake Bay, which would be reflected in the nitrogen isotope values of local organisms. However, the observed differences in nitrogen isotope values of eelgrass at the two sites were not statistically different. $\delta^{34}S$ values of eelgrass in the natural site were lower than those of eelgrass in the restored meadows, indicating greater uptake of ^{34}S – depleted sedimentary sulfides.

Relatively few species (seven) were collected at both the natural eelgrass meadow and in the restore eelgrass plots in sufficient numbers for statistical comparison. The isotope values of several species collected in both 2004 and 2005 in the restored meadows were significantly different between the two years. Four species had significantly different nitrogen isotope values in the natural meadow. Four species also had distinct sulfur isotope values, while two had significantly different carbon isotope values.

6.4. Conclusions

The first hypothesis - consumer diets in the restored eelgrass meadows differed from those of the same species from the eelgrass-free sites – is supported by both stable isotope and fatty acid analysis. Fish carbon isotope values, which approximate those of primary production sources, were different depending on the consumer habitat, particularly in the late summer. This conclusion in supported by earlier analyses that found differences in stomach contents of consumers between eelgrass and eelgrass-free sites (van Montfrans *et al.*, 2006).

Also, for several species, notably blue crab , the fatty acid profile combined with fatty acid abundances indicated assimilation of widely different dietary sources depending on the presence of eelgrass.

The second hypothesis – eelgrass persistence provided stability to consumer nutritional sources relative to eelgrass-free sites dominated by ephemeral primary production sources – is somewhat supported by stable isotope analyses. The relatively consistent average $\delta^{13}C$ values of fish in eelgrass meadows throughout the study suggests that the continuous presence of eelgrass provided stability to consumer nutritional sources in eelgrass meadows compared to the sharp decline noted in eelgrass-free sites. It is believed that the widespread loss of macroalgae biomass in 2005 resulted in a shift in dietary carbon sources utilized in eelgrass-free sites. Additional fatty acid analyses of fish collected in eelgrass-free sites in September 2005 are needed to compare with analyses presented here to more conclusively identify carbon sources utilized by fish.

The third hypothesis – eelgrass primary productivity comprised a measurable dietary component of consumer diets – is supported for a few species, though the extent is unclear from the current data. There was little evidence of direct uptake of live eelgrass tissues. However, the seagrass fatty acid biomarkers 18:3ω3 and 18:2ω6 were found in blue crab, skeleton shrimp, and pigfish in eelgrass sites. Rather, the data indicated that detritus is a major

organic matter source to the South Bay food web. Bacterial biomarkers were found in tissues of all consumers analyzed from all sites; further analyses are needed to conclusively determine whether the organic matter utilized by bacteria includes seagrass detritus.

The fourth hypothesis – isotope values of consumers at the natural meadow at Fisherman's Island differed from those of organisms in the restored meadows – was supported by the current data. $\delta^{34}S$ values of eelgrass in the natural site indicated greater use of ^{34}S – depleted sedimentary sulfides than at the restored meadows. Isotope values of seven species collected at both sites were compared; four species had significantly different nitrogen isotope values, four also had distinct sulfur isotope values, while two had significantly different carbon isotope values. However, $\delta^{15}N$ values of consumers did not reflect influence of high rates of anthropogenic nutrient loading from the Chesapeake Bay, which had been expected.

The restored eelgrass meadows that were the focus of this study were shown to provide several important functions for local consumers. Though this study has shown that live eelgrass tissues are of limited nutritional importance to consumers in the restored eelgrass meadows in South Bay, there is some evidence that dead and decaying eelgrass tissues are incorporated into the local food web via detritivores. We have also shown that eelgrass presence and seasonal persistence affects the nutritional sources of consumers that inhabit the

meadows. There are distinct differences in dietary sources for fish collected in eelgrass-free sites and in restored eelgrass meadows, which appear to be related to the structure and stability eelgrass provides.

6.5. References

Childers, D.L., Day, J.W. Jr., McKellar, H.N. Jr. 2000. Twenty more years of marsh and estuarine flux studies: Revisiting Nixon (1980). Pages 391-423 in: Weinstein, M.P., and Kreeger, D.A. (eds.), *Concepts and controversies in tidal marsh ecology.* New York: Kluwer Academic Publishers. 875 pages.

Fonseca, M.S., and Uhrin, A.V. 2009. The status of eelgrass, Zostera marina, as bay scallop habitat: consequences for the fishery in the western Atlantic. Marine Fisheries Reviews 71 (3): 20-33.

Fourqurean, J.W., Moore, T.O., Fry, B., and Hollibaugh, J.T. 1997. Spatial and temporal variation in C:N:P ratios, $\delta^{15}N$, and $\delta^{13}C$ of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA. Marine Ecology Progress Series 157: 147-157.

Harris, L.A., Buckley, B., Nixon, S.W., and Allen, B.T. 2004. Experimental studies of predation by bluefish *Pomatomus saltatrix* in varying densities of seagrass and macroalgae. Marine Ecology Progress Series 281: 233-239.

Hughes, A.R., Williams, S.L., Duarte, C.M., Heck, K.L. Jr., and Waycott, M. 2009. Associations of concern: declining seagrasses and threatened dependent species. Frontiers in Ecology and the Environment 7 (5): 242-246.

Klumpp, D.W., Howards, R.K., and Pollard, D.A. 1989. Trophodynamics and nutritional ecology of seagrass communities. Pages 394-457 in: A.W.D. Larkum, A.J. McComb and S.A. Shepherd (eds.) *Biology of Seagrasses: A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region.* Amsterdam: Elsevier Science Publishing. 841 pages.

Kwak, T.J., and Zedler, J.B. 1997. Food web analysis of southern California coastal wetlands using multiple stable isotopes. Oecologia 110 (2): 262-277.

Minello, T.J., Able, K.W., Weinstein, M.P., and Hays, C.G. 2003. Salt marshes as nurseries for nekton: testing hypotheses on density, growth and survival through meta-analysis. Marine Ecology Progress Series 246: 39-59.

Moore, K.A., and Jarvis, J.C. 2008. Environmental factors affecting recent summertime eelgrass diebacks in the lower Chesapeake Bay. Journal of Coastal Research special issue 55: 135-147.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L. Jr., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., and Williams, S.L. 2006a. A global crisis for seagrass ecosystems. Bioscience 56 (12): 987-996

Orth, R.J., Luckenbach, M.L., Marion, S.R., Moore, K.A., and Wilcox, D.A. 2006b. Seagrass recovery in the Delmarva Coastal Bays, USA. Aquatic Botany 84: 26-36.

Orth, R.J., Marion, S.R., Moore, K.A., and Wilcox, D.J. 2010. Eelgrass (*Zostera marina* L.) in the Chesapeake Bay region of mid-Atlantic coast of the USA: Challenges in Conservation and Restoration. Estuaries and Coasts 33: 139-150.

Short, F.T., Carruthers, T., Dennison, W., and Waycott, M. 2007. Global seagrass distribution and diversity: a bioregional model. Journal of Experimental Marine Biology and Ecology 350: 3-20.

Vähätalo, A.V., and Søndergaard, M. 2002. Carbon transfer from detrital leaves of eelgrass (*Zostera marina*) to bacteria. Aquatic Botany 73: 265-273.

van Montfrans, J., Macko, S.A., Harbeson, S.A., and Combs, D. 2006. Trophic Interactions of fish predators utilizing restored seagrass beds in Virginia's Coastal Bays. Final Report for FY 2003, Task 12.05. 7 pages.