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Factors mediating structure and trophic interactions of estuarine nekton communities

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FACTORS MEDIATING STRUCTURE AND TROPHIC INTERACTIONS OF ESTUARINE NEKTON COMMUNITIES

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CO-AUTHORSHIP DECLARATION

I hereby declare that this dissertation includes original research reprinted from co-authored, submitted and published manuscripts. In all chapters A.T. Fisk contributed intellectually by providing consultation, and facilities and materials required for completion of the research. For chapter 2, P.W. Stevens, S.A. Rush and N.E. Hussey contributed to the research with sample collection, statistical guidance and manuscript consultation. For chapter 3, N.E. Hussey, M. Fritts, M.R. Heupel, C.A. Simpfendorfer and G. Poulakis contributed to the research with sample collection, sample preparation and consultation on the manuscript. For chapter 4, S.A. Rush and M.A. MacNeil contributed to the research with statistical guidance and manuscript consultation. For chapter 5, S.A. Rush and N.E. Hussey contributed to the research with statistical guidance and manuscript consultation, M.R. Heupel, C.A. Simpfendorfer and G.R. Poulakis contributed to the research with sample collection. The writing of all chapters included in this dissertation, however, was completed entirely by the author, Jill A. Olin.

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This dissertation includes 3 original manuscripts that have been published or submitted for publication in peer reviewed journals, as follows:

CHAPTER 2 Olin JA, Stevens PW, Rush SA, Hussey NE, Fisk AT. Loss of seasonal variability in nekton community structure in an altered tidal river: evidence for homogenization in a flow-altered system. (*Manuscript in Review: Ecological Applications* 17 October 2011).

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ABSTRACT

Understanding how communities and species assemblages persist is among the most fundamental objectives in ecology, particularly as human modifications to the landscape increase. Through application of traditional community metrics with emerging biochemical tracers in combination with community/food web ecology theory, I provide an evaluation of the effects of anthropogenically-altered freshwater flow disturbance on estuarine nekton community structure and trophic interactions. These two parameters are central toward understanding the functioning of aquatic communities and ensuring their persistence.

This dissertation provides data regarding the effects of human-altered freshwater flow on estuarine nekton communities in tidal rivers and, in doing so, has fostered valuable findings regarding the application of stable isotopes to estuarine fishes and large vertebrates. Specifically, this research demonstrates that losses of estuarine nekton community biodiversity (Chapter 2), the shift in resource availability to lower trophic level species (Chapter 5), and changes to energy flow pathways leading to higher trophic level consumers (Chapter 6), are all associated with high flow events. This dissertation further demonstrates that the application of stable isotopes requires consideration of a species life history characteristics, as interpretation of a species diet and trophic roles can be complex (Chapters 3 and 4).

Collectively, these findings suggest that high flow events affect the structure and trophic interactions of estuarine nekton communities and provide a greater understanding of the impacts of such anthropogenic-mediated stressors on these complex ecosystems. Whether altered high-flow disturbance events result in adverse or beneficial effects on the

persistence of estuaries remains to be established. However, in order to maintain and/or restore the integrity of an ecosystem requires that conservation and management actions be firmly grounded in scientific understanding. This becomes especially relevant as worldwide changes to hydrologic connectivity continue with increasing anthropogenic pressures.

This research demonstrates the potential for the simplification of food webs and changes to dominant trophic assemblages that are associated with flow alteration. For the commercially, recreationally and ecologically valuable species that define estuarine nekton communities, these observations emphasize the necessity of research and management programs aimed at maintaining the integrity of these highly-valued ecosystems.

DEDICATION

GBO, NAO and TJO

xoxo

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Let the next one begin!

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GENERAL INTRODUCTION

The food web is one of the central and unifying concepts in ecology (Lindeman 1942; Martinez 1995) representing an integration of all ecological relationships within a community (Elton 1927). The food web concept provides the framework to test and quantify ecosystem processes such as population dynamics, predator - prey relationships, feeding ecology, and responses to disturbance. Food webs are modeled on the unifying theory of energy transfer (Lindeman 1942) which provides a mechanism for characterizing the trophic interactions and exchanges within and between communities (Odum 1968). As such, understanding the factors regulating food web structure is critical. This is especially relevant in aquatic food webs, where species extinction rates are increasing as a result of multiple anthropogenic stresses (Ricciardi and Rasmussen 1999; Jackson et al. 2001).

DISTURBANCE

Periodic disturbances are a natural component of nearly all ecosystems and are important determinants of community structure and dynamics (Sousa 1984; Pickett and White 1985). A disturbance as defined by Pickett and White (1985) is a relatively discrete event in time that disrupts community or population structure, and changes resource availability or the nature of the physical environment.

Some ecological models predict that species mortalities as imposed by occasional natural disturbances, such as fires, are integral components of most ecosystems and can be vital for maintaining biological diversity as well as renewing essential nutrients (Pickett and White 1985; Webster and Halpern 2010). However, many of these same models also predict a decrease in diversity when the frequency or severity in magnitude

of the disturbance is too great (intermediate disturbance hypothesis; Connell 1978). In aquatic systems, this is best illustrated by drought and storm events, where reductions in species complexity i.e., decrease in diversity, in stream (Walters and Post 2011), estuarine (Livingston et al. 1997; Greenwood et al. 2006; Baptista et al. 2010) and coastal marine (Byrnes et al. 2011) communities have been documented to coincide with these events. Understanding how such reductions in diversity impact community functioning is critical for regulating anthropogenic-mediated effects of habitat degradation (Mora et al. 2007), urbanization (Marchetti et al. 2006), species invasions (Lodge 1993) and species overexploitation (Pauly et al. 1998). This is especially relevant as climate change models predict increased frequency and severity of many forms of large abiotic disturbances, such as tropical storms (Easterling et al. 2000; Meehl et al. 2000). As such, simplification of food webs is an expected consequence.

Recently, it has been argued that some of the most fundamental aspects behind the persistence and functioning of complex systems may be manifested in their ability to adapt in the face of disturbance (Levin 1998). McCann and Rooney (2009) argue that temporal and spatial variability in food web structure and the ability of species to rapidly respond to such variation are critical to the persistence of food webs. McCann (2007) and McCann and Rooney (2009) advocate the empirical examination of food web variability by evaluating how communities, specifically those with relatively consistent species assemblages, respond and/or change across resource gradients in natural and anthropogenic altered systems. Such evaluations will enable predictions regarding the consequences of human modifications on the structure and functioning of ecosystems (McCann 2007). In this manner, food web dynamics are fundamentally based on the

premise of predicting species interactions and thereby understanding predator-prey relationships. This permits the ability to determine the magnitude of energy available to a consumer and also facilitates an understanding of the extent of resource exploitation that may be influenced by disturbance events. The loss of individual species and subsequent biodiversity is known to impact the functioning of both organisms and ecosystems (Cardinale et al. 2006). Should natural or anthropogenic-mediated disturbance events function similarly to alter species abundance and diversity, such changes can lead to significant impairments to ecosystem structure and functioning (McCann and Rooney 2009). Given the increasing frequency of anthropogenic-mediated disturbances such as species invasions, habitat loss and climate change, there is a need to understand how species and ecosystems respond to such events (McCann 2000).

ESTUARIES: ECOLOGY AND IMPORTANCE

Cowardin et al. (1979) formally defined estuaries as "deep-water tidal habitats and adjacent tidal wetlands which are usually semi-enclosed by land, but have open, partially obstructed, or sporadic address to the open ocean and in which water is at least occasionally diluted by freshwater runoff from the land." As such, estuaries are thus viewed as transition zones between terrestrial, and freshwater and marine aquatic systems (Dardeu et al. 1992). This confluence of freshwater and marine aquatic environments results in a wide spectrum of abiotic and biotic characteristics that influence estuarine physical and biological community structure (Dardeu et al. 1992; Rush et al. 2010). Consequently, estuaries are valued as highly productive environments that provide important spawning, nursery, refuge and foraging habitats for a number of species,

including commercial and recreational fishes during one or more of their life history stages (Beck et al. 2001). For example, a recent estimate of U.S. fisheries indicated that approximately 46% of the commercial and 80% of the recreational fisheries harvests are derived from the communities of Gulf Coast estuaries (Lellis-Dibble et al. 2008).

Estuaries, however, are among the most intensely modified ecosystems as a consequence of extensive hydrological alteration, habitat alteration and chemical and organic pollution (Lotze et al. 2006). Globally, there are few estuarine systems that remain unaffected by upstream manipulation of their freshwater flow (Dynesius and Nilsson 1994; Nilsson et al. 2005). River regulation by dams has fragmented hydrological and ecological processes (Nilsson et al. 2005) often restricting or severing connectivity to estuaries and coastal marine systems, as well as facilitating the introduction and establishment of invasive species which can modulate flows of energy and nutrients (Bunn and Arthington 2002). Such anthropogenic-mediated alterations can be detrimental to downstream communities, as freshwater inflow from riverine sources provides nutrients, sediment and organic matter essential for primary and secondary production in these systems (Mallin et al. 1993; Chanton and Lewis 2002).

Anthropogenic-mediated alterations to freshwater flow indirectly affect the physicochemical characteristics of the system by shifting the salinity and dissolved oxygen gradients, and increasing turbidity, among other impacts (Sklar and Browder 1998; Gillson 2011). Predicting the response of estuaries to changing environmental conditions is challenging, as it necessitates understanding interactions among several trophic levels and among multiple nutrient sources (Rush et al. 2010). Many life-history stages of estuarine species from juveniles to adult are intimately tied to water flow (Bunn

and Arthington 2002; Rehage and Trexler 2006). For example, larval stages of many estuarine fishes are reliant on freshwater flow as a cue for migration into estuaries (Strydom et al. 2002; Gillanders et al. 2011). Thus, disruption of this natural event affects recruitment, and thus growth and mortality of these species (Purtlebaugh and Allen 2010). Consequently, the effects of altered flow on estuarine communities are expected to be revealed not only by the presence or absence of certain species (Hofmann and Powell 1998) but also by changes in food web interactions (Akin et al. 2005).

STUDY SITES

The Charlotte Harbor Estuary is a large (~700 km^2) relatively shallow estuary on the southwest coast of Florida that serves as a forage and/or nursery area for more than 255 species of resident, migrant, recreational and commercial fishes of the Gulf of Mexico (Poulakis et al. 2004), as well as to federally-protected species (e.g., manatees, sea turtles and dolphins). The Caloosahatchee and Myakka Rivers (see Figure 2.1; details on the study areas can be found in the following chapters) are major tributaries of Charlotte Harbor Estuary. These rivers are subject to different anthropogenic influences, regarding land-use development, shoreline modification and freshwater flow. Specifically, the Myakka River has been subjected to relatively minor anthropogenic modifications and experiences relatively natural flow regimes. In contrast, the artificial connection of Lake Okeechobee to the Caloosahatchee River represents a unique anthropogenic manipulation of riverine hydrology (Doering and Charmberlain 1998), whereby substantial seasonal discharge from Lake Okeechobee occurs for flood control and water supply, as well as to flush algal blooms and salt water intrusion (Flaig and

Capece 1998). Accompanying these hydrologic changes is a decrease in water quality, marked by increases in turbidity and nutrient loading, changes in water residence time in the estuary, and alteration in the natural salinity gradient (Barnes 2005). These flow characteristics of the Caloosahatchee and Myakka provide a unique opportunity to test how disturbance, in the form of altered flow regimes, affects food webs and provides the context by which this dissertation was developed.

DISSERTATION OBJECTIVES

The objective of this dissertation was to apply the principles and foundations of community/food web ecology to understanding estuarine community response to anthropogenically-altered freshwater flow disturbance. Patterns of community response to flow variability were investigated in a framework that encompassed both temporal and spatial scales and addressed changes in community characteristics associated with a human-driven disturbance. Specifically, this dissertation investigates the effects of altered freshwater flow on community structure and trophic interactions of estuarine communities by comparing the Myakka and Caloosahatchee Rivers and their contrasting flow regimes.

To investigate the effects of altered flow disturbance on estuarine communities, I applied a combination of traditional community metrics with biochemical tracers to demonstrate how altered *vs*. natural flow affect temporal estuarine community structure and function. Traditional metrics included estimates of species density, diversity, richness, and evenness. The biochemical tracers included stable isotopes of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$), and fatty acid biomarkers.

Application of biochemical tracers, especially stable isotopes and fatty acids, have become increasingly prevalent for investigations of diet, trophic interactions and foraging habitats (Peterson and Fry 1987; Iverson et al. 1997; Post et al. 2000; Rubenstein and Hobson 2004) which has allowed for broad evaluation and inference regarding the changing structure and function of food webs (Vander Zanden et al. 1999; Hebert et al. 2006). The stable isotope approach is based on the fact that the ratios of the stable isotopes of nitrogen ($^{15}N/^{14}N$), carbon ($^{13}C/^{12}C$) and sulfur ($^{34}S/^{32}S$) in consumers' tissues reflect (1) isotopic composition of their dietary resources and (2) isotopic fractionation during diet assimilation (DeNiro and Epstein 1978, 1981). Enrichment of isotopes within tissues of a consumer over that of its diet arises as a result of the greater retention of the heavier over the lighter isotope during the process of protein amination and deamination for ^{15}N and ^{34}S, and respiration for ^{13}C (DeNiro and Epstein 1978; 1981). This produces ratios in a consumer's tissues, between approximately 0 and 2‰ for $\delta^{13}C$ and $\delta^{34}S$, and 2 and 5‰ for $\delta^{15}N$, higher than those of its diet (DeNiro and Epstein 1981; Minagawa and Wada 1984; Post 2002; Vanderklift and Ponsard 2003). Specifically, $\delta^{15}N$ values have found application in determining the relative trophic position of a consumer (Minagawa and Wada1984; Post 2002), and $\delta^{13}C$ and $\delta^{34}S$ values have found application in determining basal organic matter sources incorporated into a consumer's diet (Peterson and Fry 1987), species habitat use (Herzka 2005) and dependence on marine and/or terrestrial/freshwater energy pathways (Simenstad and Wissmar 1985; Darnaude et al. 2004; McLeod and Wing 2009).

Fatty acids are the main constituents of many types of lipid and are required for normal growth and development of an organism (Arts 1999). Essential fatty acids are

fatty acids that cannot be efficiently synthesized by consumers in amounts sufficient for optimal growth and development, instead originate in primary producers and need to be acquired through diet (Arts et al. 2001). The utility of fatty acids as biochemical tracers of food web pathways stems from the fact that they are highly conserved during trophic interactions (Iverson et al. 2004) and incorporated into consumers' tissue in largely unmodified form (Falk-Peterson et al. 2002; Hall et al. 2006), thereby allowing inferences to be made regarding consumer diet composition (Iverson et al. 2004; Hebert et al. 2009). For example, the ratio of ω3/ω6 polyunsaturated fatty acids (PUFA) is a useful indicator of the relative contribution of aquatic *vs*. terrestrial-derived resources in a consumers' diet (Smith et al. 1996; Hebert et al. 2009).

OVERVIEW OF CHAPTERS

In Chapter 2 (Loss of seasonal variability in nekton community structure in a tidal river: evidence for homogenization in a flow-altered system), I evaluated seasonal trends (i.e., the transition of dry to wet season) of estuarine nekton trawl and seine assemblages from the Myakka and Caloosahatchee estuaries, with the prediction that the these estuaries would exhibit contrasting responses to the onset of the wet season, i.e., the Caloosahatchee Estuary would exhibit loss in diversity, whereas the Myakka would exhibit an increase. By comparing, nekton density, diversity, richness, and evenness within and between estuaries, this chapter provides unique evidence regarding nekton community response to altered high-flow. This chapter provides a baseline by which hypotheses in subsequent chapters regarding effects of high-flow on the nekton community were posed.

Stable isotope analysis has proven to be a powerful tool for the study of estuarine food webs (Peterson and Fry 1987). Despite the prevalence of stable isotope analyses in ecological studies of diet and food webs, there are still a number of confounding factors that can complicate interpretations of stable isotope data and studies have recommended establishing species-specific criteria for accurate isotopic assessment of an organism (Sweeting et al. 2007). I tested assumptions regarding (1) tissue stable isotope values of young individuals reflecting their current diet and (2) estuarine fishes exhibiting ontogenetic or body-size based shifts in dietary resources. In this manner, Chapter 3 and Chapter 4 allowed me to determine whether a species, sampled over variable time periods and over a range of sizes, would be suitable for use in subsequent community analyses, without confounding size-based effects.

In Chapter 3 (Maternal meddling in neonatal sharks: implications for interpreting stable isotopes in young animals), I examined the relationships between several size metrics and stable isotope values of $\delta^{13}C$ and $\delta^{15}N$ measured from muscle and liver tissues of two species of placentatrophic shark to determine the length of time tissues of young individuals are influenced by their mothers' isotopic signal. This chapter provides guidance regarding estimation of trophic position and characterization of carbon sources and diet of young sharks using stable isotopes.

In Chapter 4 (Isotopic ratios reveal mixed seasonal variation among fishes from two subtropical estuarine systems), I examined temporal and spatial relationships between body size and $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values for fish species across multiple trophic levels, with the expectation that temporal variability would be manifested in changes to $\delta^{13}C$ and $\delta^{34}S$ with season, and that $\delta^{15}N$ would scale with body size. This chapter

supports previous observations in estuarine fishes regarding body size and in that context allows for inclusion of estuarine fishes in subsequent food web analyses.

In Chapter 5 (Going with the flow: reduced inter-specific variability in stable isotope ratios of nekton in response to altered high flow), I evaluated the effect of altered high-flow on food web structure by comparing seasonal isotopic trends ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) in consumer species sampled over four trophic levels in the Caloosahatchee and Myakka estuaries. From the community perspective, I hypothesized that extreme high flows would be most evident among lower trophic level species (i.e., primary and secondary consumers). Specifically, we expected species sampled following the dry season to be enriched in ^{13}C and ^{34}S relative to those sampled following the wet season, reflecting a polyhaline estuarine status (i.e., tidally influenced). In contrast, those sampled following the wet season would be depleted in ^{13}C and ^{34}S reflective of an oligohaline estuarine status (i.e., terrestrial/freshwater influenced). Additionally, the magnitude in the seasonal isotopic shifts would be expected to be greater in the Caloosahatchee as opposed to the Myakka. This chapter demonstrates the effect that altered high flow has on isotopic values of consumer species and in so doing, demonstrates the effect on the overall food web structure, regarding relative trophic position and carbon resources of estuarine consumers.

In Chapter 6 (Changes in resource exploitation by estuarine consumers in response to altered high flow as inferred from fatty acid biomarkers), I used fatty acid biomarkers to evaluate the main trophic pathways and relative importance of different energy sources to the diet of estuarine consumers under different flow regimes. I hypothesized that the contribution of allochthonous carbon sources (i.e., terrestrially-derived) would be more

important during the wet season than the dry season and would be especially evident during extreme high flow. Fatty acid biomarkers and specific fatty acid ratios (i.e., ω3/ω6) indicative of marine *vs*. terrestrial/freshwater resource use were measured in species that constitute several trophic guilds, sampled seasonally from both estuaries. This chapter provides a novel application of fatty acid biomarkers to track altered flow events in estuaries and provides a compliment to Chapter 5, for determining flow-related changes to carbon source and energy pathways of estuarine consumers.

In light of escalating human water demand, urbanization, and climate change that will ultimately lead to increased frequency of extreme flow events, in chapter 7, I summarize the chapters presented here and discuss their contribution to understanding of how altered flow effects estuarine nekton communities in the context of maintaining structure and stability of these productive systems.

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CHAPTER 2

LOSS OF SEASONAL VARIABILITY IN NEKTON COMMUNITY STRUCTURE IN A TIDAL RIVER: EVIDENCE FOR HOMOGENIZATION IN A FLOW-ALTERED SYSTEM[*]

[*] Olin JA, Stevens PW, Rush SA, Hussey NE, Fisk AT. Loss of seasonal variability in nekton community structure in a tidal river: evidence for homogenization in flow-altered system. *Ecological Applications*, In Review: 17 October 2011.

INTRODUCTION

Extensive fragmentation of riverine systems by dams, and associated modifications to fluvial processes (e.g., flux of water, nutrients and sediment) represent a pervasive alteration of the landscape (Nilsson et al. 2005; Poff et al. 2007). These human modifications, which alter the timing and magnitude of freshwater flow, have led to unprecedented changes in natural seasonal and inter-annual hydrologic connectivity, reducing the natural seasonal variability in flow regimes (Poff et al. 2007). This disturbance to natural flow dynamics poses a significant threat to riverine and downstream estuarine and coastal community composition and biodiversity, and as a consequence, compromises the overall structure and function of these important ecosystems (Rozas et al. 2005; Poff and Zimmerman 2010; Carlisle et al. 2011).

Freshwater flow is known to be an important factor structuring nekton communities of estuarine reaches within tidal rivers (Peterson and Ross 1991; Sklar and Browder 1998), with the nekton assemblages changing most rapidly at the oligohaline-mesohaline boundary (Greenwood et al. 2007). Because many estuarine species have evolved life history strategies in response to natural seasonal flow regimes (Bunn and Arthington 2002; Lytle and Poff 2004), alterations to the magnitude and timing of flow can be detrimental (Drinkwater and Frank 1994; Gillson 2011). For example, a reduction in species growth rates (Edeline et al. 2005; Rypel and Layman 2008) and recruitment dynamics (Jenkins et al. 2010), and changes to the overall structure of estuarine food webs (Adams et al. 2009) have been documented in response to altered flow regimes.

Periodic disturbances are a natural component of nearly all ecosystems and are important determinants of community structure and dynamics (e.g., Sousa 1984; Pickett

and White 1985). However, extreme events where the frequency or severity of the disturbance becomes too great result in a decrease in species diversity (Connell 1978). This is best illustrated by drought and storm events in aquatic systems, where a reduction in complexity (i.e., decreases in diversity and abundance) in stream (Walters and Post 2011), estuarine (Livingston et al. 1997; Greenwood et al. 2006; Baptista et al. 2010) and coastal marine (Byrnes et al. 2011) communities have been documented. More specifically in estuarine environments, a decrease in the diversity of estuarine resident, and marine nekton and macrofaunal species, have been associated with prolonged periods of freshwater inflow resulting from human alteration (Rutger and Wing 2006; McLeod and Wing 2008). As well, Chamberlain and Doering (1998) indicated that seagrasses, oyster beds, juvenile fish abundance, and richness decreased, partly in response to rapidly changing salinities and sediment loads as a result of heavy freshwater flows. The consequences of altered flow for the complexity of estuarine communities however, can be unpredictable. For example, Kimmerer (2002) observed that lower trophic levels (i.e., plankton) negatively responded to high flow (i.e., decreased abundance), whereas higher trophic level (i.e., fishes) responded positively to flow (i.e., increased abundance). Nevertheless, reduced species diversity and abundance following extreme disturbance events have the potential to destabilize food webs (McCann et al. 1998; Rooney et al. 2006).

Contention over flow regime management arises not only from competition among water uses, but also from the difficulty of specifying flow requirements, i.e., management measures, that will maintain ecological integrity in aquatic systems (Freeman et al. 2001). Highly altered ecosystems can therefore serve as endpoints for

examining how changes in assemblage structure influence food web function, a study of which can aid the development of key management and restoration strategies (Cross et al. 2011). Understanding the biotic response to altered flow regimes is required to effectively manage aquatic ecosystems and is critical in estuarine systems, as escalating human water demand, urbanization, and climate change will ultimately lead to increased frequency of extreme flow events (Vörösmarty et al. 2000). To address this question, we sampled nekton assemblages of two tidal rivers in the Charlotte Harbor Estuary, Florida; one that has undergone major human development and experiences altered flow regimes, and one that is relatively natural. By comparing the seasonal nekton assemblage trends in these two systems, this study aimed to determine the response of estuarine nekton communities to altered flows, specifically anthropogenic-induced high flows. Because periods of moderate flow have resulted in the highest abundance of species (Idelberger and Greenwood 2005; Cross et al. 2011), we predict the natural estuary would exhibit an increase in nekton density and diversity with the seasonal progression of dry to wet conditions. Additionally, we predict that species composition would reflect the conditions of the estuary, i.e., dry and wet seasons. For example, density and diversity of freshwater species would increase during the wet season, whereas the opposite trend would be observed in marine species. In contrast, within the altered estuary we predicted that extreme high flows would negatively disturb the nekton community, whereby the density and diversity would decrease with the seasonal progression of dry to wet conditions. As physicochemical conditions (i.e., salinity, temperature) have been demonstrated to be important determinants of spatial and temporal fish assemblage structure (Akin et al. 2005; Greenwood et al. 2007) and are commonly correlated with flow, we expect that this

disturbance would largely be evidenced in a decrease in marine and less tolerant estuarine species.

MATERIALS AND METHODS

The Caloosahatchee River (26°30' N, 81°54' W) is a major tributary of Charlotte Harbor Estuary, a large (~700 km^2) relatively shallow estuary on the southwest coast of Florida, USA (Fig. 2.1). The artificial connection of Lake Okeechobee to the Caloosahatchee River represents a unique anthropogenic manipulation of hydrology (Doering and Charmberlain 1998), whereby substantial seasonal discharge from Lake Okeechobee occurs for flood control and water supply, as well as to flush algal blooms and salt water intrusion (Flaig and Capece 1998). Major modifications to the hydrology, along with land-use transformations and dredging for navigation (e.g., ~70% of shoreline is hardened with seawalls and rip-rap) have resulted in large-scale alterations within the estuary (Barnes 2005). The volume of the Caloosahatchee estuary is approximately 105 x 106 m^3, while the median annual discharge is 870 x 106 m^3 (Flaig and Capece 1998). During periods of low freshwater discharge (i.e., during winter/spring months), salt water regularly intrudes to S-79, the most downstream water control structure, often exceeding 10‰ (Fig. 2.1). High freshwater discharge (i.e., during summer/fall months) can cause salinity to drop below 5‰ at the mouth and the transition between the two states can be rapid, sometimes occurring in less than a week (Doering et al. 2002). These fluctuations observed at the head and mouth of the estuary, exceed the salinity tolerances of most oligohaline and marine species (Barnes 2005). These alterations to flow patterns of the Caloosahatchee are particularly relevant, in light of implications for the Comprehensive

Everglades Restoration Plan, thereby creating an ideal system to document the effects of altered flow on community dynamics (RECOVER 2008).

Trends in abundances of nekton can be influenced by myriad factors, including recruitment and/or stochastic climactic events (Greenwood et al. 2007*a*). To minimize variability associated with these factors, we chose the Myakka River (82°12' W, 26°57' N) for comparison with the Caloosahatchee, as it is proximately located (< 100 km; Fig. 2.1) and therefore is accessible to fishes of Charlotte Harbor. Additionally, the Myakka estuary experiences relatively natural flow periods and it's shoreline has been subjected to relatively minor anthropogenic modification (i.e., ~40% of shoreline area is hardened; estimated from 2007 Digital Ortho Quad County Mosaic, USDA, Geospatial Data Gateway in ArcGIS (ESRI ArcGIS version 9)). The natural shoreline areas of the Myakka estuary are characterized by mangroves and saltmarsh, principally *R. mangle*, black mangrove *Avicennia germinans*, saltmarsh cordgrass *Spartina alterniflora* and black needlerush *Juncus roemerianus*. Based on similar trends in fish and macrofaunal abundance among proximate estuaries in Chesapeake Bay (Kraus and Secor 2004), and among three river-estuaries along the Texas coast (Palmer et al. 2011), the Myakka provides a control by which comparisons of nekton community dynamics to the Caloosahatchee can be made.

Nekton community composition

Data on nekton assemblages in the Myakka and Caloosahatchee estuaries were obtained from a long-term fisheries-independent monitoring (FIM) program in the Charlotte Harbor Estuary. Between 2004 and 2009, monthly stratified-random sampling

was conducted in the estuarine reaches of the Myakka and Caloosahatchee rivers using a 6.1-m trawl (38-mm stretch mesh, 3.2-mm stretch mesh liner) and using a 21.3 m seine (3.2-mm stretch-mesh, center-bag). Sampling locations were chosen randomly each month from all possible sites that contained adequate depth for trawling (1.8–7.6 m) and seining (0.3–1.8 m). The sampling effort implemented within the areas used in this study were 3 trawls and 4 seines/month for the Myakka and 4-5 trawls and 10-12 seines/ month for the Caloosahatchee. The trawl was towed for 5 minutes at 0.6 $m \bullet s^{-1}$, providing a tow length of ~180 m. Trawl width averaged ~4 m, providing an approximate area of 720 m^2 sampled by a typical tow. The seine was deployed from a boat in a shallow arc parallel to shore and hauled directly along the shoreline. The two ends of the seine were pulled together, sampling an area of ~68 m^2.

During each sampling event, environmental parameters—including temperature (°C), salinity (ppt) and dissolved oxygen (mgl^{-1})—were profiled with a Hydrolab water quality datasonde (measurements taken at 0.2 m, 1.0 m if applicable, and at the bottom). Fishes and select invertebrates collected during each sample event were identified to the lowest practical taxonomic level (nomenclature for fishes follows Nelson et al. 2004), measured (standard length (SL) for fishes and carapace width (CW) for crabs), counted and released. Representative subsamples of organisms were retained for laboratory verification. For specific details on site selection and sampling technique refer to Idelberger and Greenwood (2005) and Idelberger et al. (2011).

Each sampled species was categorized into an ecological guild according to Elliot et al. (2007) and Nordlie (pers. comm.) (Table 2.S1 *Supplemental Material*): freshwater species (FW); estuarine species (ES) [i.e., estuarine resident—those that complete their

life history in the river and estuarine dependent—those that spawn at sea and recruit to rivers as juveniles], and marine migrants (MM) [i.e., species that spawn at sea and use estuarine and nearshore waters]. Based on primary dietary resources, species were then further classified according to trophic or feeding guild (e.g., Froese and Pauly 2009; Table 2S.1 *Supplemental Material*): primary consumer, diet composed largely of algae and detritus (>70%); secondary consumer, diet composed primarily of invertebrate species; tertiary consumer, diet composed of both fishes and invertebrates; and piscivore, diet composed primarily of fishes (> 80%). For the overall nekton community, and each ecological and trophic guild, density (individuals•100 m^{-2}), diversity (Shannon index, *H′*), richness and evenness (Pielou index, *J′*) was calculated for each unique sampling event (i.e., trawl and seine) from both dry and wet seasons in both estuaries. The Sorenson Similarity Index (C_s) was calculated to compare beta diversity (β) between seasons in each estuary. Open water species with extreme abundances that form large schools with patchy distributions (i.e., *Anchoa mitchilli* and *Membras martinica*; Clark and Warwick 2001) were excluded prior to the calculations of the community metrics (e.g., Tsou and Matheson 2002; see Table 2S.1 *Supplemental Material*).

Statistical analysis

In southwest Florida, many rivers are categorized as having the southern river flow pattern, i.e., a significant proportion of riverine annual flow (~60%) is concentrated in the wet season, which occurs during the months of June-September (Kelly and Gore 2008). In the case of the Caloosahatchee, a fundamental premise in our analysis is that the wet season is further exaggerated by altered discharges from Lake Okeechobee, while

the Myakka experiences a relatively natural hydrological cycle. For both rivers, data for all trawl and seines from dry and wet seasons were therefore grouped for the months of May–June (low flow) and August–September (high flow), respectively, from 2006, 2008 and 2009. Data from 2004 and 2005 were excluded from the analyses because of known hurricane effects to fish assemblages that occurred throughout Charlotte Harbor (Greenwood et al. 2006). Data from 2007 were excluded from the analyses because of severe drought that led to minimal differences in flow between dry and wet seasons in the rivers (see Fig. 2.2).

Each unique sampling event (i.e., a single trawl or seine) was considered as the sample unit for all analyses. To assess if the environmental parameters of the two estuaries differed, flow and environmental parameters (i.e., salinity, temperature, dissolved oxygen) recorded for each unique sampling event were compared by estuary (Myakka and Caloosahatchee), season (spring–dry and autumn–wet) and their interaction (estuary x season) using a two-way factorial analysis of variance (ANOVA). Two sets of analyses were then conducted for the trawl and seine nekton community data for both the Myakka and the Caloosahatchee. First, linear mixed-effect models were constructed to investigate the effects of altered freshwater flow on the nekton assemblages of the two estuaries, by comparing dry and wet seasons. Second, multivariate techniques were applied to investigate the differences in nekton community structure between the dry and wet seasons among estuaries.

Linear mixed-effect models, with year as the random effect, were applied to test for differences in the dependent variables (i.e., density, diversity, richness and evenness) between seasons in each estuary. This was based on the premise that we were testing for

the effects of altered flow on the nekton communities, not annual variation in the magnitude of flow. For each mixed-effects model, we applied orthogonal linear contrasts (*glht* in the multcomp package available in the statistical program R: R Development Core Team 2011) to compare dependent variables between seasons in each estuary. Separate analyses were conducted to test for differences in the dependent variables among nekton assemblages, ecological guilds and trophic guilds, for trawl and seine data.

To evaluate differences among the nekton communities of the Myakka and Caloosahatchee estuaries, a multivariate ANOVA based on dissimilarities (*adonis* function in R) was performed on density data across estuaries and seasons. To reduce the influence of rare species, only the twenty most abundant species collected from either estuary for trawl and seine data were included. Non-metric multidimensional scaling (NMDS; *metaMDS* function in R) ordination was used to graphically coordinate the patterns in community structure and composition among estuaries. Data from a Bray-Curtis similarity matrix were used to construct the ordination plots. NMDS data, reflecting dry and wet seasons within each estuary were fitted with 95% confidence ellipses to depict the distribution patterns of the season-estuary communities. In addition, environmental parameters (i.e., flow, salinity and DO) that had low correlation values (< 0.6) were log-transformed and fit to the NMDS (*envfit* function in R) to determine the influence of these variables on the distribution patterns of the season-estuary communities.

Prior to all analyses, environmental parameters were tested for normality using Shapiro-Wilk tests and quantile-quantile probability plots. Data were then log-transformed where appropriate. To reduce the influence of highly abundant species, the

density estimates for each species were square-root transformed. An examination of the probability plots of residuals from linear mixed-effect models indicated that models fit adequately, and quantile-quantile plots showed data to be generally described by normally distributed errors for all comparisons. All statistical analyses were performed in R 2.13.0 (R Development Core Team 2011) with a criterion for significance of $P < 0.05$ used for all comparisons. Diversity, richness and evenness estimates, and NMDS were performed using the *vegan* package (Oksanen et al. 2011) and linear mixed-effect models were fit using the *lme4* package (Bates and Maechler 2010).

RESULTS

Environmental parameters

Mean daily freshwater flow significantly increased while salinity, measured during both trawl and seine surveys significantly decreased in both estuaries, between dry and wet seasons (Table 2.1). As expected, the magnitude of flow in the Caloosahatchee was significantly greater during both seasons relative to the Myakka (Fig. 2.2). Water temperatures ranged between ~25 and 33°C with each estuary exhibiting a similar seasonal pattern from both trawl and seine surveys; a consistent temperature in the Caloosahatchee across survey periods, and an increase in water temperature during the wet season in the Myakka (Table 2.1). Dissolved oxygen exhibited a decrease during the wet, relative to the dry season in both estuaries for both sampling gear types (Table 2.1).

Nekton community: Trawl

A total of 5,162 individuals of 52 species were sampled from trawl surveys; 37 species from the Myakka and 43 species from the Caloosahatchee. The majority of these species were characterized as estuarine species (20 and 21 species) and secondary consumers (25 and 23 species) from the Myakka and Caloosahatchee, respectively (Table 2S.1 *Supplemental Material*). Nekton assemblages were more similar between seasons in the Caloosahatchee (22 common species; $C_s = 0.67$) relative to the Myakka (15 common species; $C_s = 0.54$).

For trawl data in the Myakka, linear mixed-effect models found that both mean nekton density (Fig. 2.3A) and mean nekton richness (Table 2.2) significantly increased during the wet season compared with the dry season. In contrast, in the Caloosahatchee there were no statistically significant trends for nekton density (Fig. 2.3C), diversity or richness (Table 2.2) among seasons although a trend of declining nekton density was observed.

In the Myakka, the density, diversity and richness of estuarine species significantly increased in the wet season, while there was no change in these metrics for freshwater and marine migrants between seasons (Table 2.2; Fig. 2.3B). During the wet season in the Caloosahatchee, there was a significant decrease in the density and richness of marine migrants and an increase in density and richness of freshwater species, but no observed effect on estuarine species (Table 2.2; Fig. 2.3D).

The density of secondary and tertiary consumers (Fig. 2.3C), and the diversity, richness and evenness of tertiary consumers (Table 2.2) increased in the Myakka during the wet season. Primary, secondary, and tertiary consumers of the Caloosahatchee showed no significant change in density (Fig. 2.3F) or diversity (Table 2.2) with

increased flow, but secondary consumers were observed at higher densities during the dry season (Fig. 2.3F). Results of Tukey contrasts from linear mixed-effect models are presented in Table 2S.2 in *Supplemental Material*.

Nekton community: Seine

A total of 33,105 individuals of 70 species were sampled from seine surveys; 49 species from the Myakka and 62 species from the Caloosahatchee (Table 2S.1 *Supplemental Material*). Similar to the trawl surveys, the majority of these species were characterized as estuarine species (25 and 28 species) and secondary consumers (26 and 34 species) from the Myakka and Caloosahatchee, respectively (Table 2S.1 *Supplemental Material*). In contrast to the trawl surveys, the seine surveys exhibited less similarity between the dry and wet seasons (Caloosahatchee, 28 common species, $C_s = 0.31$; Myakka, 20 species, $C_s = 0.29$).

For seine data, linear mixed effects models found no statistical change in nekton density for either the Myakka (Fig. 2.4A) or Caloosahatchee (Fig. 2.4C). For the Myakka, there was a significant increase in nekton diversity, richness and evenness (Table 2.2) between seasons indicating that a greater number of species, with more evenly distributed abundances were present following high flow.

In the Myakka, the diversity and richness of freshwater and estuarine species increased during the wet season (Table 2.2), even though no significant change in density was identified in either ecological guild (Fig. 2.4B). Similarly, in the Caloosahatchee, density did not significantly change for any of the ecological guilds between seasons (Fig. 2.4E), but there was a decrease in the diversity and richness of marine migrants and

a corresponding increase in richness of freshwater species during the wet season (Table 2.2). In terms of trophic guilds, in the Myakka, there was an increase in the density (Fig. 2.4C) and richness of primary consumers and the diversity, richness and evenness of secondary consumers during the wet season (Table 2.2). In the Caloosahatchee, tertiary consumers exhibited a significant decrease in all community metrics during the wet season (Table 2.2; Fig. 2.4F). Results of Tukey contrasts from linear mixed-effect models are presented in Table 2S.2 in *Supplemental Material*.

Comparisons of nekton communities between estuaries

MANOVA testing between the Myakka and the Caloosahatchee found significant differences in nekton density between estuaries (Trawl: $F_{1,82}$=1.835, R^2=0.02, P = 0.02; Seine: $F_{1,131}$ = 1.629, R^2 = 0.013, P = 0.0421), seasons (Trawl: $F_{1,82}$ = 3.863, R^2 = 0.044, P = 0.01; Seine: $F_{1,131}$ = 6.062, R^2 = 0.044, P = 0.005), and their interaction (Trawl: $F_{1,82}$ = 2.562, R^2 = 0.02, P = 0.01; Seine: $F_{1,131}$ = 1.710, R^2 = 0.013, P = 0.0389) and supported the relationships depicted in the NMDS plots.

The trawl assemblages of the Myakka during dry and wet seasons were different based on non-overlapping 95% confidence ellipses (Fig. 2.5A). In contrast, the 95% confidence ellipses of the dry and wet seasonal trawl assemblages in the Caloosahatchee overlapped (Fig. 2.5A), in agreement with the linear mixed model analysis. In contrast, the confidence ellipses of the dry and wet seine assemblages of both estuaries showed separation (Fig. 2.5B). Considering vector length, salinity (best correlated with axis 1, r = -0.827, R^2 = 0.242, P = 0.001) and flow (best correlated with axis 2, r = -0.644, R^2 = 0.084, P = 0.037) were the most important environmental parameters influencing the

trawl assemblages of both the Myakka and the Caloosahatchee (Fig. 2.5A). Similarly, salinity and flow (best correlated with axis 2, salinity: r = -0.917, R^2 = 0.171, P = 0.001 and flow: r = 0.884, R^2 = 0.094, P = 0.005) exhibited the greatest influence on the seine assemblages of both rivers (Fig. 2.5B). The dry-season trawl assemblages of the Myakka estuary clearly separated from the dry-season trawl assemblage of the Caloosahatchee, whereas the seine data showed overlap between the two, and in the case of the Myakka, were positively associated with salinity (Fig. 2.5A). The wet-season assemblages of the Caloosahatchee were positively associated with flow (Fig. 2.5A, 2.5B).

DISCUSSION

Given the extent of current hydrological alteration to riverine systems, understanding the effects of altered high-flow disturbance on estuarine nekton assemblage structure is critical for maintaining highly productive estuarine habitats worldwide. Our comparison of seasonal nekton assemblage dynamics in two estuaries with different hydrological patterns suggests that human altered high-flow resulted in a loss of seasonal variability in community structure. In the Myakka Estuary where the hydrology is more natural, there were clear changes in nekton community metrics between dry and wet seasons. These were represented by a marked increase in the density and species richness of larger-bodied deeper water fishes (i.e., trawl sampled) and increased diversity, species richness and evenness (but not density) of small-bodied shoreline fishes (i.e., seine sampled). These trends are what would be expected in a natural system (Greenwood et al. 2007; Sheaves et al. 2010). In the modified Caloosahatchee Estuary, these seasonal trends were not apparent. In contrast, declines in

diversity and species richness of the nekton assemblages were observed with altered high-flow, although the response was more subtle than predicted (i.e., lack of significant negative trends). These findings are consistent with the premise that high level disturbance in estuaries result in less diverse and more simplified communities (Cross et al. 2011) and importantly we identify that this type of disturbance was most influential on estuarine dependent species.

Unraveling the influences of altered freshwater inflow patterns on nekton communities presents a challenge. The difficulty arises from distinguishing the direct effects of altered flow regimes from indirect effects associated with land-use change that often accompany urbanization and water resource development. Greater urbanization in the Caloosahatchee relative to the Myakka River is exemplified by the presence of major cities, artificial connections to adjacent inland systems, and greater amount of hardened shoreline (70% *vs*. 40%). Fish assemblages respond to urbanization gradients with sensitive fishes disappearing as urbanization increases and heterogeneity of habitat decreases (Pease 1999; Morgan and Cushman 2005; Walters et al. 2003). To isolate if altered high flow is the factor driving nekton assemblage differences it is therefore necessary to identify cross comparison reference points (Mayer and Galatowitsch 2001; Tsou and Matheson 2002). In the dry season, diversity, richness and evenness estimates for trawl assemblages were similar between the two estuaries, and to a lesser degree, between the seine assemblages. The similarity between these defined reference points between estuaries during the dry season provides confidence that altered flow was the likely cause for divergence of nekton community metrics that occurred during the wet season. Moreover, similar trends in fish abundance and assemblage composition have

been observed in proximate estuaries (Kraus and Secor 2004; Idelberger and Greenwood 2005), lending further support to our cross-system comparisons through defined reference points.

Seasonal changes observed in nekton community metrics in the natural flow, Myakka Estuary were largely driven by estuarine species, a pattern not observed in the altered-flow Caloosahatchee. This provides important insights into altered river flow on a critically important ecological guild. Seasonal variation in estuarine fish assemblages is strongly influenced by biological factors including the spawning and recruitment patterns of the individual species within or outside the estuary (King et al. 2003; Sheaves et al. 2010). Additionally, the physical and chemical qualities of freshwater are known to be important drivers of species migratory processes into estuaries (Champalbert and Koutsikopoulos 1995; Barbin 1998). Idelberger and Greenwood (2005), observed recruitment of the majority of estuarine fish species, those that spawn in the estuary and recruit to rivers as juveniles (e.g., *Bairdiella. chrysoura, Cynoscion arenarius*), into the Myakka between May/June and September/October, potentially suggesting these species take advantage of such factors as abundant food resources and shelter (in the form of enhanced turbidity and access to complex shoreline habitats) associated with increased river discharge. Moreover, Purtlebaugh and Allen (2010) not only demonstrated a positive relationship between relative abundance and river flow for juveniles of estuarine species (i.e., age-0 *C. nebulosus* and *C. arenarius)* in the lower Suwannee River, but that these fishes experienced increased growth rates during the wet season (i.e., period of increased flow). Our cross-system comparison lends support to the importance of natural variation in river flow to estuarine ecosystems and as a consequence, the importance of

natural flow variation for maintaining fisheries stocks of ecologically and recreationally important estuarine species.

High flow events are known to impact estuarine ecosystems causing, for example, declines in the catches of estuarine and coastal fisheries (Drinkwater and Frank 1994), and decreases in abundances of estuarine fishes and invertebrates (Costa et al. 2007; McLeod and Wing 2008). The significant declines observed in the nekton community metrics with high flow extremes in the Caloosahatchee Estuary would therefore be expected. Although declines in community metrics in the Caloosahatchee did occur, they were non-significant and to a lesser extent than expected. These results demonstrate that extreme flow events likely create a physical barrier to recruitment of fishes into estuaries (Purtlebaugh and Allen 2010 and references therein). The lack of change of community metrics suggests that the magnitude and duration of the high-flows in the Caloosahatchee may be beyond optimum for supporting natural system variability between seasons, however not great enough to result in significant decreases in community metrics, which are apparent after major freshwater inflow events associated with hurricanes for example (Greenwood et al. 2006, 2007; Stevens et al. 2006).

The geomorphology of a river system is also an important factor to consider when examining the effects of altered flow on nekton community assemblages (Visintainer et al. 2006; Allen et al. 2007). It is possible that the effect of high flow extremes on nekton community structure of the Caloosahatchee River were dampened through the geomorphologic characteristics creating a balance between individuals leaving and entering the system. As the Caloosahatchee River descends from the Franklin Lock, it abruptly widens to 2.5 km and remains wide for ~30 km to its mouth. This relatively long

mixing zone in the Caloosahatchee, combined with the exaggerated high-flow event, could result in clearly defined isohalines within the estuarine reach of the river that would not necessarily be so apparent under natural flow regimes. With distinct isohalines the distribution of ecological guilds and their centers of abundance would be expected to shift, e.g., freshwater species move downstream with freshwater flow (Kimmerer 2002; Greenwood et al. 2007). Such distributional responses following high inflow events could account for the increases in density of the trawl-sampled freshwater guild in the estuarine portion of the Caloosahatchee coincident with decreases in the density of the marine guild, as they were displaced downstream and potentially out of the system. This overall effect would therefore be interpreted as no change in the nekton assemblage, when indeed shifts did occur. Given the different dynamics in estuaries and the balance between marine and freshwater guilds, it is important to reiterate that there was no seasonal change in the community metrics of the estuarine species, which conflicts with the results of the natural dynamics of tidal rivers.

In terms of the trophic guilds, primary consumers did not exhibit marked changes in community metrics, with the exception of the high-flow altered Caloosahatchee — a likely result of the high flow event driving a movement of freshwater primary consumers into the estuarine component of the system. Changes in the trophic guilds of the Myakka were largely driven by increases in secondary and tertiary trophic guilds during the wet season. These trophic guilds were composed of predominantly estuarine species (e.g., *Bairdiella chrysoura* and *C. arenarius*) and the increase in abundance is likely a result of natural recruitment dynamics in tidal rivers (Greenwood et al. 2007; Sheaves et al. 2010). In contrast, the Caloosahatchee exhibited a marked decrease in density, diversity and

richness of tertiary consumers, specifically in the seine assemblage with high-flow. This decline likely reflects the mobile nature of the tertiary consumers sampled here and the fact that they are marine species (e.g., belonids and sparids); species that are unable to remain in these extreme physicochemical conditions and are therefore forced to move out of the system. The lack of tertiary consumers in the Caloosahatchee may have important implications for community structure, particularly high trophic level species, such as the bull shark, *Carcharhinus leucas*, that is dependent on estuarine habitats during the first years of life. Layman et al. (2007) demonstrated a collapse in the trophic niche of the grey snapper (*Lutjanus griseus*), a top predator in Bahamian tidal creeks, as a consequence of reduced prey diversity in an anthropogenically-fragmented tidal creek. Reduction in diversity and richness of a particular trophic guild within a community can result in a loss and/or overall homogenization of energy flow pathways and ultimately a less stable and more simplified food web structure (Layman et al. 2007).

Management implications

Modified flow regimes are known to diminish the abundance of fish and invertebrates in estuarine and coastal systems (Gillson 2011). Understanding how other estuarine taxa (i.e., macro-invertebrates and top-level predators) and processes (e.g., primary and secondary production) respond to flow variability could enhance our ability to modify flow so as to increase the ecological integrity of altered systems. Our analysis of the composition of nekton assemblages between low *vs.* high flow periods were based on overall trends in assemblage structure between contrasting seasons and the use of ecological and trophic guilds to provide context on the distribution and structure of these

assemblages faced with variable hydrological conditions. For that reason, the present study focused on the assemblage-level response to altered high-flow and we recognize that other attributes of these assemblages (e.g., relative change in species biomass) may be important for understanding full effects of altered high-flow.

In order to maintain and restore the integrity of any ecosystem requires that conservation and management actions be firmly grounded in scientific understanding. However, current management approaches often fail to recognize the scientific principle that the integrity of flowing water systems depends largely on their natural dynamic character; as a result, these methods frequently prevent successful maintenance. Management strategies of flow-altered rivers have focused on provision of minimal flows intended to prevent deleterious biological impacts of frequent or extreme water depletions or additions (Poff et al. 1997). Pulse release, for example has proven positive in other estuaries (Odum et al. 1995; Day et al. 2009; Piazza and Le Peyre 2011). Managers of the Caloosahatchee River recognize these strategies for minimizing prolonged and excessive low and high flows and have attempted to mitigate discharge effects by implemented policies of minimal flow and pulsed release (Barnes 2005). However policies of pulse release were not in practice at the time of this study and as a consequence our results characterize the effects of extreme high flow events.

Given that studies monitoring altered flow focus on different biological aspects (i.e., taxonomic identities *vs.* overall community) and the often difficult task of standardizing data across multiple systems that differ in size and scale of urbanization, there is an increasing demand to devise classification schemes or strategies which best represent the structure and functioning of biological communities, that can be comparable

both regionally and on a global basis (Whitfield and Elliott 2002). Applying ecological and trophic guilds (sensu Elliott et al. 2007), as community classifications provides a context in which to draw broad distributional and structural community comparisons in response to altered flow. By indirectly comparing across systems, we provide a benchmark as to what we expect to occur seasonally in natural systems and are able to observe any departures from these reference conditions. To advance the management of altered riverine systems, a management framework for monitoring these systems could be developed which builds on the current analysis through integrating the ecological and trophic measures, i.e., examining ecological guilds within trophic guilds (sense Elliott et al. 2007). Through this type of standardized monitoring framework, managers would have transparent guidelines with which to monitor the effects of flow alteration on estuarine community structure and to modify management plans to mitigate against deleterious effects.

Estuaries are complex, composed of species that have variable biotic and abiotic requirements. Susceptibility to altered high-flow varied in this study, suggesting that some ecological (e.g., estuarine species) and trophic guilds (e.g., tertiary consumers) that exhibited a marked negative response, might be good indicators of the potential impacts associated with extreme flow alteration. Understanding these changes in nekton community assemblages are therefore of particular importance in light of predictions of global climate change models that show a future characterized by increased frequency and severity of abiotic disturbances (Easterling et al. 2000; Meehl et al. 2000), particularly rainfall events (Wolock and McCabe 1999). What remains to be better understood is how management of the timing and magnitude of flow interact with

community composition and how these effects can alter energy flow and food web interactions of estuarine-associated species (Kimmerer 2002; Piazza and La Peyre 2011).

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Table 2.1 Flow[1] and environmental parameters[2] measured from each sampling event in the Myakka and Caloosahatchee estuaries during the dry (spring—May and June) and wet (autumn—August and September) seasons of 2006, 2008 and 2009. Data are mean (± SE) and range. Bold values reflect significant differences at $\alpha = 0.05$.

TRAWL							
	Myakka		Caloosahatchee		$F_{1,72}$		
	Dry (n = 17)	Wet (n = 18)	Dry (n = 20)	Wet (n = 20)	Estuary	Season	Estuary x Season
Flow (m^3s^{-1})	1.3 ± 0.1 (0.04–3.1)	13.5 ± 0.4 (7.1–28.6)	25.9 ± 0.5 (18.3–46.1)	131.0 ± 1.5 (77.5–165.1)	**300.328** ***P* = 0.000**	**169.745** ***P* = 0.000**	**11.892** ***P* = 0.000**
Salinity (ppt)	27.8 ± 0.1 (24.4–32.9)	5.9 ± 0.2 (0.2–10.5)	21.1 ± 0.4 (14.8–33.2)	2.7 ± 0.2 (0.1–15.5)	**20.799** ***P* = 0.000**	**69.286** ***P* = 0.000**	0.703 *P* = 0.404
Temperature (°C)	28.7 ± 0.1 (25.5–30.9)	29.7 ± 0.03 (28.7–30.6)	29.5 ± 0.1 (27.4–32.0)	29.1 ± 0.1 (26.3–33.3)	0.007 *P* = 0.933	0.3669 *P* = 0.546	2.031 *P* = 0.057
DO (mgl^{-1})	6.3 ± 0.1 (5.3–7.5)	5.9 ± 0.1 (4.0–6.7)	6.1 ± 0.1 (2.8–8.6)	5.1 ± 0.1 (2.5–7.0)	0.231 *P* = 0.632	0.463 *P* = 0.712	0.272 *P* = 0.604
SEINE							
	Myakka		Caloosahatchee		$F_{1,124}$		
	Dry (n = 24)	Wet (n = 24)	Dry (n = 42)	Wet (n = 42)	Estuary	Season	Estuary x Season
Flow (m^3s^{-1})	1.3 ± 1.3 (0.0–3.3)	12.6 ± 1.8 (7.7–24.7)	23.8 ± 2.4 (0.0–42.3)	148.7 ± 22.9 (9.1–401.0)	**16.785** ***P* = 0.000**	**30.382** ***P* = 0.000**	**8.425** ***P* = 0.004**
Salinity (ppt)	24.8 ± 0.5 (17.7–28.4)	2.9 ± 0.6 (0.2–10.3)	15.0 ± 1.4 (0.9–35.7)	2.8 ± 0.6 (0.1–20.7)	**7.804** ***P* = 0.006**	**154.854** ***P* = 0.000**	0.265 *P* = 0.608
Temperature (°C)	28.7 ± 0.4 (26.0–31.0)	30.0 ± 0.2 (28.5–32.0)	29.4 ± 0.2 (27.2–32.1)	29.0 ± 0.2 (26.7–32.3)	0.661 *P* = 0.418	0.6961 *P* = 0.408	1.518 *P* = 0.234
DO (mgl^{-1})	6.6 ± 0.2 (5.0–7.7)	5.2 ± 0.3 (3.7–7.8)	6.5 ± 0.2 (4.4–10.5)	5.8 ± 0.3 (2.5–11)	0.890 *P* = 0.347	**18.577** ***P* = 0.000**	1.205 *P* = 0.274

[1] Flow data was obtained from the South Florida Water Management District for the Caloosahatchee and the USGS for the Myakka, and represents the mean daily flow recorded for each unique sampling event combined over the complete sampling period; 2008-2009 for the Myakka; 2006, 2008 and 2009 for the Caloosahatchee.

[2]Environmental parameters are mean data collected from all trawls and seines within each estuary for each sampling period combined from 2006, 2008 and 2009.

Table 2.2 Community metrics estimated (mean ± SD) from trawl and seine sampling events for the nekton assemblages, and the ecological and trophic guilds, sampled from the Myakka and the Caloosahatchee estuaries during the dry and wet seasons.[1] Bold values reflect significant differences at $\alpha = 0.05$ (see Table S2.2 *Supplemental Material* for results of the analyses).

			TRAWL				SEINE			
			Myakka (*n* = 18)		Caloosahatchee (*n* = 20)		Myakka (*n* = 24)		Caloosahatchee (*n*= 42)	
			Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Nekton		Diversity	1.3 ± 0.6	1.3 ± 0.5	1.2 ± 0.5	1.0 ± 0.5	**0.8 ± 0.4**	**1.3 ± 0.4**	1.0 ± 0.6	0.8 ± 0.5
Community		Richness	**5.2 ± 3.1**	**7.4 ± 2.7**	5.2 ± 2.5	5.0 ± 2.8	**5.1 ± 2.8**	**7.1 ± 3.0**	6.1 ± 3.7	5.8 ± 3.5
		Evenness	0.8 ± 0.2	0.6 ± 0.3	0.8 ± 0.2	0.6 ± 0.3	**0.4 ± 0.3**	**0.7 ± 0.2**	0.6 ± 0.3	0.5 ± 0.3
	Freshwater species	Diversity	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	**0.0 ± 0.0**	**0.1 ± 0.2**	0.0 ± 0.0	0.1 ± 0.3
		Richness	0.0 ± 0.0	0.0 ± 0.0	**0.0 ± 0.0**	**0.3 ± 0.6**	**0.1 ± 0.2**	**0.3 ± 0.8**	**0.1 ± 0.2**	**0.6 ± 1.1**
		Evenness	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.2	0.01 ± 0.0	0.1 ± 0.3
	Estuarine species	Diversity	**0.7 ± 0.6**	**1.0 ± 0.5**	0.9 ± 0.5	0.8 ± 0.5	**0.4 ± 0.4**	**0.8 ± 0.5**	0.4 ± 0.4	0.4 ± 0.4
Ecological		Richness	**2.8 ± 1.7**	**5.2 ± 1.7**	3.7 ± 1.7	3.5 ± 1.7	**2.7 ± 1.7**	**4.1 ± 1.9**	2.8 ± 2.4	3.2 ± 2.0
Guild		Evenness	0.5 ± 0.5	0.6 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	**0.3 ± 0.3**	**0.5 ± 0.3**	0.3 ± 0.3	0.3 ± 0.3
	Marine migrants	Diversity	0.7 ± 0.5	0.5 ± 0.5	0.5 ± 0.4	0.2 ± 0.4	0.5 ± 0.4	0.7 ± 0.4	**0.7 ± 0.5**	**0.5 ± 0.4**
		Richness	2.5 ± 1.9	2.2 ± 1.8	**2.0 ± 1.5**	**1.0 ± 1.3**	2.6 ± 1.5	2.7 ± 1.3	**3.2 ± 2.0**	**2.2 ± 1.8**
		Evenness	0.6 ± 0.4	0.4 ± 0.4	**0.5 ± 0.4**	**0.2 ± 0.3**	**0.5 ± 0.4**	**0.7 ± 0.3**	0.6 ± 0.4	0.4 ± 0.4
	Primary consumers	Diversity	0.0 ± 0.0	0.0 ± 0.0	0.04 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.03 ± 0.1	0.01 ± 0.1
		Richness	0.6 ± 0.5	0.7 ± 0.5	0.5 ± 0.7	0.3 ± 0.5	**0.2 ± 0.4**	**0.8 ± 0.8**	0.5 ± 0.7	0.3 ± 0.5
		Evenness	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.03 ± 0.1	0.01 ± 0.1
	Secondary consumers	Diversity	1.0 ± 0.7	0.8 ± 0.5	0.8 ± 0.4	0.7 ± 0.5	**0.6 ± 0.4**	**1.1 ± 0.4**	0.8 ± 0.5	0.7 ± 0.4
Trophic		Richness	3.9 ± 2.8	3.8 ± 2.0	3.2 ± 1.7	3.0 ± 1.1	**3.7 ± 1.9**	**5.1 ± 2.0**	4.2 ± 2.5	4.6 ± 2.6
Guild		Evenness	0.7 ± 0.4	0.6 ± 0.3	0.7 ± 0.3	0.5 ± 0.4	**0.5 ± 0.3**	**0.7 ± 0.2**	0.6 ± 0.3	0.5 ± 0.3
	Tertiary consumers	Diversity	**0.1 ± 0.4**	**0.6 ± 0.4**	0.3 ± 0.4	0.3 ± 0.4	0.3 ± 0.4	0.3 ± 0.4	**0.4 ± 0.4**	**0.2 ± 0.4**
		Richness	**0.7 ± 1.0**	**2.9 ± 1.2**	1.5 ± 1.3	1.7 ± 1.5	1.4 ± 1.3	1.3 ± 1.2	**1.7 ± 1.1**	**0.9 ± 1.1**
		Evenness	**0.1 ± 0.3**	**0.6 ± 0.3**	0.4 ± 0.4	0.3 ± 0.4	0.3 ± 0.4	0.3 ± 0.4	**0.4 ± 0.4**	**0.2 ± 0.4**

[1] For the Caloosahatchee, values are 2006 and 2009 for the dry season, and 2006 and 2008 for the wet season. For the Myakka, values are 2006, 2008 and 2009.

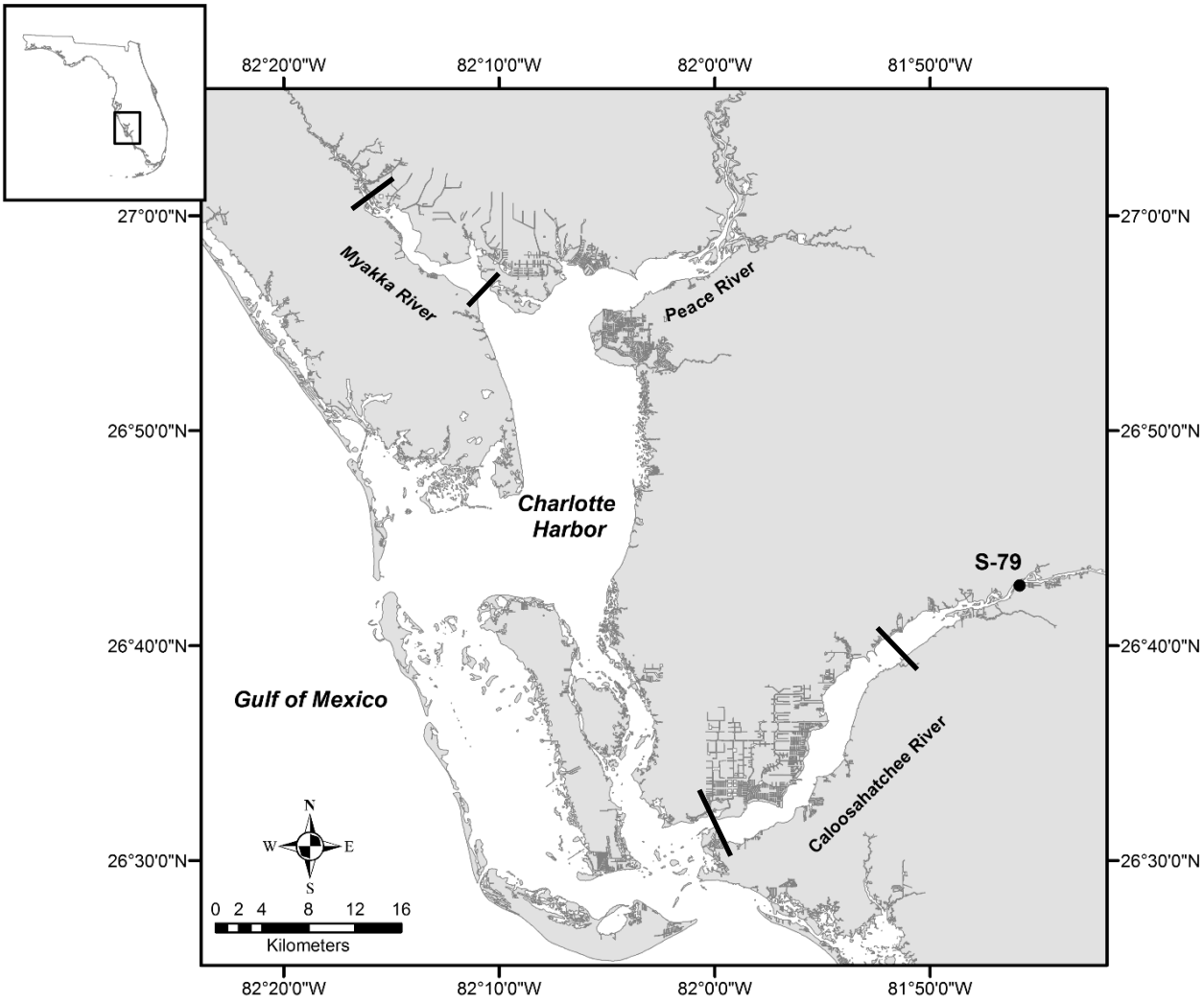

Figure 2.1 Map of the study sites showing the estuarine reaches of the Myakka and Caloosahatchee Rivers with respect to the south western coast of Florida.

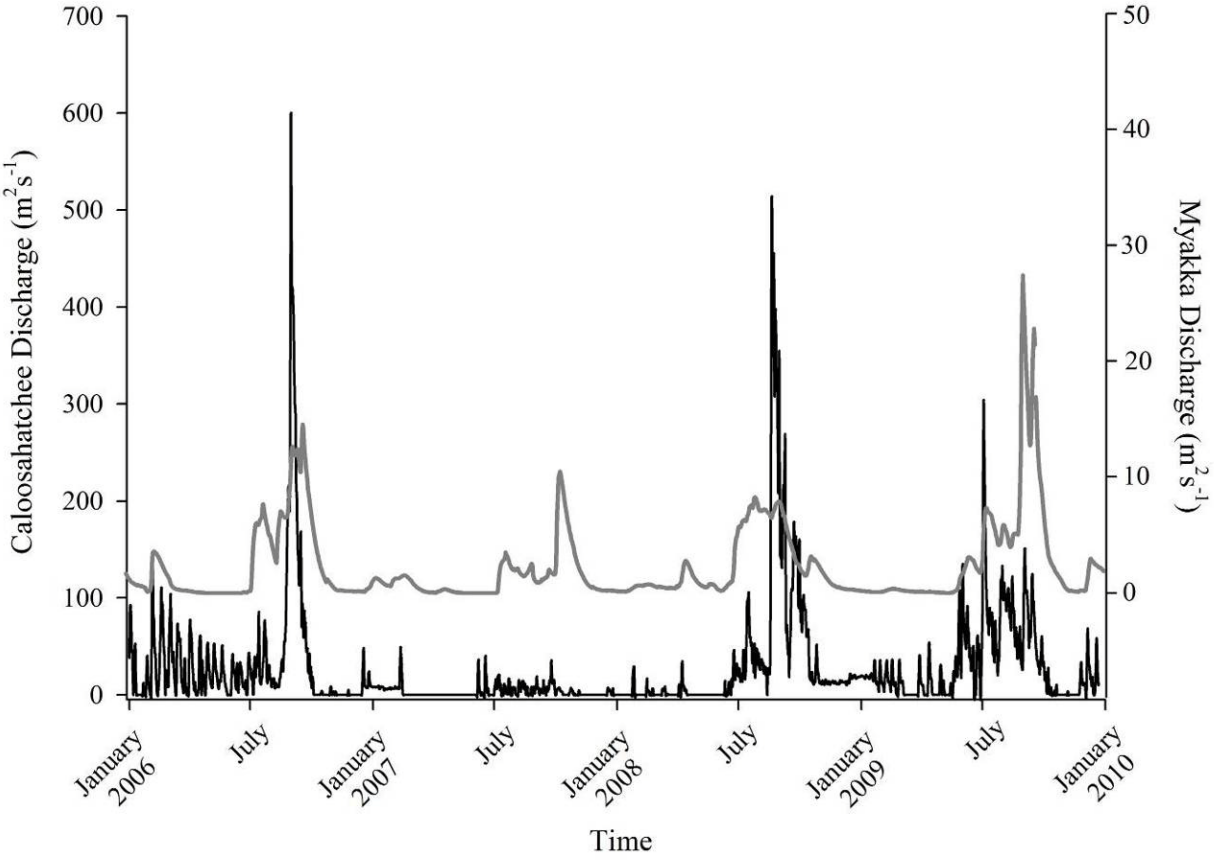

Figure 2.2 Mean daily river discharge recorded in the Caloosahatchee (black) and the Myakka (gray) from 2006 to 2010. River discharge data were obtained from the U.S. Geological Survey web site (http://water.usgs.gov/data.html) for the Myakka River at Myakka River near Sarasota (Station 02298830), and from the South Florida Water Management District web site (http://my.sfwmd.gov) for the Caloosahatchee River at the Cape Coral Bridge (Station CCORAL).

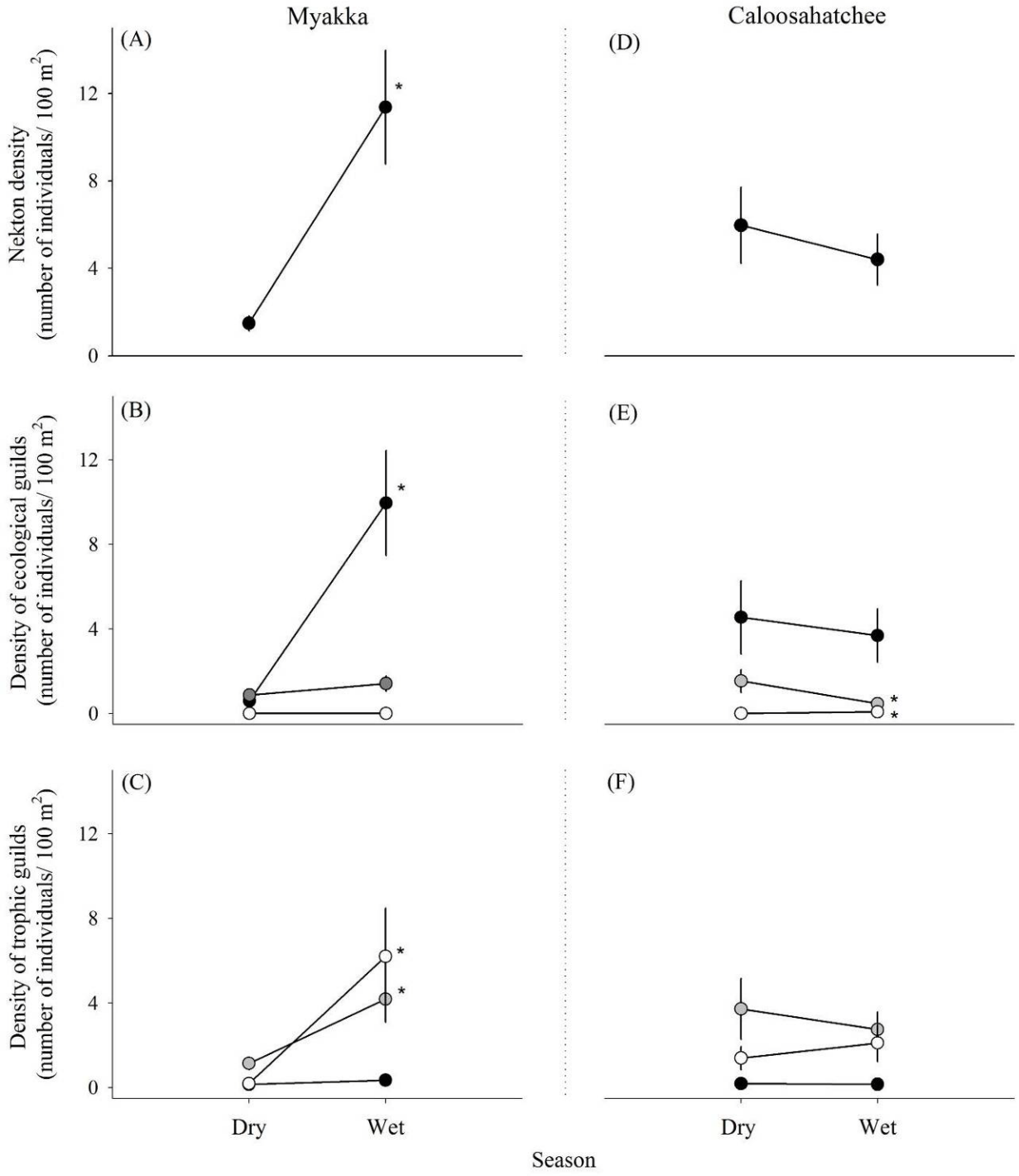

Figure 2.3 (A), (D) Nekton assemblage, (B), (E) ecological guild (estuarine species, black points; marine migrant species, gray points; freshwater species, white points) and (C), (F) trophic guild density from trawl sampling (primary consumers, black points; secondary consumers, gray points; tertiary consumers, white points) against season (data are mean density ± SE). Asterisks (*) indicates significant differences between dry and wet season at $\alpha = 0.05$.

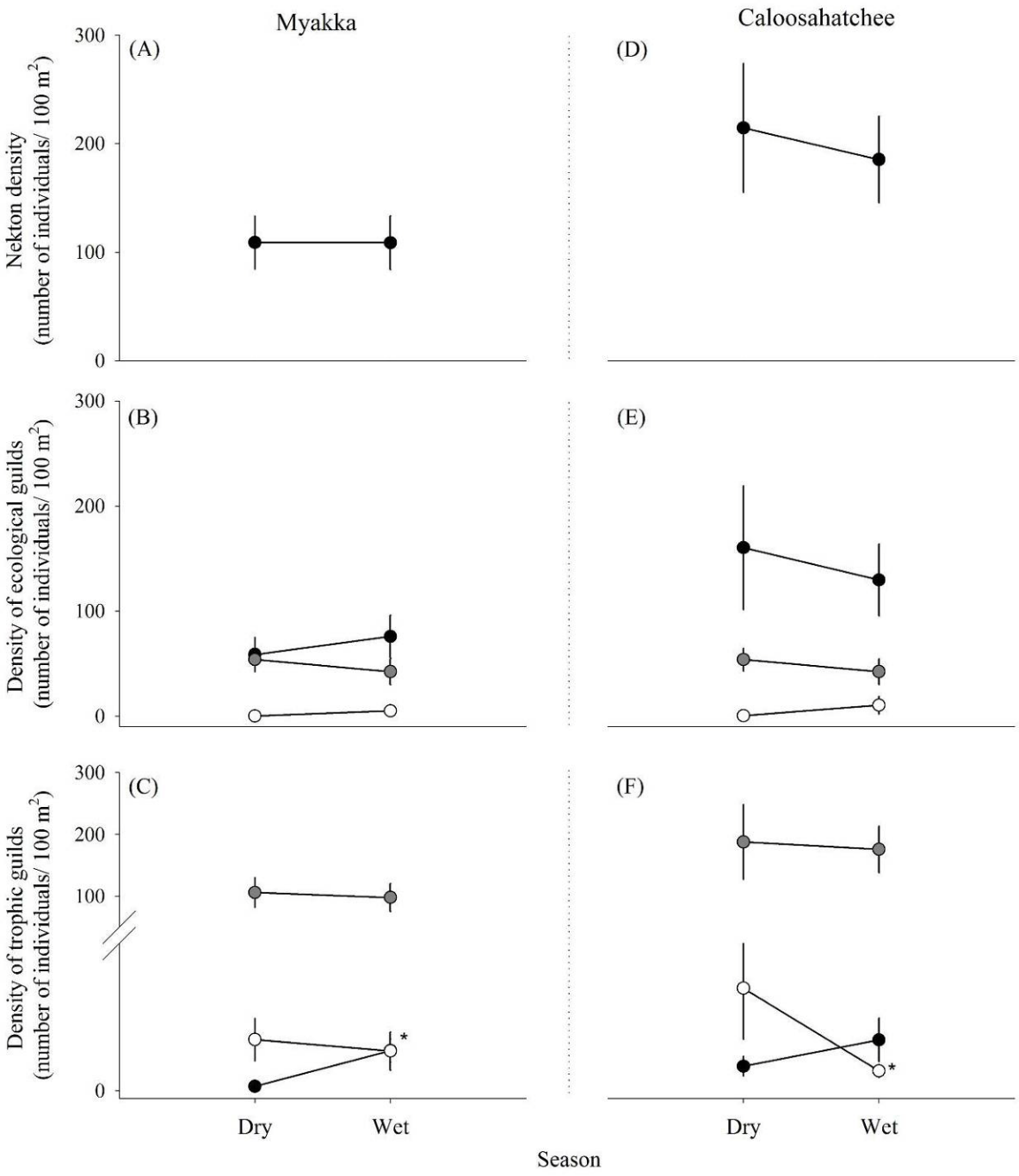

Figure 2.4 (A), (D) Nekton assemblage, (B), (E) ecological guild (estuarine species, black points; marine migrant species, gray points; freshwater species, white points) and (C), (F) trophic guild density from seine sampling (primary consumers, black points; secondary consumers, gray points; tertiary consumers, white points) against season (data are mean density ± SE). Asterisks (*) indicates significant differences between dry and wet season at $\alpha = 0.05$.

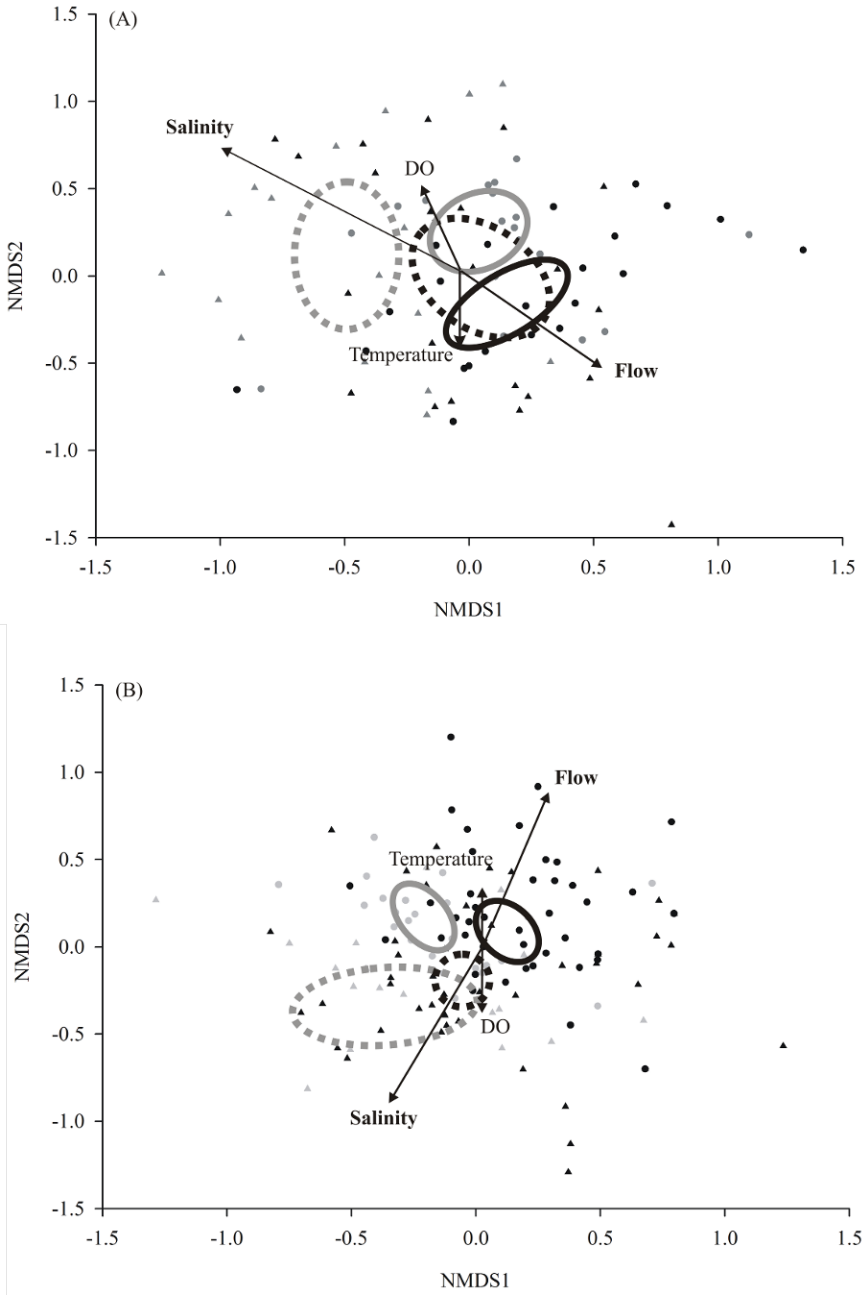

Figure 2.5 Nonmetric multi-dimensional scaling (NMDS) depicting assemblage differences between the Myakka (dry: gray triangles; wet: gray circles) and Caloosahatchee (dry: black triangles; wet: black circles) estuaries. Data are density estimates of species collected via (A) trawl (stress: 0.12) and (B) seine (stress: 0.14), fitted with 95% confidence interval ellipses to represent the season-estuary differences. Strength of the environmental parameters is indicated in bold. Dotted lines represent the dry season and solid lines represent the wet season.

SUPPLEMENTAL MATERIAL

Table 2S.1 Summary of species and total abundance collected from trawl and seine surveys from the Myakka (MR; *n* = ~6 trawls and ~4 seines each season) and Caloosahatchee (CR; *n* = ~5 trawls and ~10 seines each season) estuaries during the dry and wet seasons of 2006, 2008 and 2009. Species are categorized using ecological guild (EG) and trophic guild (TG) designations[1].

Family	Species	EG	TG	TRAWL				SEINE			
				MR		CR		MR		CR	
				Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Achiridae	*Achirus lineatus*	ES	TC	0	0	2	1	1	1	0	2
	Trinectes maculatus	ES	SC	27	335	92	435	1	30	8	5
Ariidae	*Ariopsis felis*	ES	TC	1	81	47	167	0	0	0	2
	Bagre marinus	ES	TC	0	16	1	6	0	0	0	0
Atherinopsidae	*Menidia* spp.	ES	SC	0	0	1	0	546	838	4,080	3,428
	Labidesthes sicculus	FW	SC	0	0	0	0	0	0	0	1
	Membras martinica	FW	SC	0	0	0	0	1,086	0	60	2
Batrachoididae	*Opsanus beta*	ES	TC	6	0	1	2	0	0	0	0
Belonidae	*Strongylura marina*	MM	TC	0	0	0	0	0	0	2	0
	Strongylura notata	MM	TC	0	0	0	0	22	3	102	5
	Strongylura spp.	MM	TC	0	0	0	0	1	0	46	3
	Strongylura timucu	MM	TC	0	0	0	0	4	0	14	0
Bothidae	*Ancylopsetta quadrocellata*	MM	SC	0	0	1	0	0	0	0	0
Carangidae	*Caranx hippos*	MM	TC	0	0	0	1	0	0	0	2
	Chloroscombrus chrysurus	MM	TC	0	0	0	3	0	0	0	0
	Oligoplites saurus	ES	TC	0	0	0	0	60	11	31	23
	Trachinotus falcatus	MM	TC	0	0	0	0	0	0	5	0
Centrarchidae	*Lepomis macrochirus*	FW	SC	0	0	0	0	0	0	0	273
	Lepomis microlophus	FW	SC	0	0	0	0	0	0	0	1
	Lepomis spp.	FW	SC	0	0	0	0	0	0	0	7
Centropomidae	*Centropomus undecimalis*	ES	TC	0	0	0	0	0	3	3	2
Cichlidae	*Hemichromis letourneuxi*	FW	SC	0	0	0	0	0	3	0	0
	Cichlasoma urophthalmus	FW	SC	0	0	0	0	0	0	7	0
	Oreochromis aureus	ES	PC	0	0	0	0	0	0	2	0
	Tilapia mariae	ES	PC	0	0	0	0	0	0	0	9
Clupeidae	*Dorosoma petenense*	FW	PC	0	0	0	1	0	11	0	6
	Harengula jaguana	MM	SC	0	0	0	0	1	0	0	8
	Opisthonema oglinum	ES	SC	0	0	0	0	24	0	0	216
	Brevoortia spp.	MM	PC	0	0	0	0	0	12	0	0
Cynoglossidae	*Symphurus plagiusa*	MM	SC	3	10	0	0	0	0	3	0
Cyprinodontidae	*Cyprinodon variegatus*	ES	SC	0	0	0	0	0	2	5	0
Dasyatidae	*Dasyatis sabina*	ES	SC	2	6	8	1	19	0	1	0
Diodontidae	*Chilomycterus schoepfii*	MM	SC	1	0	0	0	0	0	0	0

Elopidae	*Elops saurus*	MM	TC	1	0	19	0	0	0	25	0
Engraulidae	*Anchoa hepsetus*	ES	SC	0	0	3	0	79	3	58	9
	Anchoa mitchilli	ES	SC	0	348	1,110	153	3,654	505	6,020	6,404
Ephippidae	*Chaetodipterus faber*	MM	SC	3	5	0	0	0	0	0	0
Fundulidae	*Adinia xenica*	ES	PC	0	0	0	0	0	4	0	0
	Fundulus grandis	ES	SC	0	0	0	0	0	23	0	0
	Fundulus similis	ES	SC	0	0	0	0	1	42	0	0
	Fundulus seminolis	FW	SC	0	0	0	0	0	0	0	1
	Lucania parva	ES	SC	0	0	0	0	1	0	252	55
Gerreidae	*Eucinostomus gula*	MM	SC	5	4	18	33	24	3	157	12
	Eucinostomus harengulus	MM	SC	0	12	14	63	206	128	557	95
	Eucinostomus spp.	MM	SC	12	2	7	10	463	213	385	673
	Eugerres plumieri	ES	SC	0	54	1	75	0	204	16	118
Gobiesocidae	*Gobiesox strumosus*	ES	SC	1	0	0	0	0	0	1	0
Gobiidae	*Bathygobius soporator*	ES	SC	0	0	0	1	0	0	0	0
	Gobionellus oceanicus	ES	PC	0	0	1	0	0	0	0	0
	Gobiosoma bosc	ES	SC	0	0	0	2	0	1	8	15
	Gobiosoma robustum	ES	SC	2	0	0	0	1	0	3	0
	Gobiosoma spp.	ES	SC	6	0	0	0	1	4	11	35
	Microgobius gulosus	ES	SC	8	4	22	8	110	46	143	168
	Microgobius thalassinus	ES	SC	1	0	1	0	0	0	0	0
Haemulidae	*Orthopristis chrysoptera*	MM	SC	27	0	60	0	0	0	1	0
Hemiraamphidae	*Hyporhamphus* spp.	MM	SC	0	0	0	0	0	0	3	0
Ictaluridae	*Ameiurus catus*	FW	TC	0	0	0	7	0	0	0	0
	Ameiurus natalis	FW	TC	0	0	0	1	0	0	0	0
	Ictalurus punctatus	FW	SC	0	0	0	6	0	0	0	0
Lutjanidae	*Lutjanus griseus*	MM	TC	1	0	0	2	0	1	1	2
Monacanthidae	*Stephanolepis hispidus*	MM	SC	1	0	0	0	0	0	0	0
Mugilidae	*Mugil cephalus*	MM	PC	0	0	0	0	1	12	65	6
	Mugil curema	MM	PC	0	0	0	0	0	0	4	0
	Mugil gyrans	MM	PC	0	0	0	0	6	0	4	0
Paralichthyidae	*Paralichthys albigutta*	MM	SC	0	0	3	1	0	0	0	0
Penaeidae	*Farfantepenaeus duorarum*	MM	PC	20	47	31	27	2	22	22	179
Poeciliidae	*Gambusia holbrooki*	FW	PC	0	0	0	0	1	41	0	2
	Poecilia latipinna	FW	PC	0	0	0	0	0	26	0	2
Portunidae	*Callinectes sapidus*	ES	SC	10	15	50	47	1	1	25	8
Sciaenidae	*Bairdiella chrysoura*	ES	TC	6	332	22	135	0	41	216	4
	Cynoscion arenarius	ES	TC	14	447	132	62	6	12	2	3
	Cynoscion nebulosus	ES	TC	0	4	4	1	5	12	31	25
	Leiostomus xanthurus	MM	SC	0	1	97	0	3	0	2	0
	Menticirrhus americanus	MM	SC	14	107	19	6	6	0	46	0
	Micropogonias undulatus	MM	TC	0	0	1	0	0	0	0	0
	Sciaenops ocellatus	ES	TC	0	0	0	0	1	0	0	0

Sparidae	*Archosargus probatocephalus*	MM	TC	0	1	2	1	0	4	7	3
	Lagodon rhomboides	MM	SC	27	1	48	1	24	14	189	0
Syngnathidae	*Hippocampus erectus*	ES	SC	1	0	0	0	0	0	0	0
	Microphis brachyurus	ES	SC	0	0	0	0	0	0	2	3
	Syngnathus louisianae	ES	SC	3	1	0	0	0	0	1	0
	Syngnathus scovelli	ES	SC	2	0	1	0	2	0	7	3
Synodontidae	*Synodus foetens*	MM	TC	4	0	5	0	3	0	5	1
Tetraodontidae	*Sphoeroides nephelus*	ES	TC	1	0	2	1	2	0	1	0
Triglidae	*Prionotus scitulus*	MM	SC	1	0	2	0	0	0	0	0

[1]Ecological guilds: FW freshwater species; ES estuarine species; MM marine migrant species. Trophic guilds: PC primary consumer; SC secondary consumer; TC tertiary consumer.

Table 2S.2 Results of the Tukey contrasts from the linear mixed-effect models. Comparisons between seasonal estimates of nekton diversity, richness and evenness for each estuary are presented. Bold values reflect significant differences at $\alpha = 0.05$.

			Myakka				Caloosahatchee			
TRAWL			Density	Diversity	Richness	Evenness	Density	Diversity	Richness	Evenness
	Nekton Community		**t = -5.354, *P* = 0.000**	t = -0.415, *P* = 0.678	**t = -3.223, *P* = 0.001**	t = 1.775, *P* = 0.076	t = 0.403, *P* = 0.687	t = -0.309, *P* = 0.758	t = -0.146, *P* = 0.884	t = 1.071, *P* = 0.284
	Ecological Guilds	*Freshwater species*					**t = -1.996, *P* = 0.046**	t = -1.384, *P* = 0.166	**t = -2.186, *P* = 0.029**	t = -1.384, *P* = 0.166
		Estuarine species	**t = -6.016, *P* = 0.000**	**t = -2.423, *P* = 0.015**	**t = -5.336, *P* = 0.000**	t = -1.004, *P* = 0.315	t = -0.545, *P* = 0.586	t = 0.343, *P* = 0.732	t = 0.058, *P* = 0.954	t = -0.099, *P* = 0.921
		Marine migrants	t = -0.963, *P* = 0.336	t = 1.303, *P* = 0.192	t = 0.545, *P* = 0.586	t = 1.729, *P* = 0.083	**t = 2.360, *P* = 0.018**	t = 1.692, *P* = 0.091	**t = 2.037, *P* = 0.042**	**t = 2.181, *P* = 0.029**
	Trophic Guilds	*Primary consumers*	t = -1.669, *P* = 0.095	t = 1.474, *P* = 0.141	t = 0.449, *P* = 0.653	t = 1.474, *P* = 0.141	t = 0.441, *P* = 0.659	t = 0.780, *P* = 0.435	t = 0.087, *P* = 0.931	t = 1.801, *P* = 0.071
		Secondary consumers	**t = -3.629, *P* = 0.000**	t = 0.416, *P* = 0.678	t = -0.632, *P* = 0.527	t = 0.345, *P* = 0.738	t = 0.585, *P* = 0.559	t = 0.538, *P* = 0.590	t = -0.436, *P* = 0.663	t = 1.074, *P* = 0.283
		Tertiary consumers	**t = -5.007, *P* = 0.000**	**t = -3.900, *P* = 0.009**	**t = -5.635, *P* = 0.000**	**t = -4.098, *P* = 0.004**	t = -0.122, *P* = 0.903	t = 0.087, *P* = 0.931	t = -0.309, *P* = 0.757	t = 0.314, *P* = 0.753
SEINE	Nekton Community		t = 0.341, *P* = 0.733	**t = -3.747, *P* = 0.000**	**t = -2.232, *P* = 0.026**	**t = -3.163, *P* = 0.002**	t = -1.359, *P* = 0.174	t = 0.905, *P* = 0.366	t = 0.018, *P* = 0.985	t = 0.601, *P* = 0.548
	Ecological Guilds	*Freshwater species*	t = 1.841, *P* = 0.066	**t = 3.537, *P* = 0.000**	**t = -2.074, *P* = 0.038**	t = -1.397, *P* = 0.163	t = 0.551, *P* = 0.480	t = 0.047, *P* = 0.063	**t = -2.246, *P* = 0.024**	t = -1.629, *P* = 0.103
		Estuarine species	t = 1.011, *P* = 0.312	**t = -3.690, *P* = 0.000**	**t = -3.318, *P* = 0.000**	**t = -2.292, *P* = 0.022**	t = -0.695, *P* = 0.487	t = -0.800, *P* = 0.424	t = -1.375, *P* = 0.169	t = -1.220, *P* = 0.223
		Marine migrants	t = 0.907, *P* = 0.365	t = -1.943, *P* = 0.052	t = -0.185, *P* = 0.853	**t = -2.739, *P* = 0.006**	t = 1.115, *P* = 0.265	**t = 2.520, *P* = 0.011**	**t = 2.559, *P* = 0.010**	t = 1.576, *P* = 0.115
	Trophic Guilds	*Primary consumers*	**t = -3.419, *P* = 0.000**	t = -1.607, *P* = 0.108	**t = -3.079, *P* = 0.002**	t = -1.693, *P* = 0.090	t = -0.598, *P* = 0.550	t = 0.128, *P* = 0.898	t = 0.867, *P* = 0.386	t = 0.211, *P* = 0.833
		Secondary consumers	t = 1.779, *P* = 0.075	**t = -3.575, *P* = 0.000**	**t = -2.082, *P* = 0.037**	**t = -3.124, *P* = 0.001**	t = -0.900, *P* = 0.368	t = 0.089, *P* = 0.929	t = -1.495, *P* = 0.135	t = 0.381, *P* = 0.704
		Tertiary consumers	t = 0.529, *P* = 0.597	t = -0.100, *P* = 0.920	t = 0.193, *P* = 0.847	t = 0.017, *P* = 0.987	**t = 3.277, *P* = 0.001**	**t = 2.957, *P* = 0.003**	**t = 3.775, *P* = 0.000**	**t = 2.233, *P* = 0.025**

CHAPTER 3

MATERNAL MEDDLING IN NEONATAL SHARKS: IMPLICATIONS FOR INTERPRETING STABLE ISOTOPES IN YOUNG ANIMALS[*]

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INTRODUCTION

The stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in different animal tissues provide a tool to examine species trophic interactions as they are dietary integrators across variable time scales (Peterson and Fry 1987). Enrichment of isotopes within tissues of a consumer over that of its diet arises as a result of the greater retention of the heavier over the lighter isotope during the process of protein amination and deamination for ^{15}N and respiration for ^{13}C, respectively (DeNiro and Epstein 1978; DeNiro and Epstein 1981). This produces ratios in a consumer's tissues, between approximately 0-2‰ for $\delta^{13}C$ and 2-5‰ for $\delta^{15}N$, higher than those of its diet (DeNiro and Epstein 1978; Minagawa and Wada 1984; Post 2002), but see the recent review (Caut et al. 2009).

Size and season-based shifts in diet that reflect the changing role of an organism within an ecological community are common and often explain variation in stable isotope composition between species and among individuals within a population. However, changes in diet are not instantly manifested in the isotopic composition of a consumer's tissues but require a period of time to achieve equilibrium (Tieszen et al. 1983; MacNeil et al. 2006). A consumer's tissue will reflect a combination of effects aside from diet (i.e. metabolism, growth, isotopic routing, and tissue protein composition) thereby potentially masking other factors that can cause a shift in isotopic composition as an animal grows (Vander Zanden et al. 2000). When considering newborn animals, interpreting stable isotope values is further complicated by (i) the mother-young transfer of maternal resources and hence isotopic signature, either during gestation and/or through post-parturition survival on maternal reserves (Jenkins et al. 2001) and; (ii) known isotopic

discrimination between placental connected young and their mothers (Sare et al. 2005; McMeans et al. 2009).

In light of the documented declines in some predator populations, raising concerns over ecosystem effects (Heithaus et al. 2008), understanding the trophic role of young age classes of sharks, assumed to be top predators within coastal habitats (Cortés 1999) is important. Carcharhinid sharks bear live young and even though parental care is absent, young are provisioned with maternal resources in the form of an enlarged liver (Hussey et al. 2009, 2010). Although, neonatal sharks begin to feed soon after parturition, it is expected that the stable isotope composition of their tissues will reflect that of the mother and/or provisioned reserves. Indeed, it has been observed that embryos of the placentatrophic Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) were enriched in both ^{15}N and ^{13}C in muscle and liver tissues relative to their respective mothers' tissues (McMeans et al. 2009). At birth, the $\delta^{15}N$ and $\delta^{13}C$ values of neonates are therefore higher than those of young-of-year sharks whose postpartum feeding habits would have restructured their stable isotope profiles to reflect that of their postembryonic diet. Similar to other placental species (e.g., pinnipeds, ursids and viperids), stable isotope analysis of neonatal sharks is therefore confounded by variable mixtures of mother and own diet signals (Hobson et al. 1997; Pilgrim 2007; Ducatez et al. 2008), which if not accounted for, will distort the true nitrogen and carbon sources leading to misinterpretation of data.

Values of $\delta^{13}C$ and $\delta^{15}N$ were measured in liver and muscle tissue of two species of sharks, the bull (*Carcharhinus leucas*; Valenciennes, 1839) and the Atlantic sharpnose (*Rhizoprionodon terraenovae*; Richardson, 1836), to measure the loss of the maternal

isotopic signal on the stable isotope values of growing neonate (< 4 weeks) and young-of-year (< 1 year old) sharks. Three measures, considered to be proxies for age, were used to quantify this relationship; total length, date sampled, and umbilical scar stage. Umbilical scar stage is a unique characteristic among fishes, and was included in these analyses as it affords advantages over date sampled and total length by providing a quantifiable measure of the age of young animals (Duncan and Holland 2006). Inter- and intra-species variation in birth date and size at birth, are well documented (Parsons 1985; Neer et al. 2005). Here we tested the prediction that isotopic values of neonatal/young-of-year sharks would decline with increasing total length and date sampled, and reduced umbilical scar presence, until they reached equilibrium with their diet, i.e., when the isotopic values of a young shark reflects its own diet. This prediction was based on (i) the known enrichment in ^{15}N and ^{13}C of neonates relative to their mothers (McMeans et al. 2009), and (ii) the premise that the young sharks of both study species inhabit isotopically distinct habitats from adults; bull sharks remain in low-salinity estuaries for several years (Heupel and Simpfendorfer 2008) and Atlantic sharpnose sharks inhabit nearshore coastal environments (Carlson et al. 2008). Tissues of both bull and Atlantic sharpnose sharks will therefore adopt a more ^{13}C and ^{15}N depleted estuarine diet compared to their mothers' marine signature, which will result in the predicted decline in $\delta^{13}C$ and $\delta^{15}N$ values over time. Moreover, because variable tissue turnover and growth rates influence isotopic values, we predicted that $\delta^{13}C$ and $\delta^{15}N$ values in neonatal/young-of-year sharks would decline (i) at a faster rate in liver than muscle, and (ii) more quickly in the faster growing Atlantic sharpnose shark.

MATERIALS AND METHODS

Liver and muscle (~5 g) were sampled from 39 bull and 42 Atlantic sharpnose sharks collected from nursery habitats of the Caloosahatchee and Myakka Rivers of Florida (USA) between May and October of 2006-2008, and from Georgia (USA) estuaries between May and August of 2005, respectively. Total length (TL), date sampled and umbilical scar stage (USS) was recorded for all individuals. A qualitative 6-point USS scale was devised where (i) open wound with umbilical remains attached (USS1), (ii) open wound without remains (USS2), (iii) wound partially open (USS3), (iv) wound completely closed (USS4), (v) faint scar present (USS5), and (vi) no scar present (USS6)). A limited amount of information is available on the time required for the umbilical scar to heal completely, but the majority of estimates range from 4 to 6 weeks Bass et al. 1973). Duncan and Holland (2006) estimated ~ 2 weeks for the umbilical scar of neonate scalloped hammerhead (*Sphyrna lewini*) to be healed, which corresponds to our USS4 descriptor. Furthermore, Duncan and Holland (2006) suggest ~1 year for complete disappearance of the scar. Only sharks estimated to be≤ 1 year old were included in the statistical analyses.

Tissues were sub-sampled (~1.0 g), freeze-dried for 48 hrs, pulverized and lipid extracted by twice agitating the pulverized tissue in 2:1 chloroform: methanol solution for 24 h and decanting the solvent (modified method outlined by Bligh & Dyer (1959)). Relative abundances of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were determined on ~0.5-1.0 mg sub-samples on a Thermo Finnigan Delta^{Plus} mass-spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled with an elemental analyzer (Costech, Valencia, CA, USA) at the Chemical Tracers Laboratory, Great Lakes Institute for Environmental

Research, University of Windsor and at the Odum School of Ecology, University of Georgia. Results are expressed in standard delta notation (δ), defined as parts per thousand as follows: $\delta = [(R_{sample}/R_{standard})-1] \times 10^3$, where R is the ratio of heavy to light isotopes in the sample and standard, respectively (Peterson and Fry 1987). The standard reference material was Pee Dee Belemnite carbonate for CO_2 and atmospheric nitrogen for N_2. The analytical precision based on the standard deviation of two standards (NIST 8414 and internal lab standard; $n = 76$) for $\delta^{13}C$ ranged from 0.06‰ to 0.09‰ and for $\delta^{15}N$ ranged from 0.10‰ to 0.21‰. Accuracy of analysis based on NIST standards (sucrose (NIST 8542) and ammonium sulfate (NIST 8547); $n = 3$ for each) that were analyzed in-conjunction with the shark tissue samples were within 0.01‰ and 0.07‰ of certified values for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Stable isotope data were found to be normally distributed based on probability plots consequently no data transformations were performed. We ruled out the possible effects of sex, season, sampling location and year on stable isotope values of both shark species (see Table 3S.1 *Supplemental Material*). Data were therefore grouped per species for all following analyses.

To test the prediction that $\delta^{13}C$ and $\delta^{15}N$ values of neonatal/young-of-year sharks of each species declined with age; (1) the relationships between date sampled and tissue $\delta^{13}C$ and $\delta^{15}N$ values; and (2) the relationship between total length and tissue $\delta^{13}C$ and $\delta^{15}N$ values of both liver and muscle tissue were fitted with polynomial models (*lm* in R). This was based on the premise that polynomial models often produce the best fit for determining the relationship between stable isotope values and either total length and date sampled, as isotope assimilation of new diet into an individual's tissues is expected to

experience a lag-time with the loss of maternal isotopic signal (Matich et al. 2010). In addition, the use of polynomial models best fit our prediction that isotopic values of neonate/young-of-year sharks will decline (e.g. representing the loss of the maternal signal), reach an asymptote or equilibrium with their diet (i.e. complete turnover of the maternal isotopic signal), and subsequently respond to the new diet (e.g. remain stable, increase or decrease). Consideration of an exponential decay model (Hobson and Clark 1992) to characterize our predictions was taken; however we were unable to sample the required endpoints (i.e. mothers and new diet) (see Discussion). Because USS is an ordinal variable, one-way analyses of variance (ANOVA) was used to test for differences among umbilical scar stages. As the sample sizes were unbalanced, significance of pair-wise comparisons was tested using adjusted Bonferroni tests. Statistical analyses were conducted using program R (R Development Core Team, 2009), with a criterion for significance of $p < 0.05$ used for all analyses. All mean values are presented ± one standard error.

RESULTS

For the Atlantic sharpnose, there was a significant decline in muscle $\delta^{13}C$ values between USS1 and USS5 (-14.9‰ to -17.0‰; $F_{5,36} = 4.178$, $p = 0.004$; Fig. 1a). In liver tissue, the $\delta^{13}C$ decline was more pronounced between USS1 and USS4 (-15.4‰ to -18.8‰; $F_{5,36} = 13.868$, $p < 0.0001$; Fig. 3.1c). In agreement, the $\delta^{15}N$ for the Atlantic sharpnose showed that both muscle and liver values decreased with USS (16.2 ‰ to 14.7‰; $F_{5,36} = 5.612$, $p = 0.001$ and 15.8‰ to 13.7‰; $F_{5,36} = 8.427$, $p < 0.0001$, respectively; Fig. 3.1b, d). Pair-wise comparisons found that USS4 and USS5, $\delta^{13}C$ and

$\delta^{15}N$ muscle and liver tissue values were significantly lower than USS1 and USS2 (Fig. 3.1a - d). For liver and muscle tissue, pair-wise comparisons indicated that stable isotope values of the Atlantic sharpnose do not continue to decline beyond USS4 and USS5, respectively (Fig. 3.1c and d). However, the low sample size of USS6 limits the interpretation of this result.

For the bull shark, the decline in $\delta^{13}C$ with USS was significant for both muscle and liver tissue (-15.0 to -16.2‰; $F_{4,34} = 11.120$, $p < 0.0001$ and -15.4 to -18.5‰; $F_{4,34} = 8.450$, $p < 0.0001$, respectively; Fig. 3.2a and c). USS5 muscle and liver $\delta^{13}C$ values were significantly lower than all other USSs, accepting limited data for USS6. The range of $\delta^{15}N$ values for both bull shark tissues were narrow and no significant $\delta^{15}N$-USS relationships were detected (muscle: $F_{4,34} =0.299$, $p = 0.876$; liver: $F_{4,34} =0.675$, $p = 0.614$; Fig. 3.2b and d).

For the Atlantic sharpnose, there was a significant decline in $\delta^{13}C$ and $\delta^{15}N$ values in muscle and liver tissue with increasing total length (Fig. 3.3a - d) and consecutive sampling date (Fig. 3.4a - d), yet date sampled exhibited stronger relationships than total length, based on the coefficients of determination. Despite the stronger $\delta^{13}C$ and $\delta^{15}N$ relationships, only the $\delta^{13}C$ and $\delta^{15}N$ relationship between Atlantic sharpnose liver and date sampled suggested sharks were approaching the point when the maternal stable isotope signal was no longer influencing the values seen in young-of-year sharks. Muscle $\delta^{13}C$ and $\delta^{15}N$ values vs. total length and sampling date indicated stable isotope data of sharks were still declining, and thus still potentially influenced by the mother's isotope signal. In contrast, for the bull shark only the declines in $\delta^{13}C$ with increasing total length (Fig. 3.3e and g) and consecutive sampling date (Fig. 3.4e and g) were significant but

neither tissue showed evidence of approaching the point where stable isotope values in the young-of-year were not influenced by maternal isotopes. Unlike the Atlantic sharpnose, bull shark total length exhibited a stronger relationship with muscle $\delta^{13}C$, whereas date sampled exhibited a stronger relationship with liver $\delta^{13}C$. The bull shark $\delta^{15}N$ values of liver tissue showed a small depletion with increasing total length and consecutive sampling date (Fig. 3.3h and 3.4h), while for muscle tissue there was no change (Fig. 3.3f and 3.4f). Neither date sampled nor total length were strong predictors of either bull shark tissue $\delta^{15}N$ relationships.

DISCUSSION

Our results revealed the distinct loss of enriched isotopic values commensurate with increasing total length, consecutive sampling date and healing of the umbilical scar in neonate to young-of-year Atlantic sharpnose and bull sharks. These trends, with the exception of bull shark $\delta^{15}N$, affirm the prediction that neonates of both study species have higher isotopic values than young-of-year, confirming that the interpretation of stable isotopes in young sharks is complicated as a result of the maternal isotopic signal. The expression of maternal isotopic signals in offspring has been documented in a number of non-elasmobranch species (Jenkins et al. 2001; Pilgrim 2007; Hobson et al. 2000), but this is the first study to adopt multiple age measures to document the rate of maternal isotopic signal loss of neonatal sharks as they progress through their first year. Additionally, the loss of maternal isotopic signal was variable between species and tissues highlighting the potential implications for using stable isotope data from multiple tissues to characterize diet and habitat use of < 1 year old animals.

For both the Atlantic sharpnose and bull shark, $\delta^{13}C$ and $\delta^{15}N$ muscle and liver values declined with total length and consecutive sampling date, but most relationships did not reach an asymptote as predicted. This would suggest that both these age measures are problematic for estimating when complete loss of the maternal isotopic signal occurs in young sharks. Overall, date sampled was a stronger predictor of maternal signature loss compared with total length. However, date sampled can be difficult to quantify, specifically for the species in this study, as both species pup at various times throughout the spring and early summer (Clark and von Schmidt 1965). Consequently, if a species utilizes or revisits nursery habitat for an extended period of time (i.e., >1 yr), similar to the bull shark, second year cohorts could be misclassified as neonates or young-of-year although they would have already lost their maternal isotopic signal and tissue isotope values would be reflecting their own diet. If these >1 year old sharks were categorized based on sampling date, they would likely increase the variability in isotopic values in early age classes and complicate interpretation of these relationships.

The length of sharks at birth (i.e. total length) is also highly variable and the size ranges of early age classes overlap (Parsons 1985; Neer et al. 2005). Size at birth of Atlantic sharpnose has been reported in the range of 25-41 cm TL (Parsons 1985; Branstetter and Stiles 1987; Loefer and Sedberry 2003). In this study, Atlantic sharpnose sharks collected in June had overlapping TLs but represented three umbilical scar stages and bull sharks from USS2 to USS4 included individuals ranging between 69 and 86 cm TL. Additionally, three bull sharks not included in these analyses exhibited characteristics of $\geq$ 1 year old individuals (lack of scar and different isotope values), but were of a similar length to the young-of-year sharks sampled here. It is therefore

necessary to couple TL and date sampled with USS to provide a more reliable estimate of the true age of the shark to assess if the maternal isotopic signal is still present. Atlantic sharpnose liver $\delta^{13}C$ and $\delta^{15}N$ values exhibited a significant change at USS4 from earlier scar stages suggesting these young sharks had replaced the maternal isotopic signal with that of their own diet. Reported $\delta^{15}N$ turnover rates in liver tissue of freshwater stingrays (*Potamotrygon motoro*) of ~166 days (MacNeil et al. 2006) provides further support that USS4 of the Atlantic sharpnose shark was at or near equilibrium, considering the USS timeline of Duncan and Holland (2006). Accurate inferences on the diet and trophic ecology of young Atlantic sharpnose using stable isotopes of liver would therefore seem permissible at stages later than USS4. Muscle $\delta^{13}C$ and $\delta^{15}N$, however, did not indicate a diet switch until USS5 and USS6, respectively, which is expected as muscle tissues of juvenile sandbar sharks (*Carcharhinus plumbeus*) reached equilibrium at >500 days for $\delta^{13}C$ and >300 days for $\delta^{15}N$ (Logan and Lutcavage 2010). However, reported mother-embryo muscle tissue discrimination values for Atlantic sharpnose of 1.3‰ for $\delta^{13}C$ and 1.1‰ for $\delta^{15}N$ (McMeans et al. 2009) would suggest the USS6 shark was approaching complete maternal signal replacement and assimilation of new diet, based on the difference between USS1 and USS6, but a larger range of sizes including adults would be required to confirm this.

Bull shark muscle and liver $\delta^{13}C$ values indicated loss of maternal isotopic signal and assimilation of new diet at USS5, however limited data (n =2) for USS6 warrants caution with interpretation. The lack of a $\delta^{15}N$-USS relationship limits any inferences made about maternal isotopic influence on $\delta^{15}N$ in this species. If indeed we consider the estimates of turnover rates of muscle $\delta^{15}N$ and $\delta^{13}C$ detailed above, young bull sharks

would not be predicted to reach equilibrium with their diet, i.e. complete turnover of the maternal isotopic signal, until they were >1 year old or reached a TL of approximately 90-100 cm (Neer et al. 2005; Branstetter and Stiles 1987). However, based on liver turnover rates, we would have expected bull shark liver tissue to have reached equilibrium prior to USS6. Therefore the reported turnover rates for elasmobranch liver tissue in conjunction with the fact that neither $\delta^{15}N$ nor $\delta^{13}C$ of bull shark tissues approached equilibrium, may suggest that USS is not appropriate for this slow growing species and that sampling older individuals is necessary to fully document loss of maternal signature. Likewise, both whole blood and plasma have been shown to assimilate the stable isotope ratios of a new diet within shorter time frames than muscle tissue, ranging from several days in the case of plasma (Hobson and Clark 1992; Podlesak et al. 2005) to several weeks (Oppel and Powell 2010) or months (Logan and Lutcavage 2010) in the case of whole blood. These tissues may be more easily quantified using USS, as they will show the loss of the maternal isotopic signal more definitively in slow growing species. Hence, a combined approach, USS and TL, and/or possibly the use of blood plasma would be an appropriate method to determine when newborn animals are in equilibrium with their own diet.

The rate of loss of maternal isotopic signal was quicker in Atlantic sharpnose than bull sharks, based on the USS estimations for when the maternal isotope signal was lost. This was likely a result of the faster growth rate reported for this species and associated rate of tissue turnover. The growth coefficient (K) for bull sharks of 0.08-0.09 yr^{-1}(Neer et al. 2005; Branstetter and Stiles 1987) is much lower than that for Atlantic sharpnose (K = 0.42-0.50 yr^{-1}) (Parsons 1985; Loefer an d Sedberry 2003). The faster turnover in liver

stable isotope values of Atlantic sharpnose as opposed to muscle is consistent with trends seen for liver and muscle in fishes, birds and marine mammals (Tieszen et al. 1983; MacNeil et al. 2006; Hobson and Clark 1992; Logan and Lutcavage 2010; Guelinckx et al. 2007; Buchheister and Latour 2010). Thus, the length of time that the maternal isotopic signal will influence the stable isotope values of a young shark is inversely related to growth rate of the species and metabolic activity of the tissues.

In contrast to our expectations, the mean $\delta^{13}C$ USS2-USS4 values in both bull shark tissues were similar and did not decline until later stages. Additionally, ANOVAs revealed that three USS4 bull sharks collected furthest from the mouth of the river (26.5 km upstream) had the most enriched ^{13}C liver and muscle signatures (~13‰; see *Supplementary material*). Marine food webs are typically enriched in ^{13}C compared to terrestrial C_3 or freshwater food webs (Hobson et al. 2000) therefore neonate isotopic values were expected to diverge from their mothers as they assimilate a more $\delta^{13}C$-depleted estuarine diet (mean consumer taxa $\delta^{13}C$ ~-20.8 ± 0.19 in the Caloosahatchee and Myakka Rivers, J. Olin unpublished data). The lack of ^{13}C depletion in the youngest sharks would suggest feeding in marine as opposed to estuarine environments, yet this would seem counterintuitive as bull sharks pup in estuarine environments and inhabit riverine systems for ~1-2 yrs (Heupel and Simpfendorfer 2008). It is more probable that the constant $\delta^{13}C$ values observed in the youngest bull sharks reflect the use of liver reserves provisioned by the mother (Hussey et al. 2010). Considering the Atlantic sharpnose showed depletion in both ^{13}C and ^{15}N from birth, this may suggest greater maternal investment in bull sharks, as compared to the Atlantic sharpnose. Nevertheless, aside from variable growth rates between species, it is likely that variation in maternal

investment across shark species may also complicate establishing a single scar stage for all species at which stable isotopes reflect the actual diet of young sharks.

For the bull shark, the lack of a decline in $\delta^{15}N$ values with age could result from (1) young sharks feeding on a diet with comparable $\delta^{15}N$ values to their mothers or (2) equivalent source $\delta^{15}N$ values between young/mother habitats. Given the trend of increasing body size-trophic level relationships in large predatory fish and sharks (Scharf et al. 2000; Lucifora et al. 2009), mother-young feeding at the same trophic level would seem unlikely. A more probable explanation is equivalent source $\delta^{15}N$ values between young/mother habitats. Baseline estuarine $\delta^{15}N$ values in developed areas, like the Caloosahatchee River estuary, are reportedly higher than coastal values (Heaton 1986) therefore $\delta^{15}N$ values of young individuals would be artificially inflated.

An unanticipated result was the lack of difference in the rate of maternal isotopic signal loss among liver and muscle tissues of the bull shark. If we consider the previous argument, that baseline $\delta^{15}N$ signatures are similar between neonate and mother habitat, then it is probable that we can extend this point to explain the similar $\delta^{15}N$ values between liver and muscle tissue of the bull shark. Liver tissue $\delta^{15}N$ turns over significantly faster than muscle tissue $\delta^{15}N$ (MacNeil et al. 2006; Logan and Lutcavage 2010) Therefore, bull shark liver would reflect a diet representative of the enriched ^{15}N baseline, producing similar $\delta^{15}N$ values to the slow turnover muscle tissue which would reflect maternal reserves.

How the maternal stable isotope signal in near-term sharks and rays varies between species or families adopting different reproductive strategies (i.e. oviparous, ovoviviparous) is unknown. In teleost fishes, embryos are often depleted in ^{13}C, as a

result of feeding on lipid-rich yolk, and isotopic values increase post-hatch with assimilation of new dietary resources (Murchie and Power 2004; Witting et al. 2004). Clearly, consideration of the maternal influence through the mother-young transfer of maternal resources is thus necessary in any study using stable isotopes to assess diet, foraging behaviour and/or habitat use of young animals.

Future research should focus on determining tissue-specific turnover rates of the maternal signal in neonate to young-of-year sharks by applying exponential decay models Hobson and Clark 1992; Fry and Arnold 1982). Through sampling pregnant females (and associated newborn pups) and principal prey items in the diet of neonate/young-of-year sharks within the nursery habitat, exponential decay models would facilitate an examination of the rate of isotopic change or loss of maternal signature. This type of model would provide a predictive framework for investigators to determine when stable isotope values in tissues represent true diet and which juvenile animals could be sampled without the influence from maternal reserves. Defining the maternal and dietary endpoints of large sharks, however may be challenging when considering; (i) sampling large pregnant females within a nursery ground is inherently difficult, (ii) defining the dietary endpoint of neonatal sharks may be complex as many species undergo a rapid diet shift with size, which may overlap the dietary endpoint of interest and (iii) for certain shark species, juvenile and adult habitat overlap and therefore nursery habitat will not be isotopically distinct, which complicates the definition of neonatal/young-of-year dietary endpoints. Furthermore, the maternal isotopic signal is inherently variable (Barnes et al. 2008), both within a species and among species, and is influenced by whether the species is a generalist or specialist feeder and/or if mothers forage in the same/variable habitat. A

single estimate of maternal isotopic tissue turnover would therefore not be applicable to all species, but would guide field-sampling protocols.

It is difficult to draw definitive conclusions over the precise timing of tissue $\delta^{13}C$ and $\delta^{15}N$ values achieving equilibrium with diet (i.e. exact USS or TL) when considering that growth and maternal investment are species-specific. The declining trend of $\delta^{13}C$ values of both species for all three age measures, however, supports the hypothesis that the maternal isotopic influence on stable isotope values of young sharks is evident for an extended period of time after birth. Regardless of determining the exact stage of stable isotope diet-equilibrium, our data provide the first practical approach for understanding and measuring the loss of the maternal signal in stable isotope values of young sharks. Until a comprehensive timeline for stable isotope tissue turnover in these age classes and across species can be determined, we suggest a combination of USS and TL will enable investigators to effectively sample animals that will provide accurate data for dietary and food web studies.

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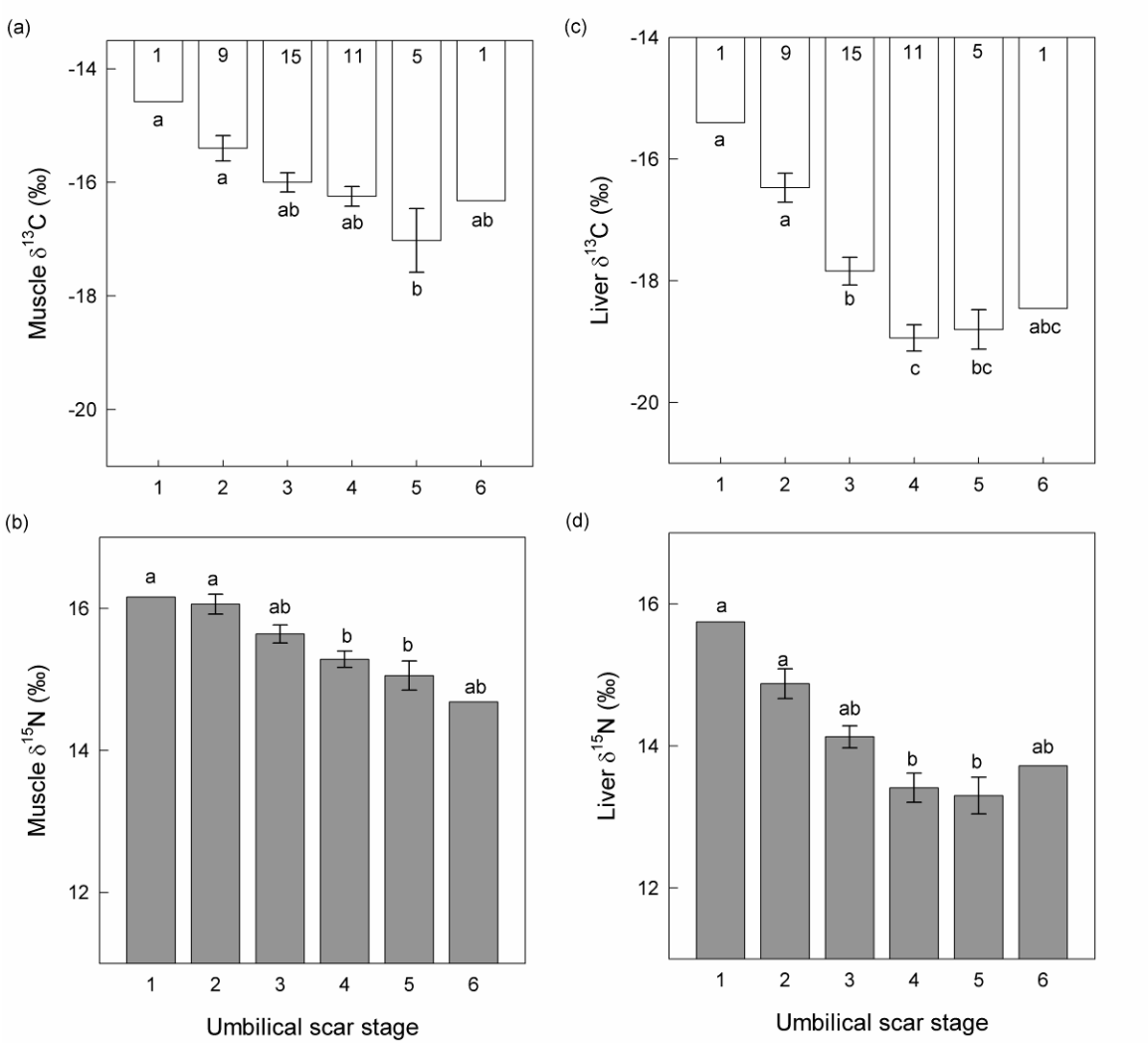

Figure 3.1 Relationships between USS and $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE) for (a), (b) muscle and (c), (d) liver of the Atlantic sharpnose (*Rhizoprionodon terraenovae*). Letters displayed above a given USS indicate the USS(s) for which pair-wise comparisons revealed significant differences. Numbers in plot (a) and (c) represent the sample size of sharks per USS.

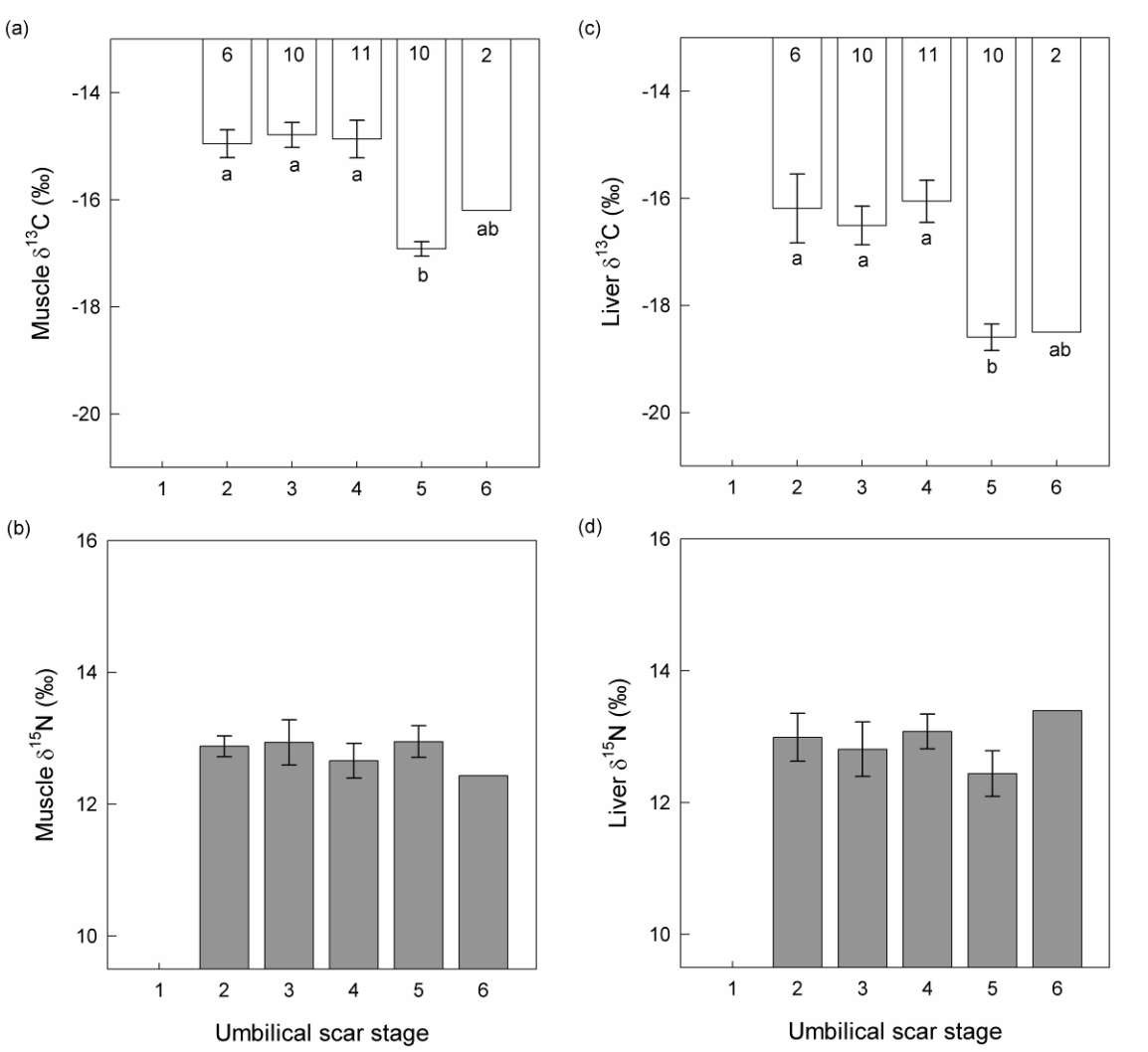

Figure 3.2 Relationships between USS and $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE) for (a), (b) muscle and (c), (d) liver of the bull shark (*Carcharhinus leucas*). Letters displayed above a given USS indicate the USS(s) for which pair-wise comparisons revealed significant differences. Numbers in plot (a) and (c) represent the sample size of sharks sampled per USS.

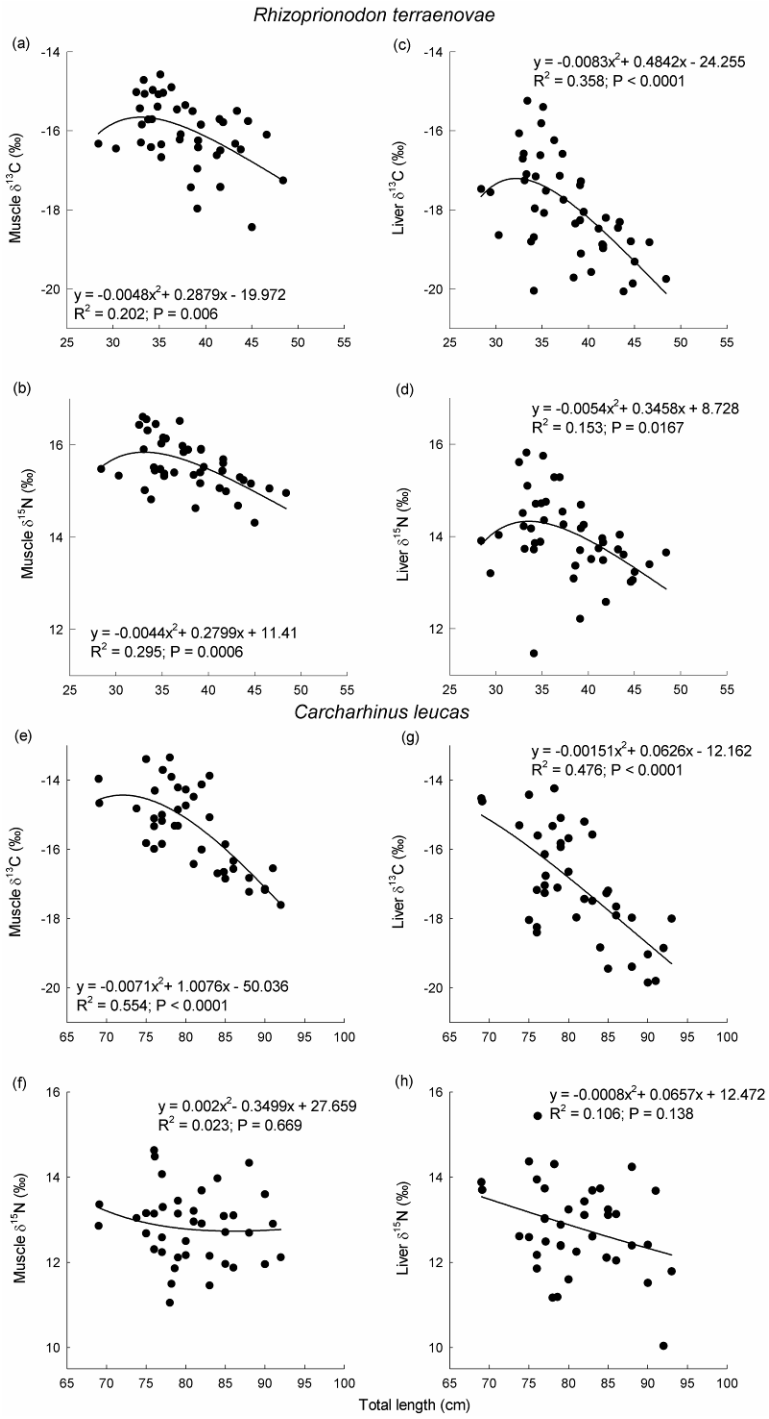

Figure 3.3 Relationships between total length (TL) and $\delta^{13}C$ and $\delta^{15}N$ values for (a), (b) muscle and (c), (d) for liver tissues of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) and (e), (f) for muscle and (g), (h) for liver of the bull shark (*Carcharhinus leucas*); curves were fitted with polynomial models.

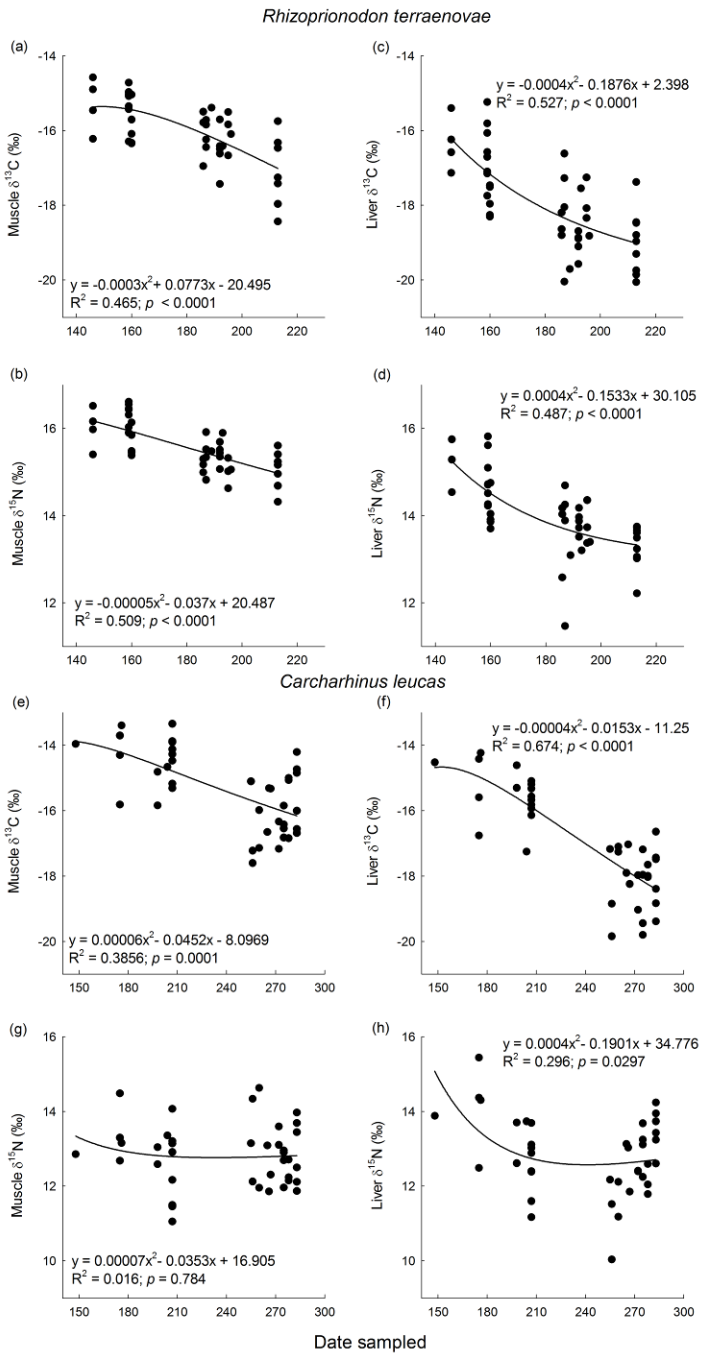

Figure 3.4 Changes in $\delta^{13}C$ and $\delta^{15}N$ values in regard to date sampled for (a), (b), muscle and (c), (d) for liver tissues of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) and (e), (f) for muscle and (g), (h) for liver of the bull shark (*Carcharhinus leucas*); curves were fitted with polynomial models.

SUPPLEMENTAL MATERIAL

Sex did not have a significant influence on $\delta^{13}C$ or $\delta^{15}N$ of either tissue in both species of shark (Table S3.1). Season, year, river and sampling location were significant parameters influencing the stable isotope values of bull shark tissues, particularly liver ($p < 0.05$; see Table S3.1). Sampling location by way of distance from mouth of river was significant for $\delta^{13}C$ of bull sharks. However, contrary to our expectations, the results of the analysis indicated that three USS4 stage individuals collected furthest from the mouth of the river (26.5 km) had enriched ^{13}C signatures in liver and muscle (~13‰). Based on distance to the mouth and work of Heupel & Simpfendorfer (2008) showing residency of bull sharks in rivers for extended periods of time, it is unlikely that these individuals are travelling 26.5 km to the mouth of the river to feed. Significant seasonal, annual or river differences are not unexpected, as inherent variability within the system is likely. Further, because individuals representing different life history stages were combined for these analyses, differences were anticipated.

Table S3.1. Results of GLMs used to test the effect of sex, season, sampling location and year of sampling on $\delta^{13}C$ and $\delta^{15}N$ of the two species of shark. Significance is denoted by bold text ($\alpha = 0.05$).

	Muscle					**Liver**			
	$\delta^{15}N$			$\delta^{13}C$		$\delta^{15}N$		$\delta^{13}C$	
Atlantic sharpnose shark	df	F	*P*	F	*P*	F	*P*	F	*P*
Sex	1,40	1.474	0.232	1.133	0.293	0.003	0.959	0.080	0.778
Sampling Location	2,40	0.001	0.976	1.891	0.177	1.137	0.293	1.530	0.223
Bull Shark									
Sex	1,36	0.189	0.665	0.426	0.517	0.027	0.870	0.671	0.417
Season	1,36	0.440	0.646	0.440	0.646	0.372	0.546	3.365	**0.019**
River	1,36	0.161	0.504	0.586	0.447	3.937	0.055	0.276	0.602
Distance from mouth	1,36	1.173	0.330	4.793	**0.005**	0.563	0.358	0.103	0.750
Year	2,36	2.466	0.122	0.757	0.388	0.323	0.726	4.932	**0.012**

CHAPTER 4

ISOTOPIC RATIOS REVEAL MIXED SEASONAL VARIATION AMONG FISHES FROM TWO SUBTROPICAL ESTUARINE SYSTEMS[*]

[*]Olin JA, Rush SA, MacNeil MA, Fisk AT. 2011. Isotopic ratios reveal mixed seasonal variation among fishes from two subtropical estuarine systems. *Estuaries and Coast*s doi: 10.1007/s12237-011-9467-6.

INTRODUCTION

Estuaries are highly productive and complex ecosystems that derive organic carbon from a combination of sources (Bouillon et al. 2004; Peterson and Howarth 1987). As a result, estuaries serve as nursery, rearing and feeding grounds for a diverse assemblage of both resident and transient fish and invertebrate species (e.g., Beck et al. 2001). This complexity makes characterizing feeding relationships and dietary resource partitioning in these systems especially challenging, particularly when considering that body sizes of some individual consumer species can range over an order of magnitude (Rountree and Able 1992) and that trophic roles can vary with ontogeny (Wilson and Sheaves 2001).

The use of stable isotopes of nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$) to characterize dietary resources has become commonplace in studies of feeding ecology, as they provide a time-integrated perspective of a consumer's diet (Peterson and Fry 1987). Specifically, $\delta^{15}N$ values are used in determining the relative trophic position of a consumer (Minagawa and Wada 1984) and $\delta^{13}C$ and $\delta^{34}S$ values have found application in determining basal organic matter sources incorporated into a consumer's diet (Peterson and Fry 1987). Changes in $\delta^{15}N$ in particular, can be attributed to either a trophic level shift (i.e. feeding on more ^{15}N enriched or depleted prey) or to a change in organic matter sources supplementing the diet (i.e. pelagic to terrestrial-derived organic matter) or both (Peterson and Howarth 1987). Thus applying $\delta^{13}C$ and $\delta^{34}S$ with $\delta^{15}N$ in combination can help to distinguish the potentially wide range of dietary resources available to consumers (Connolly et al. 2004; Peterson and Howarth 1987).

Body size has long been recognized as influential on the structural and functional complexity of aquatic food webs (Elton 1927). Size-based shifts in dietary resources, reflecting the changing role of an organism within its community, are widespread in aquatic species, including invertebrates (Cherel et al. 2009; Hoeninghaus and Davis 2007), teleosts (Deudero et al. 2004; Greenwood et al. 2010; Kolasinski et al. 2009), marine turtles (Godley et al. 1998) and marine mammals (Newsome et al. 2009). Such size-based differences often explain variation in stable isotope composition between species (Akin and Winemiller 2008), and among conspecifics within a population (Davenport and Bax 2002; Jennings et al. 2002). However, the ability to detect size-based isotopic variation is often limited (Galván et al. 2010), as sampling the range of body sizes needed to account for ontogenetic differences in the feeding ecology of consumers can be difficult. This is particularly relevant in estuarine ecosystems, as high levels of spatial and temporal variability in the physical and chemical properties (Deegan and Garritt 1997; Abrantes and Sheaves 2010) influence the age class composition of species at any particular time.

Size-dependent temporal variation in $\delta^{15}N$ and $\delta^{13}C$ has been observed in coastal and open-water marine organisms (Goering et al. 1990; Jennings et al. 2008). Although these observations were largely noted in lower trophic level species, such as zooplankton and invertebrates, body size-related temporal variation has been identified in fishes (Vizzini and Mazzola 2003). However, evidence against size- and temporal-based isotopic shifts has been reported within estuarine consumers that indicated a dietary shift with size, based on stomach content analyses (Wilson et al. 2009). Detection of temporal variation in a consumer's isotopic values however, is in part dependent on the lag

associated with processing alternative dietary resources (i.e. growth rates, and tissue turnover rates, or both; Fry and Arnold 1982; Hesslein et al. 1993). Temporal shifts in isotopic values would therefore be more likely to be detected in species or individuals (e.g., smaller fish) with fast growth and tissue turnover rates (MacNeil et al. 2006).

Using the estuarine reaches of two subtropical tidal rivers located in southwestern Florida, USA (the Caloosahatchee and the Myakka), we examine temporal and spatial relationships between body size and $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values for fish species across multiple trophic levels. Because riverine systems undergo periods of increased freshwater flow, that provides terrestrial organic matter and nutrients to the receiving estuary (e.g., Chanton and Lewis 2002) we hypothesize that small bodied relative to larger bodied fishes, will reflect the seasonal variability of the two estuaries, via their $\delta^{13}C$ and $\delta^{34}S$ values. An additional hypothesis is that $\delta^{15}N$ will scale with body size within each fish species. Our objectives were to (1) determine whether body size or season influence the isotopic values of individual fish species; (2) determine whether these relationships are consistent for multiple fish species; and (3) determine whether body size/seasonal-isotopic relationships were consistent across estuarine systems.

MATERIALS AND METHODS

Sample collection

The Caloosahatchee (26°30' N, 81°54' W) and Myakka (82°12' W, 26°57' N) Rivers are major tributaries of Charlotte Harbor, a large relatively shallow estuary on the southwest coast of Florida (Fig. 4.1). The study was completed in the estuarine reach of the two rivers, encompassing ~27 km of habitat in the Caloosahatchee and ~32 km in the

Myakka (Fig. 4.1Inset). The upper reaches of the Caloosahatchee and the shoreline areas of the Myakka are characterized by mangroves and saltmarsh, principally red mangrove *Rhizophora mangle,* black mangrove *Avicennia germinans,* saltmarsh cordgrass *Spartina alterniflora* and black needlerush *Juncus roemerianus.* The shoreline habitats closer to the Caloosahatchee River mouth, have been largely altered by urbanization, as evidenced by extensive canal developments and shoreline modifications.

From 2006 to 2008, fishes were collected during spring (i.e., May–June) and autumn (i.e., September–October) from the Caloosahatchee and Myakka estuaries, as a component of a larger study aimed at characterizing the food web dynamics of the two estuaries, using a shallow water (< 10 m) longline (800 m), seine (21.3x1.8 m at the centre bag, 3-mm-stretch mesh), gillnet (50 m) and otter trawl (6.1m with 38 mm stretch mesh and 3 mm mesh liner). Upon collection, individuals were measured (standard length (SL), to the nearest cm) and white muscle tissue was excised from the dorsal area anterior to the first dorsal fin. Muscle samples were stored on ice in the field and then stored frozen upon return to the laboratory (-20°C).

Stable isotope analysis

Muscle tissues were sub-sampled (~1.0 g), freeze-dried for 48 h, and homogenized in a SPEX CertiPrep 8000-D ball milling unit (SPEX CertiPrep, Metuchen, New Jersey). Lipids are depleted in ^{13}C relative to other major tissue components (i.e. proteins and carbohydrates; DeNiro and Epstein 1977) and their presence in muscle tissue samples can negatively skew observed δ^{13}C values (Post et al. 2007). Thus, to standardize within and among species, lipids were removed from all samples prior to isotopic

analysis using a modified method outlined by Bligh and Dyer (1959): twice vortexing the pulverized tissue in 5 ml of 2:1 chloroform: methanol solution for 24 h and decanting the solvent through filter paper (Whatman™ Grade-1, 125 mm) to isolate the muscle tissue sample.

Relative abundances of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) were determined on ~0.5 mg sub-samples sealed in tin capsules on a Thermo Finnigan Delta^{Plus} mass-spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled with an elemental analyzer (Costech, Valencia, CA, USA) at the Chemical Tracers Laboratory, Great Lakes Institute for Environmental Research, University of Windsor. Relative abundance of sulfur ($^{34}S/^{32}S$) was determined on ~2 mg and ~ 6 mg sub-samples sealed in tin capsules on an Isochrom Continuous Flow IRMS (GV Instruments / Micromass, UK) coupled with an elemental analyzer (Costech, Valencia, CA, USA), at the Environmental Isotope Laboratory, University of Waterloo and by a Thermo-Electron Delta^{Plus} Advantage IRMS at the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University, respectively.

Stable isotope results are expressed in standard delta notation (δ), which are parts per thousand differences from a standard as follows: $\delta = [(R_{sample}/R_{standard}) -1] \times 10^3$ (Peterson and Fry 1987), where R is the ratio of heavy to light isotopes in the sample and a standard reference material (atmospheric nitrogen for nitrogen, Pee Dee Belemnite carbonate for carbon, and Canyon Diablo Troilite for sulfur). The analytical precision based on the standard deviation of two standards (NIST 8414 and internal lab standard; *n* = 76) ranged from 0.10‰ to 0.21‰ in $\delta^{15}N$, 0.06‰ to 0.09‰ in $\delta^{13}C$, and 0.3‰ for $\delta^{34}S$, based on three sulfide standards (NBS-123, EIl-40 and EIL-43). Accuracy of analysis

based on the analysis of NIST standards, performed with muscle tissue sample analysis, sucrose (NIST 8542), ammonium sulfate (NIST 8547) and bovine liver and mussel (n = 3 for each), were within 0.07‰ for $\delta^{15}N$, 0.01‰ for $\delta^{13}C$, and 0.5‰ for $\delta^{34}S$ of certified values.

Data analysis

Seven common estuarine fish species representing a range of trophic guilds, i.e., primary, secondary and tertiary consumers, were chosen for this analysis (for species names and descriptions, see Table 4.1). These fishes were collected from a number of locations throughout each estuary. The authors recognize that consumers occupying different locations within an estuary often differ in their isotopic values (e.g., Chanton and Lewis 2002), specifically those sampled up-river relative to those sampled near the mouth. However, Wilson et al. (2009) and Chanton and Lewis (2002) observed no significant differences in $\delta^{15}N$ and $\delta^{34}S$ values, respectively, of consumers sampled from upper and middle reaches of the Apalachicola Bay. Therefore, because of sample size consideration in this study, we elected to group all individuals of each species, regardless of sampling location. Because fishes were sampled from the two estuaries during the same time-periods annually (i.e., 2006–2008), using the same sampling techniques, isotopic data were pooled from all years for each river (following Layman et al. 2005), to examine whether body size or environmental (i.e., seasonal) factors influence $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ muscle tissue values of individual species and whether evidence exists for size-based seasonal variability in isotopic values.

Body size and seasonal relationships were analyzed using linear mixed-effects models fit using restricted maximum likelihood in the *lme4* package in R (R Core Development Team 2009; Bates and Maechler 2010). Prior to analysis all stable isotope data were tested for normality using quantile-quantile probability plots and log-transformed where appropriate. We developed a set of three candidate models with estuary as the random effect and body size and season as fixed effects: a model with no predictors (Null model; Isotope = $\gamma_0 + \beta_0 + \varepsilon$), and models including the body size (Isotope = $\gamma_0 + \gamma_1$Body Size + $\beta_0 + \varepsilon$) and seasonal (Isotope = $\gamma_0 + \gamma_2$Season + $\beta_0 + \varepsilon$) predictors suspected of influencing isotopic values of the fishes collected during the sampling period. All candidate models were implemented for each species. An examination of the probability plots of residuals from all candidate models relating site-specific species isotopic values to species body size and season sampled, indicated that candidate models fit adequately, and quantile-quantile plots showed data to be generally-described by normally distributed errors for all fishes.

Model selection was based on Akaike's Information Criterion (AIC; Akaike 1973) with small-sample bias adjustment (AIC_c; Hurvich and Tsai 2002). In determining model AIC_c values, both random (i.e., estuary) and fixed (i.e., body size and season) effects were counted as unique parameters and the number of observations used to compute the log-likelihood were used in calculating AIC_c. Models were ranked and compared using AIC_c weights and ΔAIC_c, where AIC_c weights measure the weight in support of the model given the data and ΔAIC_c is the relative difference between the top ranked model and each alternative model. In most cases the model with the lowest AIC_c value was considered the best-supported model. However, when the AIC_c of several

models differed by≤ 2, we considered these models to be equally parsimonious. Additionally, if the number of parameters (K) in comparative models differed by 1, then model selection was based on the log-likelihood, with the best supported model having the lower log-likelihood (Burnham and Anderson 2002). Akaike weights (w_i) were calculated to interpret the weight of evidence for the best fitting model with evidence ratios used to compare among models (Johnson and Omland 2004). For the best supported model, parameter estimates and associated 95% confidence intervals (CIs) were determined using the *HPDinterval* function provided in the *lme4* package in R. For each estimated parameter, predictors were considered significant if the confidence interval did not contain zero. To test the effect of body size and season among estuaries, we calculated the intraclass correlation coefficients (ICC), reflecting the proportion of variance attributable to each level of the model (see Raudenbush and Bryk 2002; Elgee et al. 2010). The ICC approaches 1 when the between-estuary variation is large relative to the within-estuary variation and this coefficient has a 0 value when the within-estuary variation equals the between-estuary variation.

RESULTS

Results from the candidate models used to describe the relationships between $\delta^{15}N$ and season-body size effects in the fishes sampled from both the Caloosahatchee and Myakka estuaries indicated that the null model was the top-ranked model for five out of seven species (i.e., there was no effect of season or body size) (Table 4.2; see Table 4S.1 *Supplemental Material* for full model comparisons for $\delta^{15}N$). However, evidence based on model comparisons indicated that season was the most plausible model

describing the $\delta^{15}N$ values of two species; *Mugil cephalus,* and *Chaetodipterus faber* (Table 4.2). The parameter estimates for season were significant for both species (Fig. 4.2A; i.e. zero was not included within the CI) and evidence ratios estimated for these species, indicate that the model that included season was 48.5 and 47 times more likely than the model that included body size, respectively (see Table 4S.1). Model comparisons indicated depletion in ^{15}N between spring and autumn in *C. faber* (Table 4.1), whereas *M. cephalus* enriched in ^{15}N between spring and autumn.

Relationships between season and body size and $\delta^{13}C$, favored the null model for four of the seven species in this study (Table 4.2; see Table 4S.2*Supplemental Material* for full model comparisons for $\delta^{13}C$), suggesting limited evidence for size or seasonal effects in the data. The most plausible model describing the $\delta^{13}C$ values of *Bagre marinus, M. cephalus* and *Eugerres plumieri* included season (Table 4.2). However, confidence intervals that overlapped zero suggest there is only weak evidence of a seasonal effect on the $\delta^{13}C$ values of *B. marinus* (Fig. 4.2B). Carbon isotope values of *E. plumieri* were generally lower in the autumn relative to the spring, and evidence ratios indicated the model that included season was 2.8 times more likely than the model that included body size. This was also the case for the $\delta^{13}C$ values of *M. cephalus*; a clear depletion in ^{13}C in the autumn (Table 4.1; Fig. 4.2B).

Seasonal variability was identified in four of the seven species using $\delta^{34}S$ (Table 4.2; see Table 4S.3*Supplemental Material* for full model comparisons for $\delta^{34}S$). The support for *C. faber*, *B. marinus* and *Ariopsis felis* was strong (Fig. 4.2C) with the model that included season being 30, 4.1 and 32 times more likely than the model containing body size, respectively (see Table 4S.3). Moreover, depletion in ^{34}S from spring to

autumn was evident for the three species (Table 4.1) further supporting a seasonal effect in both estuaries (Fig. 2C). Alternatively, the best model describing the sulfur isotopes of *E. plumieri* indicated a general enrichment in ^{34}S in the autumn relative to spring (Fig. 4.2C). Intraclass correlation coefficients indicated that the proportion of variance attributable to the seasonal variation within-estuary (61–100%) was greater than the proportion attributable to season between-estuary (0–39%) in all isotopic comparisons in all species, suggesting that seasonal variability was similar between our study locations.

DISCUSSION

Our results provide evidence that for most species examined, season is the dominant influence on isotopic values within the Caloosahatchee and Myakka estuaries relative to body size of the fishes sampled here. Our results are in accordance with those of Wilson et al. (2009), supporting the fact that body size is not an important determinant of isotopic enrichment in estuarine fishes. However, there was evidence for seasonal variability in isotopic values in fish species that spanned several trophic levels and across spatially distinct systems. It is well known that many fishes undergo size-based or ontogenetic changes in diet, and thereby occupy a number of trophic levels in the course of their life history (Winemiller 1990). The absence of intra-specific association between $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$, and body size, suggests that these estuarine fish species do not undergo size-based dietary changes within the size ranges sampled here. However, the seasonal shift in isotopic values supports the finding of Polis and Strong (1996) in that the relative trophic positions of species, whether attributable to a change in diet or a shift in isotopic values of organic matter sources in food webs, are dynamic rather than fixed. The

estuarine fishes examined in the current study exhibit plasticity in their feeding strategy, as they are clearly responding to changes in production source. Differing $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values between seasons, suggests that seasonal variability influences the isotopic values of estuarine fishes, and thus the species interactions and the food web structure of these estuarine systems.

Body size variability

The absence of body-size based- $\delta^{15}N$ relationships in the fishes sampled likely result from (1) dietary preferences of these fishes not shifting within the range of body sizes sampled, (2) the fishes do shift to alternative diets with size, yet the isotope ratios of the new diet are similar to the former and are not reflected in isotopic distinctions, or (3) that spatial and temporal variation in isotopic signatures of prey negate any size-based relationships in higher trophic level species (Vander Zanden et al. 2000). Deudero et al. (2004) observed no size-based $\delta^{15}N$ changes in fishes that fed primarily on small benthic invertebrates, suggesting that although these fishes possess very diverse diets throughout their lives, they likely select prey of relatively similar trophic level. Given the trend of increasing body size-trophic level relationships in large predatory (Scharf et al. 2000) and piscivorous fishes (Deudero et al. 2004), the lack of size-based $\delta^{15}N$ relationships in the fishes included in our study may be a consequence of the fact they are predominantly secondary and tertiary consumers. As such, early life stages (i.e. larvae and young-of-year) generally feed in the pelagic environment on zooplankton and switch to benthic macro-invertebrates in later stages, thus significant size-based $\delta^{15}N$ relationships would likely have been evident from a broader range of sizes that including larval individuals

(Mittelbach and Persson 1998). Nonetheless, similar results for estuarine species of the Apalachicola Bay, an estuary in northern Florida, have been observed (Wilson et al. 2009).

Galván et al. (2010) raised the point that, the absence of a size-based relationship with $\delta^{15}N$ often resulted from the statistical power being too low to detect a significant relationship. This may be the case here, as both sample size and range of sizes sampled were low for a number of species. Yet, given the assumptions for estimating the minimal sample size required to analyze size based feeding relationships using $\delta^{15}N$ (Galván et al. 2010), the body-size independent $\delta^{15}N$ results for 57% of the focal species (4/7) were sampled across size ranges that exceeded Galvan's suggested cutoff. Although, we are confident in our relationships for the majority of species sampled, limited statistical power suggests further sampling may be required for some species. For species that did not meet the sample size minimum for each season, i.e., *Mugil cephalus*, *Lagodon rhomboides* and *Chaetodipterus faber*, improvement in power can be achieved by sampling a greater number of individuals over a broader size range, to confirm the absence of size relationships with $\delta^{15}N$ and seasonal shifts in isotopic values. However, it is important to note that use of estuaries by fishes is often seasonally based (Sheaves et al. 2010) and therefore sampling the entire size range of an individual species may not be possible.

Body size-dependent shifts in isotope ratios that reflect a shift in a consumer's diet can be attributed to either a trophic level shift and/or changes in organic matter source available to a consumer. However, in complex ecosystems, such as tropical floodplain rivers, size-related isotopic shifts are less common than in temperate aquatic

habitats (Jennings et al. 2002), as multiple primary production sources support highly variable trophic assemblages whose interactions may favor a diversification of size across trophic levels (Layman et al. 2005). Our finding that neither $\delta^{13}C$ nor $\delta^{34}S$ was associated with body size suggests the potential for absence of systematic shifts in organic matter source use that could potentially obscure the $\delta^{15}N$ trends with body size, lending support to the lack of evidence of size-based isotopic shifts within our study systems.

Seasonal variability

Body-size dependent diet shifts have been shown to influence temporal variation in aquatic food webs, particularly in highly seasonal systems (Winemiller 1990). Goering et al. (1990) suggested that aside from primary producers, seasonal isotopic variability is confined to relatively short-lived primary consumers because of relatively fast growth and associated tissue turnover rates. This has been supported by studies examining the influence of seasonal variation on producers and consumers (Jennings et al. 2008), attributing the lack of evidence in secondary and tertiary consumers, to weak seasonal variability of the system under examination and to the relatively slow rate of muscle turnover in vertebrate species (MacAvoy et al. 2001). Despite these potential limitations, seasonal variability was evident for all three isotopes employed in our study, a result similar to those reported by Vizzini and Mazzola (2003) from a Mediterranean coastal lagoon, and by Chanton and Lewis (2002) from the Apalachicola Bay.

Generally, with respect to $\delta^{13}C$ and $\delta^{34}S$, the most depleted values were observed in autumn. Although we did not characterize the primary producers of either estuary, overall seasonal variability in $\delta^{13}C$ (mean ± SE; spring, -19.6 ± 0.3‰ and -20.8 ± 0.4‰;

autumn, -20.6 ± 0.4‰ and -20.6 ± 0.3‰) and $\delta^{34}S$ (mean ± SE; spring, 12.9 ± 0.3‰ and 12.7 ± 0.3‰; autumn, 10.3 ± 0.4‰ and 11.1 ± 0.3‰) of all fishes combined in the Caloosahatchee and the Myakka respectively, was relatively low. Shifts in $\delta^{13}C$ and $\delta^{34}S$ are however reflected in the fishes' tissues likely indicating either movement to new habitats or a shift in organic matter source associated with the transition of dry to wet seasons in these estuaries. With the onset of the wet season, both rivers experience increased freshwater flow from natural sources such as rain and subsequent watershed drainage. This source of freshwater into the system could lead to consumers assimilating a more mangrove/upland carbon and sulfur source. The autumnal shift in the sulfur isotope ratios potentially reflects the input of upland/mangrove organic matters sources into the estuaries. The fact that this shift was more evident in $\delta^{34}S$ as opposed to $\delta^{13}C$ may be a consequence of sulfur sources being more distinguishable (i.e., sulfide *vs.* sulfate). Interpreting $\delta^{13}C$ values in estuarine organisms can often be difficult because a mixture of terrestrial (~27‰) and salt-marsh (~13‰) organic matter sources can yield a $\delta^{13}C$ value similar to marine phytoplankton (~21‰; Connelly et al. 2004; Peterson and Fry 1987).

Seasonal variation in isotopic values was prevalent in the majority of fishes, regardless of trophic position. This result has implications for the trophic roles of species in estuarine food webs and the tools we use to identify these relationships within the food web. One way that seasonal variation can influence our conceptual understanding of trophic relationships within estuaries relates to the use of stable isotopes. Because tissue turnover is related to growth and metabolism, rates can vary by species, tissue type and body size. For instance, generally accepted estimates of isotopic turnover in muscle range

from less than a week for larval red drum (*Sciaenops ocellatus*; Herzka and Holt 2000) to > 400 days in juvenile catfish (*Ictalurus punctatus*; MacAvoy et al. 2001) to > 500 days for $\delta^{13}C$ and > 300 days for $\delta^{15}N$ in muscle tissues of juvenile sandbar sharks (*Carcharhinus plumbeus*; Logan and Lutcavage 2010). Consideration of temporal variability in isotope values must be taken into account in all species of the community, despite the expected lag in tissue turnover rates, as shifts in prey resources or environmental conditions can greatly alter isotopic signals.

Spatial variability

The seasonally driven isotopic trends were similar among conspecifics of the Caloosahatchee and Myakka estuaries, as the proportion of variance attributable to seasonal effects within-estuary was greater than that attributable to seasonal effects between-estuary, despite the limitation of small sample size for some species. Arguably, there is the potential that the similar seasonal trends observed here among the estuaries is a result of small sample sizes and that more focused sampling would result in different results. Estuarine consumers however, are known to exhibit omnivory and have the ability to exploit peaks of prey abundance. Isotopic differences among conspecifics have been identified at multiple spatial scales: among habitats within an estuary (Deegan and Garritt 1997) and among neighboring estuaries (Griffin and Valiela 2001). Spatial differences in isotopic values would indicate that fishes adopt site-specific feeding strategies or the variability in the isotopic composition of prey resources. Similar trends between conspecifics of the two rivers, therefore suggests that the seasonal factors

driving the isotopic dynamics of these fish species are of similar magnitude, and that the fishes are responding to environmental factors in a comparable fashion.

We expected that the seasonal isotopic trends of the fishes examined here would have differed over these moderate spatial scales. However, within the southeastern USA, the magnitude of nutrient input entering into estuarine systems depends strongly on riverine discharge and can vary seasonally (Dardeu et al. 1992). In southwest Florida, many rivers are categorized as having the southern river flow pattern, i.e. a significant proportion of riverine annual flow (~60%) is concentrated in the rainy season, which generally occurs in the months of June-September (Kelly and Gore 2008). This is particularly relevant to the Caloosahatchee and Myakka Rivers, and provides a rationale for the similar seasonal trends exhibited between the two estuaries.

Conclusions

We have established that isotopic variation in the Caloosahatchee and Myakka estuaries is influenced by seasonal differences as opposed to size based structuring within fish species. Evidence of seasonal variability among fishes, across a range of trophic levels, suggests that these fishes exhibit plasticity in feeding strategies that may afford greater adaptive flexibility in response to specific changes in food availability resulting from changes in environmental conditions. Likewise, the response of conspecifics between the two estuaries is similar suggesting that the environmental influence on the isotopic composition ($\delta^{13}C$ and $\delta^{34}S$) of these estuarine fishes is of comparable magnitude. These results further suggest that the trophic structure of these estuarine food webs, as indicated by $\delta^{15}N$, is variable among seasons, a result that may be attributable to

the alteration in organic matter and/or nutrient sources associated with changes to hydrological regime.

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Table 4.1 Maximum recorded standard lengths (MSL[1]; cm), length (FL, mean ± SE, range; cm), sample size (*n*), and $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values for muscle tissue (‰ mean ± SE) of fish species sampled seasonally (i.e. spring May–June; autumn September–October) from the Caloosahatchee and Myakka estuaries. For $n < 3$, all values are presented.

Species			Caloosahatchee River					Myakka River				
	Season	MSL[1]	*n*	Length	$\delta^{15}N$	$\delta^{13}C$	$\delta^{34}S$	*n*	Length	$\delta^{15}N$	$\delta^{13}C$	$\delta^{34}S$
Striped mullet												
Mugil cephalus	Spring	90	5	22.8 ± 0.3 (21-24)	7.6 ± 0.3	-14.1 ± 0.8	8.3 ± 0.7	1	19.0	5.74	-14.57	5.23
	Autumn		6	19.0 ± 7.5 (2-43)	9.4 ± 0.2	-21.9 ± 1.8	9.0 ± 1.1	4	69.3 ± 11 (37-86)	7.4 ± 1.0	-19.26 ± 1.5	10.1 ± 2.3
Striped mojarra												
Eugerres plumieri	Spring	34	10	15.5 ± 1.7 (9-23)	10.2 ± 0.3	-18.4 ± 1.3	5.3 ± 1.7	1	9.5	10.0	-22.8	10.6
	Autumn		31	9.0 ± 1.0 (3-24)	10.6 ± 0.3	-21.6 ± 0.8	9.0 ± 0.7	17	4.6 ± 0.5 (1-8)	9.0 ± 0.1	-22.3 ± 0.5	10.6 ± 0.3
Pinfish												
Lagodon rhomboides	Spring	37	12	9.4 ± 0.6 (6-12)	11.2 ± 0.4	-19.4 ± 0.8	13.7 ± 0.8	11	9.7 ± 0.4 (7-12)	9.6 ± 0.2	-21.8 ± 0.5	13.4 ± 0.3
	Autumn		5	12 ± 1.9 (8-18)	10.6 ± 0.6	-18.9 ± 1.0	11.4 ±1.1	3	12.3 ± 0.9 (11-14)	10.0 ± 0.3	-22.2 ± 0.9	13.4 ± 0.4
Atlantic spadefish												
Chaetodipterus faber	Spring	85	10	12.1 ± 1.2 (7-19)	12.2 ± 0.2	-20.6 ± 0.4	13.4 ± 0.4	3	10.5 ± 4.0 (6-19)	11.3 ± 0.2	-21.3 ± 0.6	11.8 ± 0.7
	Autumn		9	17.9 ± 0.9 (11-21)	10.2 ± 0.6	-20.0 ± 0.6	10.9 ± 0.9	2	7.0, 20.0	10.7, 11.5	-22.0, -24.1	12.9, 13.5
Hardhead catfish												
Ariopsis felis	Spring	62	12	30.4 ± 0.8 (25-33)	11.8 ± 0.3	-21.1 ± 0.4	13.5 ± 0.4	10	29.7 ± 1.5 (23-38)	11.0 ± 0.3	-21.4 ± 0.5	12.7 ± 0.6
	Autumn		28	23.5 ± 1.8 (5-35)	12.1 ± 0.3	-21.0 ± 0.4	12.6 ± 0.5	13	24.4 ± 4.0 (6-40)	10.5 ± 0.6	-20.9 ± 0.8	11.0 ± 0.4
Gafftopsail catfish												
Bagre marinus	Spring	60	13	30.0 ± 2.3 (20-50)	12.4 ± 0.7	-20.5 ± 0.7	14.0 ± 0.5	6	42.2 ± 3.8 (29-57)	12.1 ± 0.1	-18.9 ± 0.5	13.6 ± 0.2
	Autumn		10	24.4 ± 4.6 (10-47)	11.1 ± 1.0	-19.1± 1.0	12.9 ± 0.6	16	38.3 ± 2.3 (13-46)	11.1 ± 0.3	-18.9 ± 0.6	12.2 ± 0.3
Bull shark[2]												
Carcharhinus leucas	Spring	180	12	91.3 ± 2.9 (81-106)	13.1 ± 0.1	-18.0 ± 0.4	11.5 ± 0.5	3	102.5 ± 3.0 (87-98)	12.6 ± 0.3	-17.8 ± 0.4	11.1 ± 0.6
	Autumn		3	127.1 ± 18.4 (95-159)	14.1 ± 0.7	-17.7 ± 0.6	13.4 ± 0.5	3	91.6 ± 13.8 (78-126)	12.7 ± 0.2	-18.5 ± 0.7	12.0 ± 0.1

[1]Maximum recorded standard lengths derived from FishBase (Froese and Pauly 2010). Maximum length recorded for *C. leucas* presented here represents size at maturity, as only individuals ranging from neonate to juvenile age classes are common to these estuaries.

[2]Only bull sharks with healed umbilical scars (c. ≥ 1 year old) were included in this study to eliminate any potential for maternal isotopic influence (Olin et al. 2011).

Table 4.2 Model selection results[1] for top-ranked models for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values of each fish species pooled across both estuaries.

Species		Model	*K*	*n*	LogLik	AIC	AIC_c	w_i
Mugil cephalus	$\delta^{15}N$	Season	4	16	-24.21	56.41	60.06	0.97
	$\delta^{13}C$	Season	4		-39.44	86.89	90.52	0.99
	$\delta^{34}S$	Null	3		-39.34	84.69	86.68	0.37
Eugerres plumieri	$\delta^{15}N$	Null	3	59	-98.83	203.70	204.10	0.69
	$\delta^{13}C$	Season	4		-161.00	330.00	330.74	0.71
	$\delta^{34}S$	Season	4		-152.40	312.90	313.54	0.96
Lagodon rhomboides	$\delta^{15}N$	Null	3	31	-49.12	104.20	105.13	0.68
	$\delta^{13}C$	Null	3		-68.04	142.10	142.97	0.57
	$\delta^{34}S$	Null	3		-66.40	138.80	139.69	0.29
Chaetodipterus faber	$\delta^{15}N$	Season	4	25	-40.47	88.93	90.94	0.93
	$\delta^{13}C$	Null	3		-45.61	97.21	98.36	0.70
	$\delta^{34}S$	Season	4		-50.59	109.20	111.18	0.77
Ariopsis felis	$\delta^{15}N$	Null	3	63	-113.00	231.90	232.41	0.73
	$\delta^{13}C$	Null	3		-138.5	283.00	283.41	0.65
	$\delta^{34}S$	Season	4		-127.10	262.10	262.89	0.80
Bagre marinus	$\delta^{15}N$	Null	3	45	-99.38	204.80	205.35	0.28
	$\delta^{13}C$	Season	4		-103.6	215.10	216.20	0.70
	$\delta^{34}S$	Season	4		-81.15	170.30	171.30	0.92
Carcharhinus leucas	$\delta^{15}N$	Null	4	21	-22.18	50.36	51.77	0.34
	$\delta^{13}C$	Null	3		-31.68	69.36	70.77	0.76
	$\delta^{34}S$	Null	3		-37.72	81.45	90.62	0.33

[1]*K*, number of model parameters; *n*, sample size; logLik, model log-likelihood; AIC, Akaike's information criterion; AIC_c, AIC with small-sample bias adjustment; w_i, Akaike's weight.

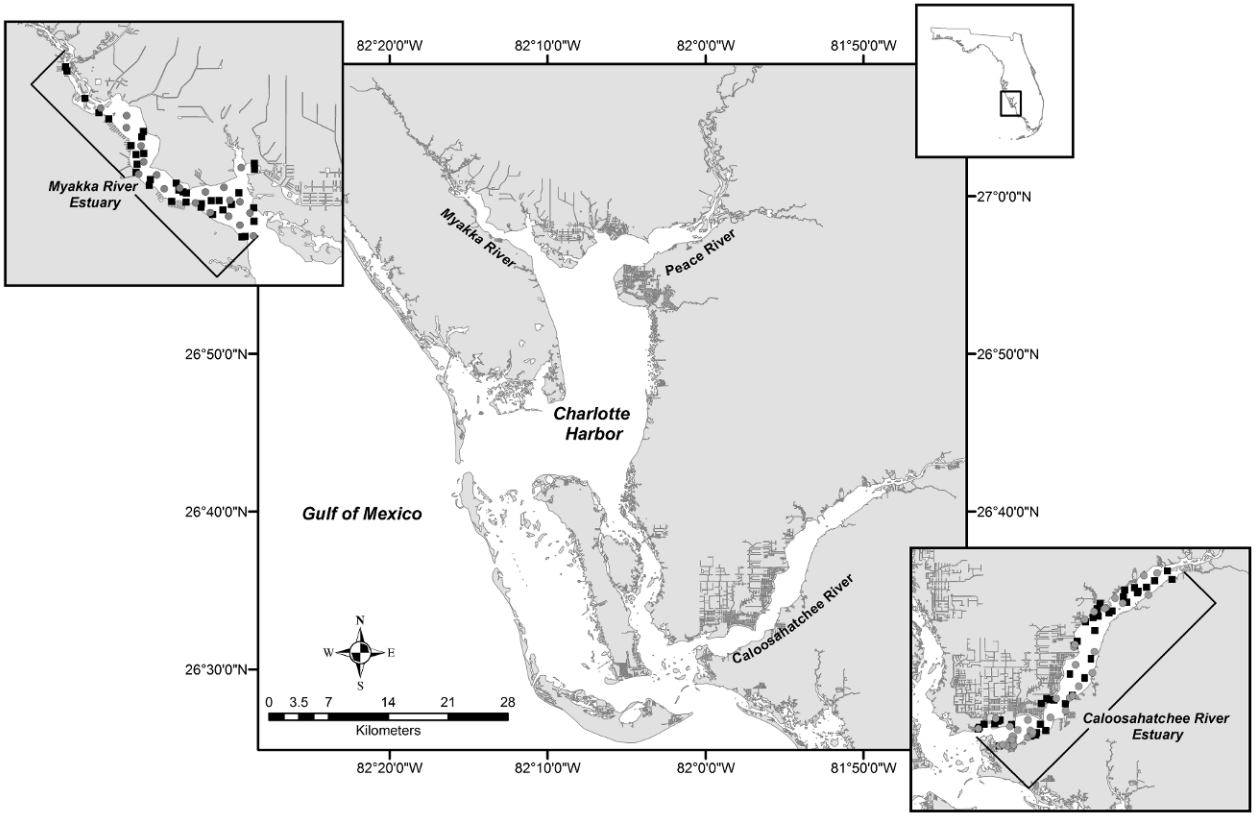

Figure 4.1 Map of the study site showing the locations of the Caloosahatchee and Myakka Rivers with respect to the south western coast of Florida. Insets: Locations of the estuarine portions of the two rivers from which fishes were sampled (black squares represent spring sample locations; gray circles represent autumn sample locations).

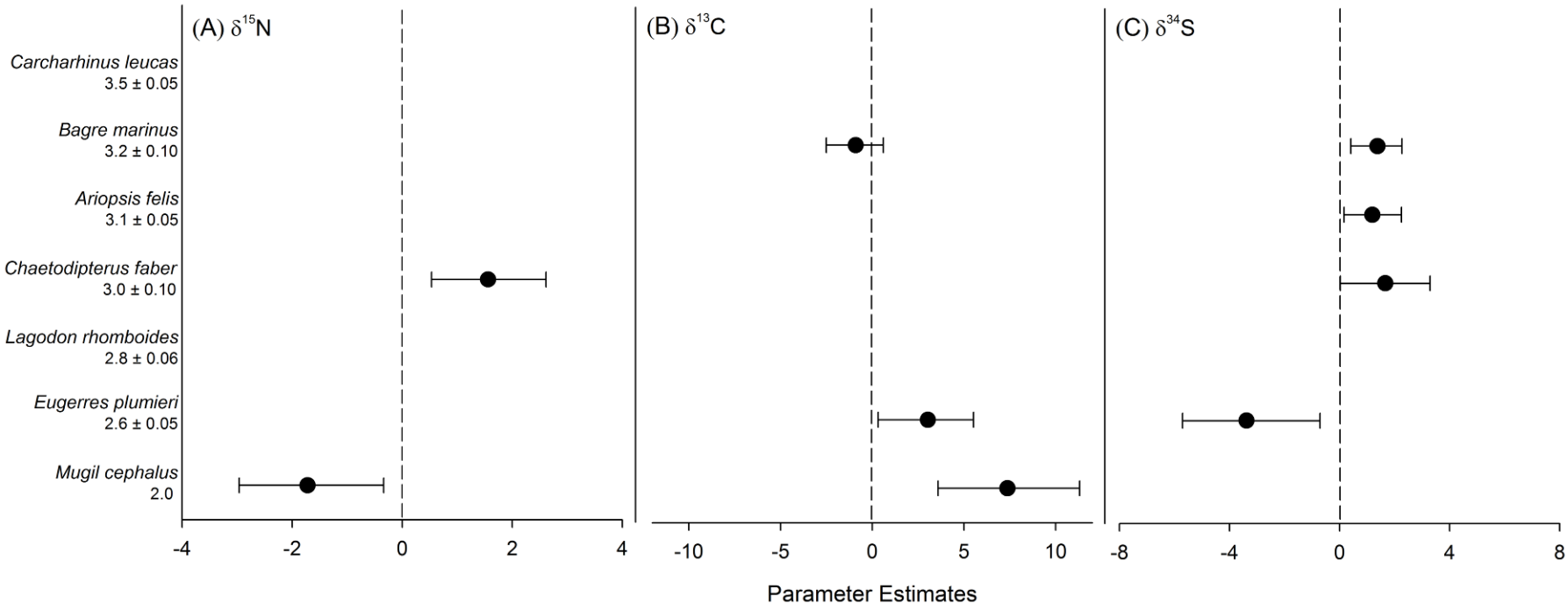

Figure 4.2 Parameter estimate results with 95% confidence intervals for the best-fit models for (A) $\delta^{15}N$, (B) $\delta^{13}C$ and (C) $\delta^{34}S$ values for each fish species sampled from the Caloosahatchee and Myakka estuaries. Symbols indicate species isotopic relationships were best described by season (●) or body size (□) where AIC_c supported such an effect. Negative parameter estimates represent enriched isotopic values in autumn and positive parameter estimates represent depleted isotopic values in autumn. Trophic position[1] is indicated along the y-axis for each species.

[1]Trophic position (TP) was estimated for all fishes using $\delta^{15}N$ as follows: $TP = TP_{baseline} + (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/\Delta^{15}N$, where $TP_{baseline}$ is the estimated TP of the baseline organism, $\delta^{15}N_{consumer}$ and $\delta^{15}N_{baseline}$ are the mean $\delta^{15}N$ of the consumer of interest and of the baseline organism, respectively, and 3.4‰ was used as the $\Delta^{15}N$ (Post 2002). Mean $\delta^{15}N$ of *Mugil cephalus*, designated as TP 2.0, was used as the baseline for all fishes, as this species is characterized as a primary consumer over the size range sampled here (Platell et al. 2006).

SUPPLEMENTAL MATERIAL

Table 4S.1 Model results for $\delta^{15}N$ values of each fish species pooled across both estuaries.

Species	Model	*K*	*n*	LogLik	AIC	AIC_c	ΔAIC_c	w_i
Mugil cephalus	Season*	4	16	-24.21	56.41	60.06	0.00	0.97
	Body Size	4		-28.09	62.19	67.82	7.76	0.02
	Null	3		-30.41	68.82	68.82	8.76	0.01
Eugerres plumieri	Null*	3	59	-98.83	203.70	204.10	0.00	0.69
	Season	4		-98.65	205.30	206.04	1.94	0.26
	Body Size	4		-100.40	208.90	209.54	5.44	0.05
Lagodon rhomboides	Null*	3	31	-49.12	104.20	105.13	0.00	0.68
	Season	4		-48.90	105.80	107.34	2.22	0.23
	Body Size	4		-49.76	107.50	109.06	3.93	0.10
Chaetodipterus faber	Season*	4	25	-40.47	88.93	90.94	0.00	0.93
	Null	3		-44.82	95.65	96.78	5.84	0.05
	Body Size	4		-44.32	96.65	98.64	7.70	0.02
Ariopsis felis	Null*	3	63	-113.00	231.90	232.41	0.00	0.73
	Season	4		-113.00	234.00	234.69	2.28	0.23
	Body Size	4		-115.00	237.90	238.69	6.28	0.03
Bagre marinus	Season	4	45	-97.30	202.60	203.60	0.00	0.69
	Null*	3		-99.38	204.80	205.35	1.75	0.29
	Body Size	4		-100.90	209.80	210.80	7.20	0.02
Carcharhinus leucas	Season	4	21	-19.98	47.96	50.46	0.00	0.66
	Null*	3		-22.18	50.36	51.77	1.31	0.34
	Body Size	4		-25.79	59.57	62.08	11.62	0.00

K, number of model parameters; *n*, sample size; logLik, model log-likelihood; AIC, Akaike's information criterion; AIC_c, AIC with small-sample bias adjustment; ΔAIC_c, estimates the relative difference between the top ranked and each alternative model; w_i, Akaike's weights. * indicates best model for each species.

Table 4S.2 Model results for $\delta^{13}C$ values of each fish species pooled across both estuaries.

Species	Model	*K*	*n*	LogLik	AIC	AIC_c	ΔAIC_c	w_i
Mugil cephalus	Season*	4	16	-39.44	86.89	90.52	0.00	0.99
	Null	3		-46.56	99.13	101.12	10.60	0.00
	Body Size	4		-48.54	105.10	108.72	18.20	0.00
Eugerres plumieri	Season*	4	59	-161.00	330.00	330.74	0.00	0.71
	Body Size	4		-162.10	332.20	332.94	2.20	0.24
	Null	3		-164.70	335.40	335.84	5.10	0.06
Lagodon rhomboides	Null*	3	31	-68.04	142.10	142.97	0.00	0.57
	Season	4		-67.22	142.40	143.98	1.01	0.35
	Body Size	4		-68.70	145.40	146.94	3.97	0.08
Chaetodipterus faber	Null*	3	25	-45.61	97.21	98.36	0.00	0.70
	Season	4		-45.18	98.36	100.36	2.00	0.26
	Body Size	4		-46.84	101.70	103.68	5.32	0.05
Ariopsis felis	Null*	3	63	-138.50	283.00	283.41	0.00	0.65
	Season	4		-138.00	284.00	284.69	1.28	0.34
	Body Size	4		-141.10	290.10	290.89	7.48	0.02
Bagre marinus	Season*	4	45	-103.60	215.10	216.20	0.00	0.70
	Body Size	4		-105.00	216.10	219.00	2.80	0.17
	Null	3		-106.50	221.00	219.59	3.39	0.13
Carcharhinus leucas	Null*	3	21	-31.68	69.36	70.77	0.00	0.76
	Season	4		-31.31	70.62	73.12	2.35	0.23
	Body Size	4		-34.79	77.58	80.08	9.31	0.01

K, number of model parameters; *n*, sample size; logLik, model log-likelihood; AIC, Akaike's information criterion; AIC_c, AIC with small-sample bias adjustment; ΔAIC_c, estimates the relative difference between the top ranked and each alternative model; w_i, Akaike's weights. * indicates best model for each species.

Table 4S.3 Model results for $\delta^{34}S$ values of each fish species pooled across both estuaries.

Species	Model	*K*	*n*	LogLik	AIC	AIC_c	ΔAIC_c	w_i
Mugil cephalus	Season	4	16	-36.99	81.97	85.62	0.00	0.63
	Null*	3		-39.34	84.69	86.68	1.06	0.37
	Body Size	4		-41.68	91.35	95.00	9.38	0.01
Eugerres plumieri	Season*	4	59	-152.40	312.90	313.54	0.00	0.96
	Null	3		-157.10	320.10	320.64	7.10	0.03
	Body Size	4		-157.20	322.30	323.14	9.60	0.01
Lagodon rhomboides	Season	4	31	-64.20	136.40	137.94	0.00	0.68
	Null*	3		-66.40	138.80	136.69	1.75	0.29
	Body Size	4		-67.32	142.60	144.18	6.24	0.03
Chaetodipterus faber	Season*	4	25	-50.59	109.20	111.18	0.00	0.77
	Null	3		-53.38	112.80	113.90	2.72	0.17
	Body Size	4		-53.71	115.40	117.42	6.24	0.03
Ariopsis felis	Season*	4	63	-127.10	262.10	262.89	0.00	0.80
	Null	3		-129.80	265.70	266.01	3.12	0.17
	Body Size	4		-130.20	268.30	269.09	6.20	0.04
Bagre marinus	Season*	4	45	-81.15	170.30	171.30	0.00	0.92
	Null	3		-85.10	178.20	176.79	5.49	0.06
	Body Size	4		-85.24	176.50	179.48	8.18	0.02
Carcharhinus leucas	Season	3	21	-35.50	78.99	81.50	0.00	0.66
	Null*	4		-37.72	81.45	82.85	1.35	0.33
	Body Size	4		-40.06	88.12	90.62	9.12	0.01

K, number of model parameters; *n*, sample size; logLik, model log-likelihood; AIC, Akaike's information criterion; AIC_c, AIC with small-sample bias adjustment; ΔAIC_c, estimates the relative difference between the top ranked and each alternative model; w_i, Akaike's weights. * indicates best model for each species.

CHAPTER 5

GOING WITH THE FLOW: SEASONAL SHIFTS IN THE FLOW OF ENERGY THROUGH AN ESTUARINE FOOD WEB EXPERIENCING ALTERED HIGH FLOW

INTRODUCTION

Hydrological connectivity or the water-mediated transfer of matter, energy and/or organisms within or between elements of the hydrological cycle, is considered to be the most influential factor driving aquatic ecosystem dynamics (Pringle 2001). Anthropogenic alterations to this connectivity, in the form of dams and diversions have resulted in habitat fragmentation and degradation, and modifications to river flow (Nilsson et al. 2005; Lotze et al. 2006). Modifications to river flow, primarily driven by appropriation of freshwater for human use, is considered the most pervasive and deleterious effect on rivers (Kingsford 2011). As few estuarine systems world-wide remain unaffected by upstream manipulation of their freshwater inflow (Dynesius and Nilsson 1994), these modifications can have major implications for individual species and thus the structure of downstream estuarine and coastal marine communities (Edeline et al. 2005; Serrano et al. 2010; Olin et al. *in review*).

The contribution of freshwater to downstream habitats is regarded as a critical landscape process in riverine systems (Sklar and Browder 1998), regulating the physical, chemical and biological properties of terrestrial, lacustrine, and marine environments (Paerl et al. 2010; Rush et al. 2010). Within estuaries, freshwater inflow from riverine sources provides nutrients, sediment and organic matter essential for primary and secondary production (Mallin et al. 1993; Drinkwater and Frank 1994; Chanton and Lewis 2002). Life history strategies (e.g., breeding, spawning and recruitment) of estuarine species are commonly synchronized with particular flow patterns (Bunn and Arthington 2002; Rehage and Trexler 2006) and variable salinity tolerances can produce

communities segregated along salinity gradients (Rakocinski et al. 1992; Gelwick et al. 2001; Montagna et al. 2002; Akin et al. 2003).

The occurrence of high freshwater flow events are considered a major form of disturbance in riverine and estuarine systems and are often influential in restructuring communities (Resh et al. 1988; Montagna et al. 2002), such as oyster reefs (e.g. Tolley et al. 2006) and nekton assemblages (e.g. Olin et al. *in review*). Specifically, Olin et al. (*in review*) demonstrated variable responses by nekton assemblages to natural and altered flow patterns, whereby an increase in nekton density, diversity and species richness was observed with the increase of flow in a natural estuary, whereas no changes in the same metrics were observed in a flow-altered estuary. Thereby, suggesting a loss of seasonal variability in these nekton assemblages with extreme high flows. This has broad implications for individual species, in terms of their feeding ecology, trophic interactions and dietary resource use, which in turn can impact ecological function and overall stability of communities. From a community perspective, alterations to the estuarine salinity gradient as a result of extreme high flows are anticipated to be most evident among lower trophic level species (i.e., primary and secondary consumers). This prediction is based on primary and secondary consumers having limited mobility, yet are capable of assimilating variable mixtures of locally-based organic matter sources (Deegan and Garritt 1997; Wainright et al. 2000; Hsieh et al. 2002), that often coincide with changes in physiochemical processes (McLeod and Wing 2008).

The aim of this study was to test the hypothesis that estuarine food webs differ between dry and wet seasons, and in so doing, specifically address the influence of anthropogenically-induced high flow. Our objectives were to, (1) determine the effect of

altered-flow, especially extreme high flow on estuarine food web structure; and (2) determine the magnitude to which low *vs*. high flow affects the relative trophic position of individual species. To accomplish this and to provide a context for our results, we compare seasonal (i.e., transition from dry to wet season) trends in stable isotopes of carbon, ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$) between estuaries of two tidal rivers; one that has undergone major human development and experiences an altered-flow regime, and one that is relatively natural. A significant proportion of annual riverine flow (~60%) is concentrated in the wet season (i.e., June-September) in the majority of rivers in southwest Florida (Kelly and Gore 2008). A fundamental premise of our analysis is that the wet season is further exaggerated by anthropogenic-altered flow in the modified river. Shifts in isotopic values of estuarine species have been observed to occur with extreme high flows, particularly those associated with heavy rains and monsoons (Abrantes and Sheaves 2010; Wai et al. 2008).With the exaggerated wet season we therefore expect that species sampled following the dry season will be enriched in ^{13}C and ^{34}S relative to those sampled following the wet season, reflecting a polyhaline estuarine status (i.e., tidally influenced). In contrast, those sampled following the wet season would have depleted ^{13}C and ^{34}S values, reflective of an oligohaline estuarine status (i.e., terrestrial/freshwater influenced; Chanton and Lewis 2002).

MATERIALS AND METHODS

Study sites

The Caloosahatchee River, located on the southwest coast of Florida, (26°30' N, 81°54' W) is a major tributary of Charlotte Harbor, Florida, USA (Fig. 5.1). The

Caloosahatchee River watershed drains an area of approximately 4,550 km^2. Prior to the artificial connection to Lake Okeechobee, the Caloosahatchee River was a smaller, meandering river originating at the west end of Lake Flirt and extending to Beautiful Island in Ft. Myers (Flaig and Capece 1998). Intensive agriculture became the major land use in the watershed with the construction of extensive drainage projects in the 1880's; additional channelization and construction have occurred at Moore Haven (S-77), Ortona (S-78) and Franklin Lock and Dam (S-79) (Flaig and Capece 1998). The Caloosahatchee River currently extends about 68 km from Lake Okeechobee to S-79. This final downstream structure defines the beginning of the Caloosahatchee Estuary and extends for approximately 42 km to San Carlos Bay. These modifications to the hydrology of the Caloosahatchee River in combination with land-use development (e.g., Ft. Myers) have resulted in large-scale alterations in the estuary (Barnes 2005). The salinity gradient of the Caloosahatchee estuary cycles annually; during the winter/spring months (dry season) the estuary ranges from mesohaline (salinity ranging from 5 to 18‰) to polyhaline (salinity range of 18 to 30‰) and during the summer/autumn months (wet season) the estuary can become exclusively oligohaline (salinity range 0 to 5‰), with minimal tidal influence (Fig. 5.2; Doering and Chamberlain 1998; Flaig and Capece 1998). This transition between dry and wet seasons can be rapid, often occurring in less than a week (Doering and Chamberlain 1998). After flows decrease, the river returns to a mesohaline gradient.

The Myakka River (82°12' W, 26°57' N), draining into the northern portion of Charlotte Harbor, was selected as a control site for comparison with the Caloosahatchee. The Myakka River was chosen for several reasons; (1) it is proximately located (< 100

km; Fig. 5.1) to the Caloosahatchee River, and therefore is accessible by fishes and macro-invertebrates of the Charlotte Harbor and; (2) in contrast to the Caloosahatchee River, it experiences relatively natural flow periods and its shoreline areas have been subjected to relatively minor anthropogenic modification. Further, although, much of the shoreline habitat of the Caloosahatchee estuary has largely been altered by urbanization, as evidenced by extensive shoreline modifications, the upper reaches and some downstream areas are composed of similar ecological communities, including saltmarsh and mangrove species. Specifically, the natural shoreline areas of both estuaries are characterized by mangroves and saltmarsh, principally *R. mangle,* black mangrove *Avicennia germinans,* saltmarsh cordgrass *Spartina alterniflora* and black needlerush *Juncus roemerianus.* Palmer et al. (2011) and Vinagre et al. (2011) conducted comparisons of community and food web structure of proximate estuaries respectively, citing similar species composition among the two study systems. In this context, the Myakka estuary provides a reference by which a comparison of food web dynamics to the Caloosahatchee estuary can be made.

Sample collection

Samples were collected during 2008 targeting the dry (May and June) and wet (September and October) seasons that occur in the Myakka and Caloosahatchee estuaries (Fig. 5.2). In an effort to sample a broad range of nekton species (see Table 5.1 for a complete list of species sampled), shallow water (< 10 m) longlines (800 m), seines (21.3 m with 3.2-mm stretch mesh, center bag), and trawls (6.1-m 3with 8-mm stretch mesh, 3.2-mm stretch mesh liner) were used for all collections. Longlines were set for periods

from 30 min to 2 h, with most set for approximately 1.5 h. The trawl was towed for 5–7 minutes at 0.6 $m \cdot s^{-1}$, providing a tow length of ~180 m. Trawl width averaged ~4 m, providing an approximate area of 720 m^2 sampled by a typical tow. The seine was deployed from a boat in a shallow arc parallel to shore and hauled directly along the shoreline. The two ends of the seine were pulled together, sampling an area of ~68 m^2.

During each sampling event, environmental parameters—including temperature (°C), salinity (ppt) and dissolved oxygen (mgl^{-1})—were recorded from depths ranging from 0.5 to 2.5 m, using an YSI water quality meter (YSI Inc., Yellow Springs, OH, USA; see Table 5S.1 *Supplemental Material*). Upon collection, all fishes and macro-invertebrates were measured; standard length for fishes, carapace width for crabs and disc width for stingrays (to the nearest mm). White muscle tissue was excised from the dorsal area anterior to the first dorsal fin from all fishes and from the dorsal surface from stingrays. Oysters and crabs were dissected prior to drying and only soft tissue was retained for stable isotope analyses. Muscle tissue samples were stored on ice in the field and then stored frozen upon return to the laboratory (-20° C).

Stable isotope analysis

Muscle tissues were sub-sampled (~1.0 g), freeze-dried for 48 h, and homogenized in a SPEX CertiPrep 8000-D ball milling unit (SPEX CertiPrep, Metuchen, New Jersey). Lipids are depleted in ^{13}C relative to other major tissue components (i.e., proteins and carbohydrates; DeNiro and Epstein 1977) and their presence in muscle tissue samples can negatively skew observed $\delta^{13}C$ values (Post et al. 2007). To standardize $\delta^{13}C$ values within and among species, lipids were removed from all samples prior to isotopic

analysis using a modified method outlined by Bligh and Dyer (1959): twice vortexing the pulverized tissue in 5 ml of 2:1 chloroform: methanol solution for 24 h and decanting the solvent through filter paper to isolate the lipid-free study sample.

Relative abundances of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) isotopes were determined on ~0.5 mg sub-samples sealed in tin capsules on a Thermo Finnigan Delta^{Plus} mass-spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled with an elemental analyzer (Costech, Valencia, CA, USA) at the Great Lakes Institute for Environmental Research. Relative abundances of sulfur ($^{34}S/^{32}S$) were determined on ~2 mg and ~ 6 mg sub-samples sealed in tin capsules on an Isochrom Continuous Flow IRMS (GV Instruments / Micromass, UK) coupled with an elemental analyzer (Costech, Valencia, CA, USA), at the Environmental Isotope Laboratory, University of Waterloo and by a Thermo-Electron Delta^{Plus} Advantage IRMS at the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University, respectively.

Stable isotope results are expressed in standard delta notation (δ), defined as parts per thousand as follows: $\delta = [(R_{sample}/R_{standard}) -1] \times 10^3$ (Peterson and Fry 1987), where R is the ratio of heavy to light isotopes in the sample and standard. The standard reference material was atmospheric nitrogen for N_2, Pee Dee Belemnite carbonate for CO_2, and Canyon Diablo Troilite for SO_4. The analytical precision based on the standard deviation of two standards (NIST 8414 and internal fish muscle lab standard; $n = 76$) for $\delta^{15}N$ were 0.10‰ and 0.21‰ and for $\delta^{13}C$ were 0.06‰ and 0.09‰, respectively, and based on three sulfide standards (NBS-123, EIl-40 and EIL-43) for $\delta^{34}S$ was 0.3‰. Analytical accuracy based on the analysis of NIST standards, performed with muscle tissue sample, sucrose (NIST 8542), ammonium sulfate (NIST 8547) and bovine liver and mussel samples (n =

3 for each), were within 0.07‰ for $\delta^{15}N$, 0.01‰ for $\delta^{13}C$, and 0.5‰ for $\delta^{34}S$, of certified values.

Data analysis

To examine the effect of season (dry *vs*. wet) on the food webs of the Myakka (11 consumer species) and the Caloosahatchee estuaries (12 consumer species), analysis of variance (ANOVA) was applied to the $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ data of each species for each estuary, separately. To further differentiate the food web response to altered flow, all species were (1) assigned to one of four groups termed "trophic guilds" (see below) and (2) to one of two groups termed "resource use categories" representing either pelagic or benthic feeders. All assignments were based on dietary data from the literature (see Table 2 for designation). Trophic guilds were defined as: primary consumer, diet composed largely of algae and detritus (>70%); secondary consumer, diet composed primarily of invertebrate species; tertiary consumer, diet composed of both fishes and invertebrates and; piscivore, diet composed primarily of fishes (> 80%). To examine the influence of altered high-flow, resource use and their interaction on the defined trophic guilds, a two-factor ANOVA was applied to the $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ data of the secondary and tertiary consumers in the Myakka, and the primary and secondary consumers in the Caloosahatchee, as those trophic guilds included both pelagic and benthic feeders.

Prior to all analyses, stable isotope data were tested for normality using Shapiro-Wilks test and for homogeneity of variance using Bartlett's test. Isotope data were log transformed to meet assumptions. All analyses were conducted in R 2.13.0 (R

Development Core Team 2011) with a criterion for significance of $P < 0.05$ used for all statistical tests.

RESULTS

For the Myakka, the natural system, a similar range and magnitude of stable isotope values of $\delta^{13}C$ in the dry and wet seasons, respectively [(absolute range: 10.5‰ (range: -14.6 to -25.1) *vs*. 11.8‰ (range: -14.1 to -25.9)] and of $\delta^{15}N$ [7.6‰ (range: 5.7 to 13.3) *vs*. 6.6‰ (range: 6.5 to 13.1)] was observed (Fig. 5.3). The range in $\delta^{34}S$ values, however, differed between dry and wet seasons [9.6‰ (range: 5.2 to 14.8) *vs*. 7.3‰ (range: 7.3 to 14.7)]. Similarly, in the Caloosahatchee, the range and magnitude of stable isotope values observed between seasons were comparable [(absolute range: 14.8‰ (range: -12.4 to -27.2) *vs*. 14.8‰ (range: -14.3 to -29.1) for $\delta^{13}C$; 9.6‰ (range: 4.5 to 14.1) *vs*. 8.3‰ (range: 6.1 to 14.4) for $\delta^{15}N$; 15.3‰ (range: -0.3 to 15.0) *vs*. 15.0‰ (range: 1.4 to 16.4) for $\delta^{34}S$, respectively (Fig. 5.4)]). However, in contrast to the Myakka, a clear shift in the range of food web values was observed in the Caloosahatchee; depletion in ^{13}C and enrichment in ^{15}N of ~2‰. For each isotope the absolute range of values in the dry and wet seasons were greater in the Caloosahatchee relative to the Myakka (Fig. 5.3, 5.4).

In the Caloosahatchee, changes to the species-level $\delta^{15}N$-, $\delta^{13}C$- and $\delta^{34}S$-season relationships were predominantly driven by primary and secondary consumers (see Table 5S.2 *Supplemental Material* for ANOVA statistics). Species whose $\delta^{13}C$ values varied significantly between season were all depleted in ^{13}C following the wet season (Fig. 5.5A). For species that did not exhibit a significant shift in $\delta^{13}C$, a declining trend in $\delta^{13}C$

values with the wet season was observed, with the exception of *Crassostrea virginica* and *Eugerres plumieri* (Fig. 5.5A). The $\delta^{15}N$ values of the majority of primary and secondary consumers were significantly enriched in ^{15}N following altered-high flow (i.e. Fig. 5.5B). The $\delta^{15}N$ values of the primary consumers, *C. virginica* and *M. cephalus*, significantly increased by approximately 2‰ and 1.5‰, respectively between seasons (Fig. 5.5B). Unlike the Myakka, the $\delta^{34}S$ values of tertiary consumers of the Caloosahatchee did not show an overall depletion in ^{34}S. On the contrary, *Lutjanus griseus* exhibited an enrichment in ^{34}S following the wet season (Fig. 5.5C).

In contrast to the Caloosahatchee, species in the Myakka exhibited a mixed response to the onset of the wet season, but overall significant shifts in isotope values were limited to only a few species (Fig. 5.5D-F; see Table 5S.2 *Supplemental Material* for ANOVA statistics). These differences were principally driven by tertiary consumers. No overall trend of depletion or enrichment was identified for ^{13}C (Fig. 5.5D) or ^{15}N (Fig. 5.5E). However significant depletion in ^{34}S was identified in the tertiary consumers, *Ariopsis felis*, *Bagre marinus*, and *Cynoscion arenarius* in the wet season (Fig. 5.5F).

When considering the relationships between season and resource use of trophic guilds, in the Myakka, both secondary and tertiary consumers exhibited significant differences in $\delta^{34}S$ between seasons (Table 5.3). The $\delta^{15}N$ values varied significantly with resource use category in the secondary consumers, and with the interaction in tertiary consumers (Table 5.3). The $\delta^{13}C$ values varied significantly with resource use in the tertiary consumers (Table 5.3). Together these results support the idea that benthic and pelagic species derive their energy from different components of the food web. In contrast, in the Caloosahatchee, the $\delta^{13}C$ and $\delta^{15}N$ values varied significantly with

season, resource use category and in the case of $\delta^{13}C$ the interaction for primary consumers (i.e., *C. virginica* and *M. cephalus*; Table 5.3), indicating that altered-high flow affects both pelagic and benthic components of the food web. For $\delta^{13}C$, this is specifically driven by the depletion observed in *M. cephalus*, whereas for $\delta^{15}N$, both primary consumers showed enriched values of ~1.5‰ in the wet season. However, no $\delta^{13}C$ or $\delta^{15}N$ effects were observed in secondary consumers. Statistically significant differences in $\delta^{34}S$ were limited to resource use, but were identified in both the primary and secondary consumer trophic guilds (Table 5.3).

DISCUSSION

As the extent of alterations to natural hydrological connectivity increases to accommodate the growing human demand for water resources, understanding the effects of freshwater flow alteration are crucial for management and sustainability of estuarine systems worldwide. Our comparison of seasonal dynamics of nekton assemblages in two tidal estuaries that experience vastly different flow patterns indicates that anthropogenically altered-high flow results in changes to isotopic values of estuarine species that are not evident in a natural system. In the Myakka estuary, where the hydrology is more natural than in the Caloosahatchee, there were no clear seasonal isotopic patterns, with the exception of more estuarine $\delta^{34}S$ values of tertiary consumers in the wet season. In the Caloosahatchee estuary, the results revealed a dichotomous response by estuarine species to altered-high flow. Specifically primary and secondary consumers exhibited a distinct shift in $\delta^{13}C$ and $\delta^{15}N$, whereas evidence of a weaker response among higher trophic level species was detected. This shift in $\delta^{15}N$ values and

$\delta^{13}C$ to a lesser extent of the primary consumers (i.e. *Crassostrea virginica* and *Mugil cephalus*), suggests that altered-high flow affected both pelagic and benthic components of this estuarine food web. Differences in the response of conspecifics in the Caloosahatchee support the assertion that high freshwater flow differentially affects organisms and could indicate that the variable responses may be related to species-specific behaviour, size or life-history characteristics (Power et al. 1996).

Estuaries that are strongly influenced by hydrologic conditions have been observed to reflect seasonal differences in their basal productivity (Kaldy et al. 2005). Our hypothesis was that species sampled following the dry season would reflect tidal influence (i.e., enriched values of ^{13}C and ^{34}S) relative to those species sampled following the compounded wet season, which would reflect freshwater influences (i.e. depleted values of ^{13}C and ^{34}S). Given the extreme high flow event, this hypothesis was supported in the Caloosahatchee by the $\delta^{13}C$ data and was particularly evident in lower trophic level species. The observed trends are consistent with our expectations of assimilation by the estuarine species of a ^{13}C-depleted source following high flow. This variation may be attributed to two effects: the increasing influence of terrestrial organic matter with extreme high flow and/or the increasing influence of ^{13}C-depleted dissolved inorganic carbon (DIC) sourcing phytoplankton in waters with decreasing salinity (Chanton and Lewis 2002). When considering the values for marine organic matter and carbon from plants that use the C_4 photosynthetic process, they are enriched in ^{13}C ($\delta^{13}C$ of marine plants, -18 to -22‰; $\delta^{13}C$ of C_4 plants, -6 to -19‰) relative to carbon sourced from C_3 plants and terrestrial sources ($\delta^{13}C$ of C_3 plants, -24 to -30‰) (Moncreiff and Sullivan 2001; Winemiller et al. 2007). Marine plankton (-22‰; Chanton and Lewis 1999) also

tends to be more enriched than riverine plankton (-28‰; Chanton and Lewis 1999). While the $\delta^{13}C$ values of the consumers sampled from the Caloosahatchee remain more similar to the marine end of the spectrum even after the wet season, there is a distinct shift in primary and secondary consumers toward the terrestrial end of the spectrum.

With known species that partition their feeding strategies and resource use between benthic *vs.* pelagic food webs (Table 5.2), our data suggest that lower trophic levels, despite feeding in the benthic or pelagic food web, appear to be assimilating a similar carbon source in the wet season. Firstly, there was a distinct depletion in ^{13}C in primary and secondary consumers in the modified estuary with altered high flow. Whether the shift to more depleted ^{13}C is a result of higher phytoplankton productivity or inputs of terrestrial organic matter remains to be understood. And, unlike data presented by Chanton and Lewis (2002), there was a general absence of ^{34}S depletion and minimal differences in $\delta^{34}S$ values of species between seasons in the Caloosahatchee. Likely, these results were observed because the mixing dynamics of estuaries favor the dominant seawater sulfate source; the contribution of marine sulfate overwhelms the signal of riverine sulfur, even at a salinity of l% (Chanton and Lewis 1999). However, the two species that generally feed on pelagic resources, *C. virginica* and *Chaetodipterus faber*, showed a similar depletion in ^{34}S following high flow, suggestive of a sulfate from a freshwater source (Fry and Chumchal 2011).

Temporal and spatial variation in basal resource $\delta^{13}C$ and $\delta^{15}N$ contributes to variation in the isotopic signatures of consumers (Vander Zanden and Rasmussen 1999; Matthews and Mazumder 2003). Therefore, ecosystem changes, such as shifts in salinity regime or availability of terrestrial organic matter are likely first evidenced in the diet of

primary consumers, such as bivalves. Because it is relatively long-lived and sedentary, the oyster, *C. virginica*—a good indicator species for ecosystem alteration—can provide an important link between terrestrial organic matter and higher trophic level consumers (Riera and Richard 1996, 1997; Chanton and Lewis 2002; Wilson et al. 2010). The fact that $\delta^{13}C$ of *C. virginica* sampled from the Caloosahatchee did not shift between seasons in this study, maintaining $\delta^{13}C$ values of ~-23‰, suggests continued use of a plankton-based organic matter; however, the depletion in ^{34}S and enrichment in ^{15}N of the muscle tissue of *C. virginica* between seasons, provides further support of a freshwater/terrestrial influence from the available water-column carbon. Moreover, the significant depletion in ^{13}C of the lower trophic level species (i.e., *M. cephalus*, *Callinectes sapidus* and *Eucinostomus harengulus*) also supports a shift to a depleted ^{13}C source. Although *C. virginica* was not sampled in the Myakka, the $\delta^{13}C$ trend of the benthic primary consumer *M. cephalus* is similar to conspecifics in the Caloosahatchee, suggesting that the wet season does alter isotopic values of lower trophic level consumers. Regardless of whether *C. virginica* in the Myakka does exhibit depletion in ^{13}C, these trends are not propagated to higher trophic levels in that system. The significant $\delta^{13}C$ shifts in the Caloosahatchee with high flow strongly supports the trend of increased terrestrial influence. However stable isotope values of primary production and organic matter sources would be required to confirm these conclusions.

Stable isotope ratios of nitrogen generally increased following altered-high flow in the modified Caloosahatchee, most notably in the primary and secondary consumers. This trend was not apparent in the natural Myakka Estuary. It is unlikely that variation in body size contributes significantly to the observed differences in $\delta^{15}N$ of conspecifics

between seasons as demonstrated by Olin et al. (*in revision*). As well, it is unlikely that prey resources shifted with altered flow, as unlike the positive response observed in the Myakka, neither density nor species richness of consumers changed between seasons in the Caloosahatchee Estuary (Olin et al. *in review*). Rather, these differences likely reflected high nutrient loads associated with freshwater inflow, similar to McClelland and Valiela (1998) who demonstrated a strong link between proximity to urbanization and elevated $\delta^{15}N$ values in estuarine species. The Caloosahatchee River and downstream estuary receive considerable urban and agricultural runoff (Flaig and Capece 1998). Although the variable levels of enrichment in ^{15}N were observed throughout the community and may be interpreted as relatively minor in the higher trophic level species, the increase in $\delta^{15}N$ values is consistent with the decrease in values of $\delta^{13}C$. This further supports the conclusion that altered-high flow influences the available production and nutrient resources available to these consumers.

A critical assumption, however, is that the species we examined exhibited site fidelity, i.e. that they were present long enough to acquire the dominant isotopic signal of the system. In our study, the $\delta^{13}C$ of all primary and secondary consumers that exhibited significant temporal differences decreased to a similar value. This result, coupled with the enrichment of ^{15}N in the majority of primary and secondary consumers, supports the contention that most species were not moving to alternative habitats and were likely integrating similar production sources. Indeed, a number of studies have demonstrated that estuarine consumer species exhibit site fidelity and their tissues reflect the organic matter close to the areas in which they inhabit (Deegan and Garritt 1997; Guest and Connolly 2004).

The magnitude of response of the upper trophic level species to extreme high flow in the Caloosahatchee was less relative to the lower trophic level species, based on stable isotope values. Specifically, the absence of significant isotopic changes in the upper trophic levels indicates that the shifts evidenced in the isotopes of the lower trophic levels were not observed throughout the food web. One explanation for this response difference could be that these upper trophic level species, which are generally more mobile, migrated out of the Caloosahatchee estuary during high flow and continued to feed on resources with similar isotope values. However the species considered were sampled within the estuary during both collection periods. Moreover, the $\delta^{13}C$ and $\delta^{15}N$ trends of these species were similar to those observed in the lower trophic levels, suggesting that movement to, and subsequent feeding in a different ecosystem is unlikely. Rather, it could be argued that the absence of a significant change in the isotopic values in the upper trophic levels of the Caloosahatchee may indicate that the duration of high freshwater flow was too short to elicit a shift in the isotope values of these species. Given these species are of relatively large body size when compared with the primary and secondary consumers, there would likely be a delay in the transfer of the new isotope values from the lower trophic levels to those of higher trophic levels as a result of (1) variable muscle tissue turnover rates in higher trophic level species and/or (2) a lag associated with movement of different isotopic values through the food web (e.g., Guelinckx et al. 2007; Jennings et al. 2008). This is not to suggest that the isotopic values of higher trophic level species do not change in a similar fashion to lower trophic level species, just that the time associated with the trophic transfer of isotopes is longer than the duration of the disturbance. This has consequences for using stable isotopes to assess

trophic ecology of species that have isotope turnover times in sampled tissues that are longer than disturbance events (i.e., events that alter isotope values at the base of the food web). Sampling of high turnover tissues, for example blood plasma (Hobson and Clark 1992), could aid in clarifying effect of high flow disturbance events on higher trophic level species. Likewise, the use of alternative chemical tracers, such as fatty acids, could provide a complementary mechanism for identifying temporal changes in prey resources to a consumer (Hebert et al. 2009), and understanding the physiological response of an organism (Arts et al. 2001) to this category of disturbance.

Although specific conclusions about diet and resource use by higher trophic level species under the different freshwater flow regimes cannot be made, these results indicated that higher trophic levels species are not as influenced by the high-flow in the Caloosahatchee when compared to lower trophic level species. Since the stable isotope values of muscle tissue reflect diet assimilated over a specific time period, minimal change in $\delta^{15}N$ and $\delta^{13}C$ of the higher trophic species residing in the Caloosahatchee through both seasons, indicated that the body composition of these animals, reflect resources assimilated during both hydrologic regimes. Importantly, it also suggests that disturbance of this magnitude does not systematically affect the upper trophic level species included here, based on stable isotopes. In contrast the tissues of the lower trophic level species of the Caloosahatchee reflected resources assimilated during each of the seasons.

It is important to note, that if this disturbance event in the Caloosahatchee was of longer duration or occurred more frequently, for example as predicted by global climate change models (Pearlstine et al. 2010), then this alteration of the salinity gradient may

have more serious consequences, particularly with respect to the physiological and dietary requirements of these species (e.g., maintaining osmotic balance; Nordlie 2006). Indeed, Olin et al. (in review) demonstrated a loss of diversity and richness of marine migrant species sampled via trawl and seine with the wet season in the Caloosahatchee. Jack et al. (2009) further demonstrated the consequences of prolonged low-salinity events which resulted in alteration to the diet of the red rock lobster, *Jasus edwardsii*, to a less preferable species. The authors attributed this diet shift to reductions in the abundance of filter-feeding invertebrates, including oysters (Pollack et al. 2011) and infaunal clams and mussels (Rutger and Wing 2006; Jack et al. 2009). It is therefore critical to understand the effects of anthropogenic modifications to hydrology on food web dynamics, as community structure may be compromised and simplified through extirpation of non-tolerant species. To advance our understanding of the species- and food web-level effects observed in this study will require future studies that focus on determining seasonal trends in primary production and organic matter sources, as well as monitoring trophic structure of food webs that experience varying flow management strategies, for example, pulse-release.

Conclusions

Establishment of freshwater inflow criteria is becoming increasingly important (e.g., Arthington et al. 2006) however, development of these criteria is dependent on understanding the response of communities to altered freshwater flow. This study highlights shifts in food web structure likely driven by resource use (i.e., production source) that occur in consumers, predominantly lower trophic level species of estuarine

communities, faced with altered flow. Shifts in resource use by primary and secondary consumers with flow, are supported by previous studies in modified systems (Jack et al. 2009; McLeod et al. 2010). Alteration to riverine flow indeed has implications for estuarine community structure (Olin et al. *in review*) and as presented here, the flow of energy to higher trophic levels through the food web. Whether these implications result in advantageous (e.g., nutrients for production) or deleterious (e.g., cause mortality) effects to estuarine species requires further research. However, the results of this study indicate that the assemblage of lower trophic level species could be influenced to a greater extent. Ultimately the frequency, intensity and duration of each disturbance dictates the characteristics that control ecosystem recovery, but the legacy effects of disturbance can leave a system more vulnerable to additional disturbances (Scheffer et al. 2001; Harris et al. 2010). Estuaries serve as nursery, rearing and feeding grounds for a diverse assemblage of fish and invertebrate species (e.g. Beck et al. 2001) that are often of recreational and commercial value. Thus, changes to natural flow regimes or anticipated precipitation patterns that modify the duration and intensity of freshwater flow, may hold significant consequences for the productivity of estuarine communities.

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Table 5.1 Stable isotope values (*n* = number of individuals sampled; ‰ mean ± SE) of species collected from the Myakka and Caloosahatchee estuaries following the dry and wet season.

Species	Season	n	Length (cm)[1]	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	n	Length (cm)[1]	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
INVERTEBRATES		MYAKKA					CALOOSAHATCHEE				
Crassostrea virginica, Eastern oyster	Dry						3		-23.5 ± 0.3	4.9 ± 0.2	13.7 ± 0.1
	Wet						3		-23.0 ± 0.6	6.6 ± 0.4	11.1 ± 0.5
Callinectes sapidus, blue crab	Dry	9	12.2 ± 1.0	-22.7 ± 0.3	8.5 ± 0.6	12.5 ± 0.6	3	19.0 ± 1.7	-20.6 ± 0.3	9.3 ± 0.3	14.0 ± 0.8
	Wet	6	15.3 ± 0.3	-18.6 ± 0.5	10.4 ± 1.1	9.9 ± 1.0	6	9.8 ± 0.1	-23.8 ± 0.7	10.7 ± 0.4	12.6 ± 0.3
FISHES											
Mugil cephalus, striped mullet	Dry	1	19.0	-14.6	5.7	5.2	4	22.9 ± 0.4	-14.7 ± 1.1	7.8 ± 0.4	8.6 ± 0.9
	Wet	3	29.4 ± 3.2	-20.7 ± 0.3	8.4 ± 0.3	12.4 ± 0.6	6	19.1 ± 7.5	-22.7 ± 1.8	9.4 ± 0.2	9. 7 ± 1.1
Trinectes maculatus, hogchoker	Dry	3	7.9 ± 0.7	-21.0 ± 1.9	9.6 ± 0.3	7.2 ± 0.6					
	Wet	3	6.9 ± 0.8	-22.4 ± 0.3	11.0 ± 0.5	9.7 ± 1.2					
Eucinostomus harengulus, tidewater mojarra	Dry						5	10.4 ± 0.4	-15.2 ± 0.5	9.4 ± 0.1	0.5 ± 0.4
	Wet						10	5.4 ± 0.4	-23.5 ± 1.1	10.5 ± 0.4	8.8 ± 1.3
Eugerres plumieri, striped mojarra	Dry	1	9.5	-22.8	10.0	10.6	5	15.5 ± 1.7	-21.7 ± 1.5	10.9 ± 0.4	9.8 ± 1.6
	Wet	17	4.6 ± 0.5	-22.5 ± 0.5	9.0 ± 0.1	9.5 ± 0.3	10	12.7 ± 1.3	-20.5 ± 1.8	10.3 ± 0.4	8.6 ± 1.5
Lagodon rhomboides, pinfish	Dry	10	9.6 ± 0.4	-21.8 ± 0.5	9.6 ± 0.2	13.5 ± 0.3	4	9.3 ± 0.6	-16.6 ± 0.3	9.7 ± 0.6	11.7 ± 0.9
	Wet	5	11.2 ± 0.9	-21.7 ± 0.6	10.0 ± 0.2	12.9 ± 0.5	5	12.0 ± 1.9	-19.7 ± 0.8	11.2 ± 0.3	12.3 ± 0.8
Dasyatis sabina, Atlantic stingray	Dry						3	23.5 ± 1.7	-14.9 ± 0.2	9.9 ± 0.1	8.2 ± 1.8
	Wet						7	13.4 ± 0.3	-19.5 ± 0.6	12.1 ± 0.4	10.8 ± 0.9
Chaetodipterus faber, Atlantic spadefish	Dry	4	10.5 ± 2.8	-21.3 ± 0.6	11.3 ± 0.2	11.8± 0.5	4	12.1 ± 1.2	-19.4 ± 0.6	11.6 ± 0.4	13.2 ± 0.3
	Wet	3	14.0 ± 3.8	-22.4 ± 0.9	11.1 ± 0.2	12.3 ± 0.9	8	17.9 ± 0.9	-20.0 ± 0.6	10.1 ± 0.7	10.7 ± 1.0
Menticirrhus americanus, Southern kingfish	Dry	3	19.7 ± 0.4	-23.3 ± 0.4	11.0 ± 0.4	11.6 ± 0.2					
	Wet	5	22.6 ± 0.2	-21.8 ± 0.2	9.9 ± 0.1	12.1 ± 0.1					
Ariopsis felis, hardhead catfish	Dry	10	29.7 ± 1.5	-21.4 ± 0.5	11.0 ± 0.3	12.7 ± 0.6	6	30.4 ± 0.8	-20.4 ± 0.7	11.3 ± 0.4	12.6 ± 0.5
	Wet	8	17.4 ± 4.9	-21.2 ± 0.4	10.7 ± 0.3	10.7 ± 0.6	24	23.5 ± 1.8	-21.2 ± 0.4	12.2 ± 0.3	12.6 ± 0.5
Lutjanus griseus, grey snapper	Dry						5	16.2 ± 1.3	-14.5 ± 0.7	11.6 ± 0.2	10.9 ± 0.5
	Wet						3	12.1 ± 4.1	-16.1 ± 0.5	11.8 ± 0.2	13.9 ± 0.5
Cynoscion arenarius, sand seatrout	Dry	3	17.8 ± 1.3	-23.9 ± 0.2	12.4 ± 0.1	13.0 ± 0.4					
	Wet	5	27.6 ± 4.4	-21.7 ± 0.2	10.4 ± 0.1	12.0 ± 0.1					
Bagre marinus, gafftopsail catfish	Dry	6	42.2 ± 3.8	-18.9 ± 0.5	12.1 ± 0.1	13.6 ± 0.2	6	40.6 ± 1.8	-19.4 ± 0.6	12.9 ± 0.4	12.9 ± 0.5
	Wet	11	36.4 ± 3.2	-19.4 ± 0.3	11.6 ± 0.2	12.2 ± 0.4	7	24.4 ± 4.6	-20.8 ± 0.5	12.8 ± 0.5	12.9 ± 0.7
Carcharhinus leucas, bull shark[2]	Dry	3	102.5 ± 3.0	-17.8 ± 0.4	12.6 ± 0.3	11.0 ± 0.6	3	94.3 ± 2.9	-16.6 ± 0.3	12.6 ± 0.2	11.6 ± 1.1
	Wet	3	91.6 ± 13.8	-18.5 ± 0.7	12.7 ± 0.2	12.0 ± 0.1	3	102.1 ± 12.6	-17.4 ± 0.5	13.4 ± 0.5	13.3 ± 1.0

[1]Length indicates standard length for fishes, disc width for stingrays and carapace width for crabs (cm). [2]Only bull sharks measuring ≥ 70 cm in standard length with healed umbilical scars (c. ≥ 1 year old) were included in this study to eliminate any potential maternal isotopic influence (Olin et al. 2011).

Table 5.2 Trophic guilds[1] and resource use categories (benthic, pelagic) based on dietary sources compiled from published literature, for consumer species sampled from the Caloosahatchee and Myakka estuaries.

Species	Resource use	Predominant prey items[2]	References
Primary consumers			
Crassostrea virginica, Eastern oyster	Pelagic	Plankton, Diatoms	Riera and Richard (1996)
Mugil cephalus, striped mullet	Benthic	Detritus, Microalgae	Platell et al. (2006)
Secondary consumers			
Callinectes sapidus, blue crab	Benthic	Crustaceans, Mollusca, Detritus, Algae	Laughlin (1982)
Trinectes maculatus, hogchoker	Benthic	Annelids, Arthropods	Derrick and Kennedy (1997)
Eucinostomus harengulus, tidewater mojarra	Benthic	Crustaceans, Polychaetes, Mollusca	Ley et al. (1994)
Eugerres plumieri, striped mojarra	Benthic	Crustaceans, Mollusca, Detritus	Austin and Austin (1971)
Lagodon rhomboides, pinfish	Benthic	Mollusca, Crustaceans, Polychaetes, Algae	Motta et al. (1995)
Dasyatis sabina, Atlantic stingray	Benthic	Crustaceans, Polychaetes, Ophiuroidea	Cook (1994)
Chaetodipterus faber, Atlantic spadefish	Pelagic	Hydrozoa, Anthozoa	Hayse (1990)
Menticirrhus americanus, Southern kingfish	Benthic	Polychaetes, Molluscs, Penaeids	Woodland et al. (2011)
Tertiary consumers			
Ariopsis felis, hardhead catfish	Benthic	Decapoda, Amphipoda, Small teleosts	Yáñez-Arancibia and Lara-Domínguez (1988)
Lutjanus griseus, grey snapper	Benthic	Teleosts (Engraulidae), Amphipoda, Decapoda	Harrigan et al. (1989)
Cynoscion arenarius, sand seatrout	Pelagic	Teleosts (Engraulidae), Penaeids	Sheridan et al. (1984)
Bagre marinus, gafftopsail catfish	Benthic	Brachyura, Stomatopoda, Small teleosts	Yáñez-Arancibia and Lara-Domínguez (1988)
Piscivore			
Carcharhinus leucas, bull shark	Benthic	Teleosts (Ariidae), Elasmobranchs (Dasyatidae)	Cortés (1999); J.A. Olin and A.T. Fisk (unpublished data)

[1] Trophic guilds were defined as: primary consumer, diet composed largely of algae and detritus (>70%); secondary consumer, diet composed primarily of invertebrate species; tertiary consumer, diet composed of both fishes and invertebrates and; piscivore, diet composed primarily of fishes (> 80%).
[2] Only the most frequently observed diet items are provided for each species (i.e., not a complete list). Predominant prey items for *C. leucas* presented here represent juvenile individuals as this age class is common to estuaries.

Table 5.3 Results of two-way ANOVAs used to test the effect of (1) season (dry *vs.* wet) and (2) resource use category (benthic *vs.* pelagic) on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of species within the designated trophic guilds (statistical significance at $\alpha = 0.05$ indicated in bold).

MYAKKA

	$\delta^{13}C$ (‰)					$\delta^{15}N$ (‰)				$\delta^{34}S$ (‰)			
Secondary consumer	df	SS	MS	*F*	*P*	SS	MS	*F*	*P*	SS	MS	*F*	*P*
Season	1	2.991	2.991	0.761	0.386	0.468	0.468	0.287	0.594	38.165	38.165	11.196	**0.001**
Resource use	1	0.192	0.192	0.049	0.826	18.077	18.077	11.057	**0.001**	2.474	2.474	0.726	0.397
Season x resource use	1	4.615	4.615	1.174	0.283	0.327	0.327	0.200	0.656	7.583	7.583	2.224	0.141
Error	65	255.565	3.932			106.271	1.635			221.583	3.409		
Tertiary consumer													
Season	1	1.138	1.138	0.448	0.507	2.77	2.770	3.550	0.066	14.587	14.587	7.061	**0.011**
Resource use	1	43.605	43.605	17.158	**0.000**	0.761	0.762	0.976	0.328	0.907	0.907	0.439	0.511
Season x resource use	1	6.355	6.355	2.501	0.121	5.791	5.791	7.424	**0.009**	0.008	0.008	0.004	0.949
Error	45	114.361	2.541			35.104	0.780			92.961	2.066		

CALOOSAHATCHEE

	$\delta^{13}C$ (‰)					$\delta^{15}N$ (‰)				$\delta^{34}S$ (‰)			
Primary consumer	df	SS	MS	*F*	*P*	SS	MS	*F*	*P*	SS	MS	*F*	*P*
Season	1	75.478	75.478	8.039	**0.015**	14.489	14.489	35.825	**0.000**	1.380	1.380	0.357	0.561
Resource use	1	65.588	65.588	6.986	**0.021**	30.319	30.319	74.965	**0.000**	36.192	36.192	9.373	**0.010**
Season x resource use	1	65.534	65.534	6.980	**0.022**	0.039	0.039	0.098	0.760	12.620	12.620	3.268	0.096
Error	12	112.670	9.389			4.853	0.404			46.337	3.861		
Secondary consumer													
Season	1	10.651	10.651	0.703	0.405	1.194	1.194	0.652	0.423	27.475	27.475	1.642	0.206
Resource use	1	0.000	0.000	0.000	0.987	0.063	0.063	0.034	0.854	75.010	75.010	4.481	**0.039**
Season x resource use	1	7.918	7.918	0.523	0.473	4.856	4.856	2.650	0.109	51.259	51.259	3.062	0.086
Error	56	848.330	15.149			102.618	1.832			920.590	16.738		

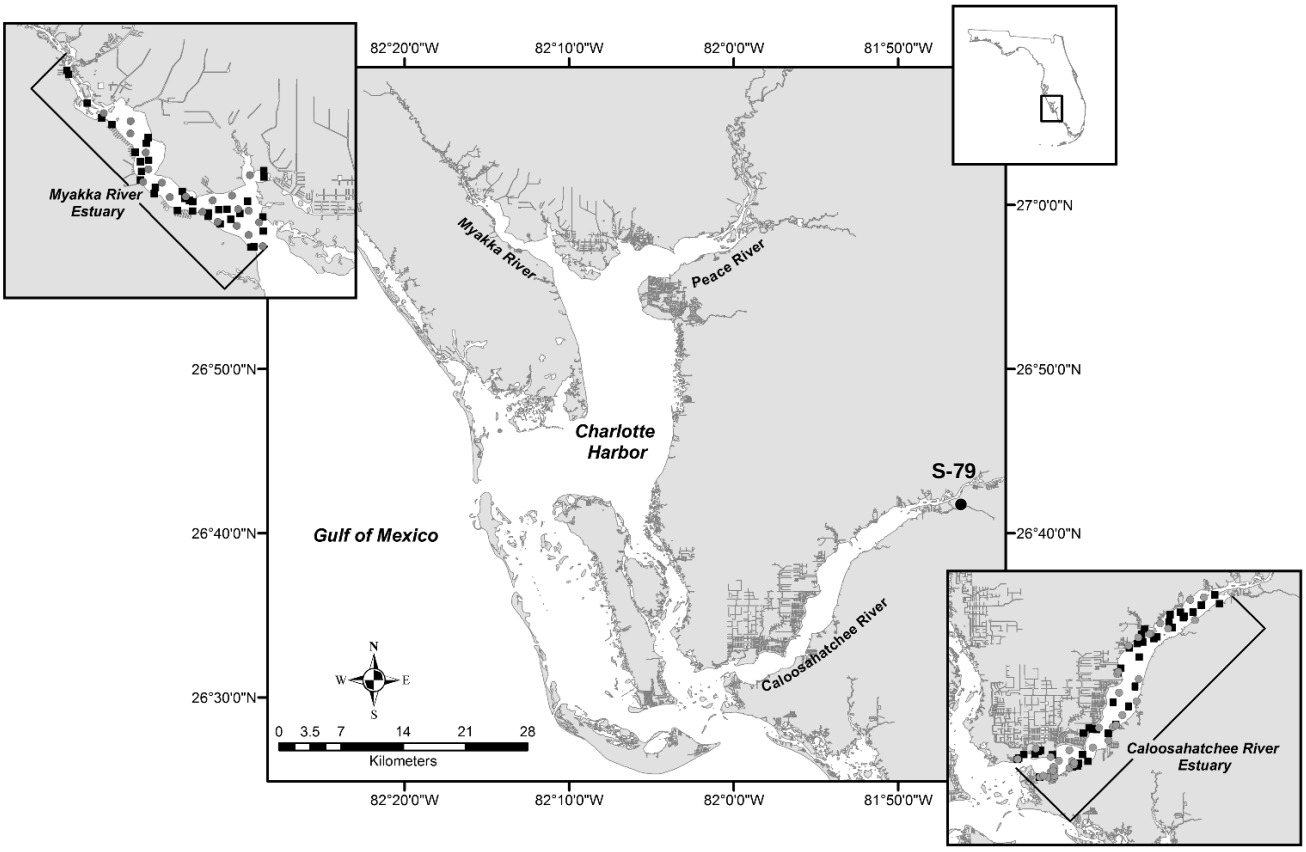

Figure 5.1 Map of the study site showing the location of the Caloosahatchee and Myakka Rivers with respect to the south-western coast of Florida. Inset: Indicates the sampling locations (e.g., water quality and consumer species; ■ spring; ▲autumn) within the estuarine portion of the rivers.

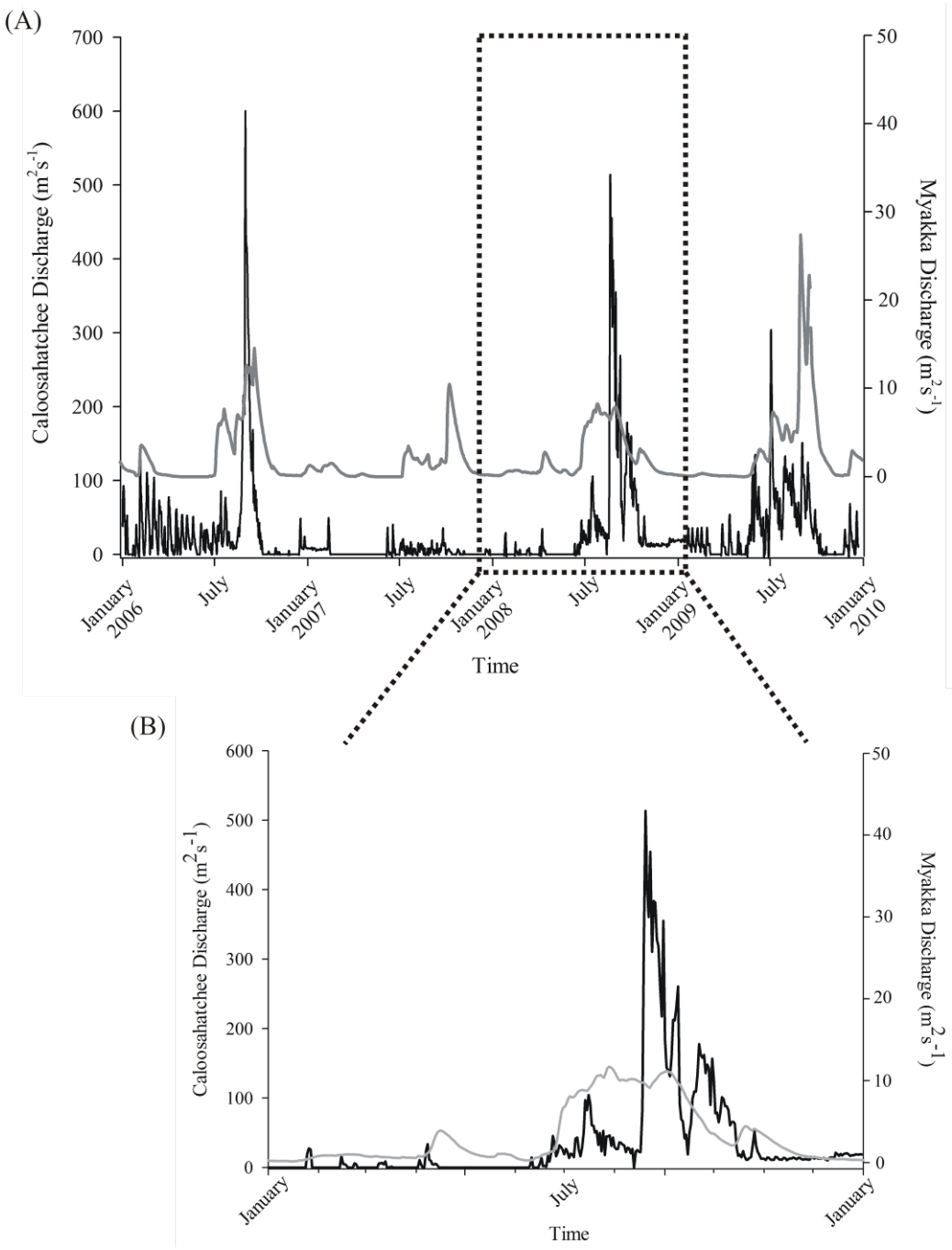

Figure 5.2 Mean daily river discharge recorded in the Myakka (gray) and the Caloosahatchee (black) from (A) 2006 to 2010, with special reference to discharge recorded from (B) 2008. River discharge data were obtained from the U.S. Geological Survey (http://water.usgs.gov/data) for the Myakka River at Myakka River near Sarasota (Station 02298830), and from the South Florida Water Management District (http://my.sfwmd.gov) for the Caloosahatchee River at the Cape Coral Bridge (Station CCORAL).

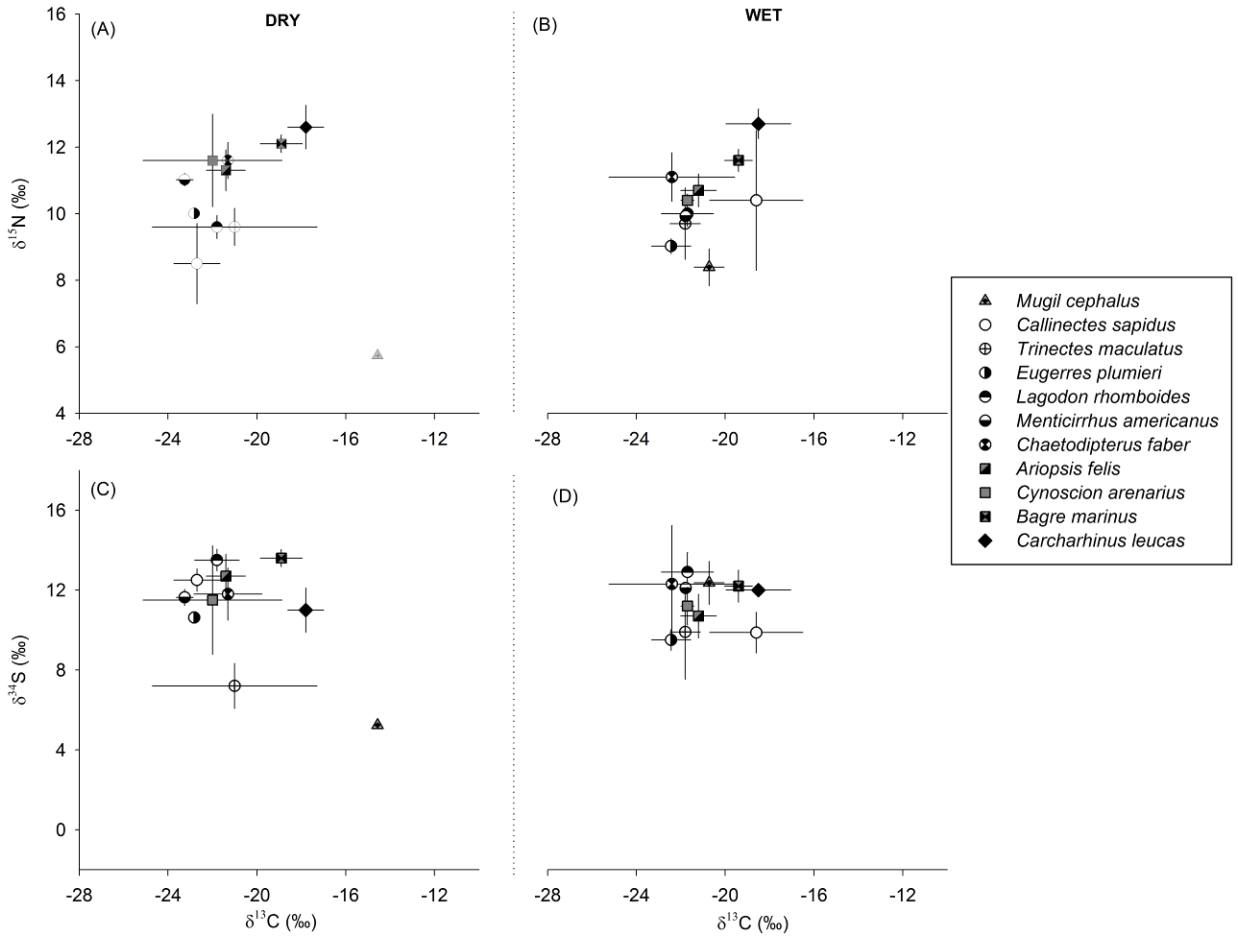

Figure 5.3 Mean (‰ ± 95% confidence interval) values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ in consumer species sampled from the Myakka estuary following the dry (A), (C) and wet (B), (D) seasons.

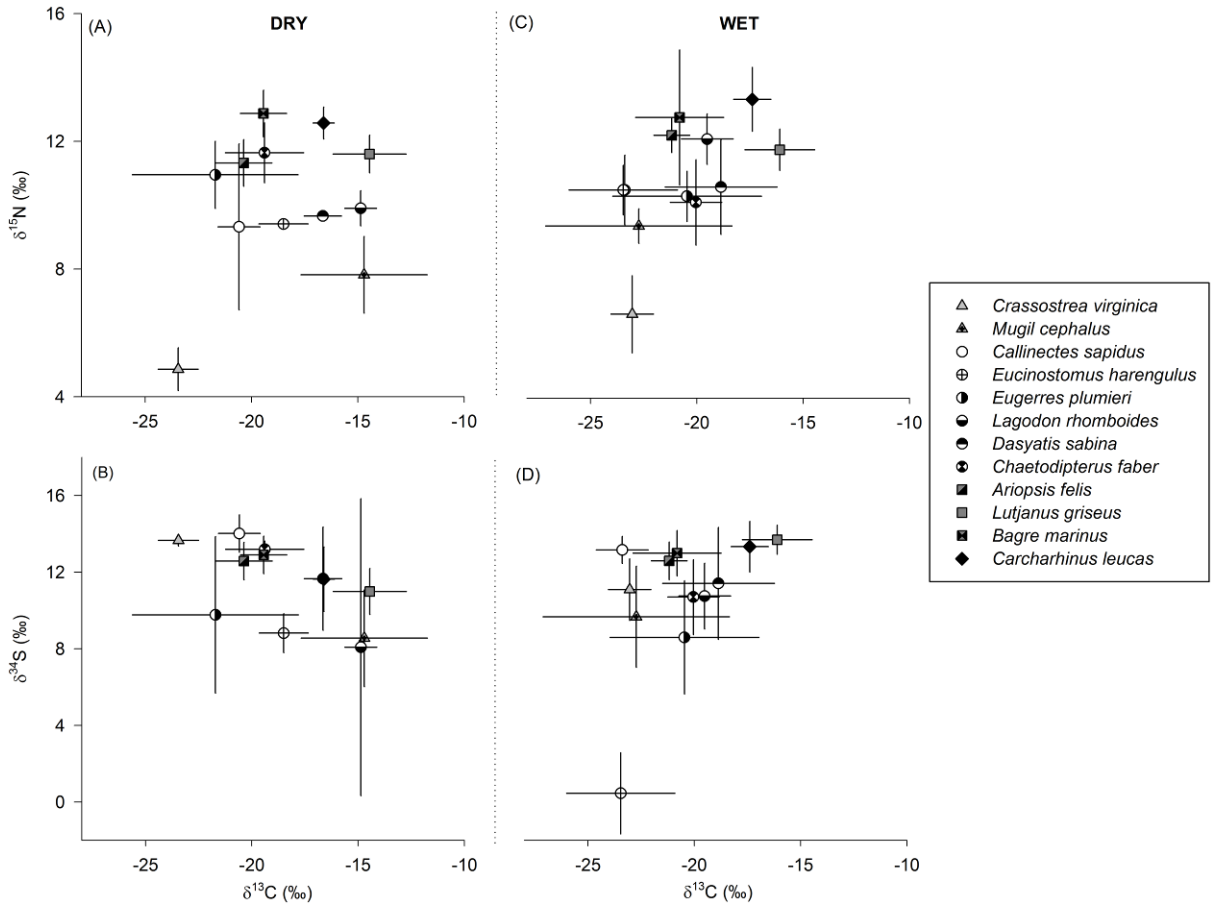

Figure 5.4 Mean (‰ ± 95% confidence interval) values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ in consumer species sampled from the Caloosahatchee estuary following the dry (A), (C) and wet (B), (D) seasons.

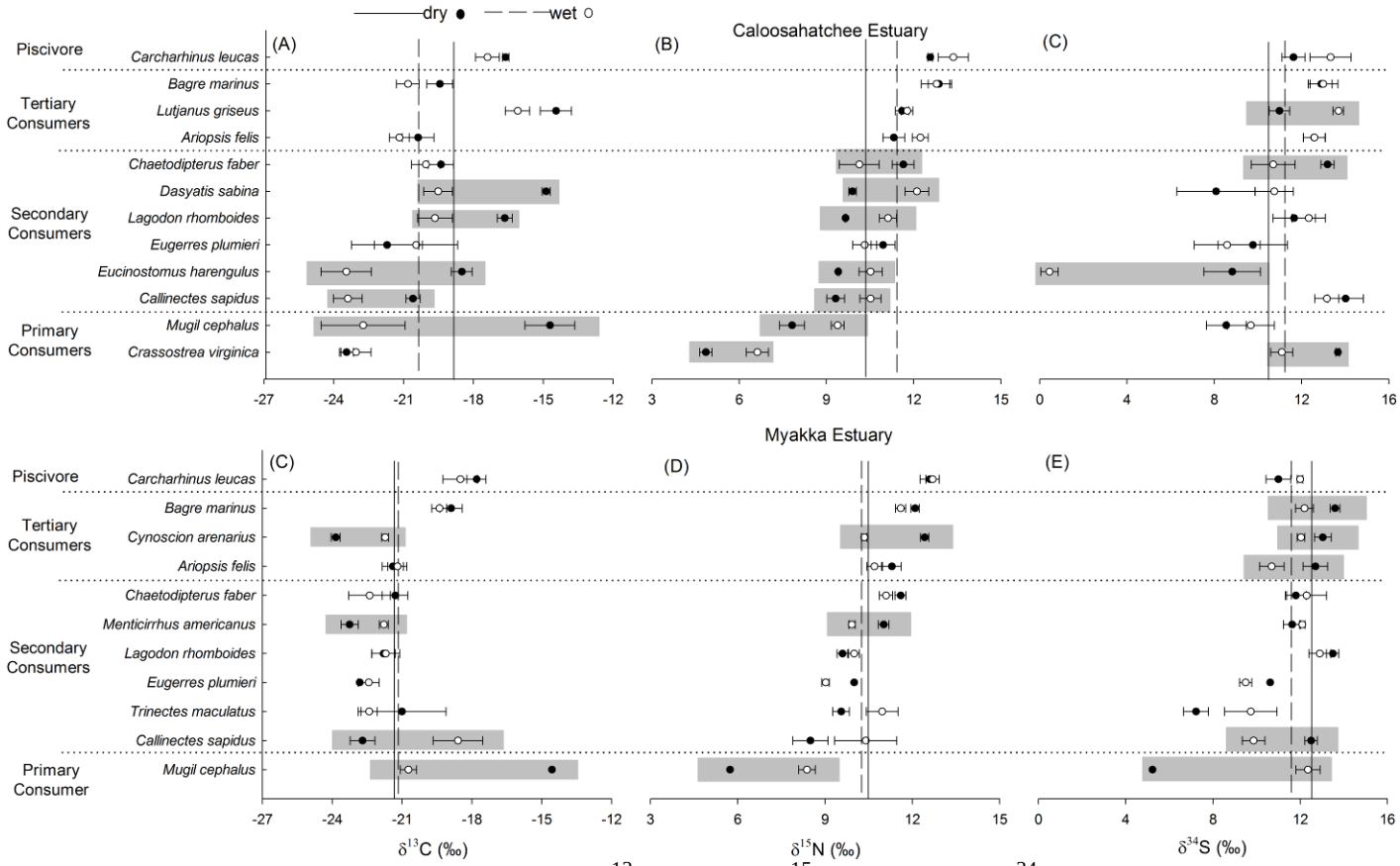

Figure 5.5 Mean (‰ ± SE) values of (A), (D) $\delta^{13}C$, (B), (E) $\delta^{15}N$ and (C), (F) $\delta^{34}S$ depicting differences between seasons (● dry; ○ wet) in consumer species sampled from the Caloosahatchee and Myakka estuaries. Vertical sold lines and broken lines represents mean isotopic values for the food web of dry and wet seasons, respectively. Significant differences in isotopic values between seasons, based on ANOVA, are highlighted in gray ($\alpha = 0.05$).

SUPPLEMENTAL MATERIAL

Table 5S.1 Environmental parameters measured from each sampling event in the Caloosahatchee and Myakka estuaries during the dry (spring—May and June) and wet (autumn—August and September) seasons of 2008. Data are mean ± SE.

	CALOOSAHATCHEE		MYAKKA	
	Dry (n = 23)	Wet (n = 36)	Dry (n = 29)	Wet (n = 30)
Salinity (ppt)	27.5 ± 7.4	3.9 ± 2.9	24.1 ± 1.2	10.0 ± 1.6
Temperature (°C)	28.9 ± 1.5	28.6 ± 1.5	29.0 ± 0.3	28.4 ± 0.2
DO (mgl^{-1})	6.3 ± 0.9	5.33 ± 0.2	5.7 ± 0.1	6.4 ± 0.2

Table 5S.2 Results of the analyses of variance (ANOVA) performed to test for differences in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values among consumer species sampled following dry and wet season (statistical significance at $\alpha = 0.05$ highlighted in bold).

Species	CALOOSAHATCHEE							MYAKKA						
		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		$\delta^{34}S$ (‰)			$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		$\delta^{34}S$ (‰)	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*df*	*F*	*P*	*F*	*P*	*F*	*P*
Primary consumers														
Crassostrea virginica, Eastern oyster	1,4	0.329	0.597	16.63	**0.015**	24.85	**0.008**							
Mugil cephalus, striped mullet	1,8	11.26	**0.009**	12.67	**0.007**	0.546	0.481	1,2	79.31	**0.012**	21.96	**0.043**	41.54	**0.023**
Secondary consumers														
Callinectes sapidus, blue crab	1,7	9.053	**0.019**	4.413	**0.040**	0.772	0.409	1,13	14.38	**0.002**	2.79	0.119	23.28	**0.000**
Trinectes maculatus, hogchoker								1,4	0.53	0.504	5.30	0.083	3.55	0.133
Eucinostomus harengulus, tidewater mojarra	1,13	10.48	**0.006**	8.168	**0.013**	19.52	**0.013**							
Eugerres plumieri, striped mojarra	1,13	0.201	0.661	0.933	0.352	0.238	0.634	1,16	0.03	0.855	4.32	0.055	0.896	0.358
Lagodon rhomboides, pinfish	1,7	3.702	**0.044**	2.875	**0.032**	0.027	0.874	1,13	0.02	0.884	2.47	0.140	1.439	0.252
Dasyatis sabina, Atlantic stingray	1,7	14.37	**0.007**	7.791	**0.027**	1.921	0.215							
Chaetodipterus faber, Atlantic spadefish	1,10	0.451	0.517	5.173	**0.036**	4.891	**0.041**	1,5	1.29	0.308	0.25	0.633	0.31	0.602
Menticirrhus americanus, Southern kingfish								1,6	16.00	**0.007**	29.34	**0.002**	1.953	0.212
Tertiary consumers														
Ariopsis felis, hardhead catfish	1,28	0.750	0.394	2.406	0.132	0.000	0.986	1,16	0.14	0.717	0.39	0.541	6.40	**0.022**
Lutjanus griseus, grey snapper	1,6	2.926	0.138	0.261	0.628	17.38	**0.006**							
Cynoscion arenarius, sand seatrout								1,6	75.00	**0.000**	161.30	**0.000**	11.51	**0.015**
Bagre marinus, gafftopsail catfish	1,11	3.515	0.087	0.014	0.909	0.012	0.913	1,15	0.78	0.398	4.12	0.061	5.96	**0.028**
Piscivore														
Carcharhinus leucas, bull shark	1,3	1.164	0.359	3.109	0.176	2.132	0.240	1,4	0.76	0.434	0.09	0.780	2.44	0.194

CHAPTER 6

CHANGES IN RESOURCE EXPLOITATION BY ESTUARINE CONSUMERS IN RESPONSE TO ALTERED HIGH FLOW AS INFERRED FROM FATTY ACID BIOMARKERS

INTRODUCTION

Production in tidal rivers represents a composite of a range of autochthonous and allochthonous resources. Allochthonous contributions to these systems can include terrestrially derived dissolved and/or particulate organic matter and leaf litter from forested catchments and mangroves (Mfilinge et al. 2005; McLeod and Wing 2009). Additionally, marine-derived organic matter from adjacent coastal habitats including seagrass meadows and offshore planktonic production also contribute to the nutrient and resource pools in estuarine food webs (Kharlamenko et al. 2001; Kang et al. 2003). It is well established that this diverse range of resources is critical to the overall structure and production of estuarine nekton communities (Chanton and Lewis 2002; Darnaude et al. 2005). However, barriers to this connectivity between the marine and terrestrial habitat extremes of estuarine systems, such as altered hydrologic regimes, have the potential to negatively impact the magnitude and timing of allochthonous contributions thereby compromising biological productivity (Livingston et al. 1997; McLeod and Wing 2009; Abrantes and Sheaves 2010).

The flow of energy and nutrients through food webs represents a complex pathway of resource acquisition and assimilation from prey to predator species (Hobson et al. 2002; Hebert et al. 2006). As such, many consumers have a high degree of feeding plasticity, as the composition and availability of resources can vary both spatially and temporally. Lipids typically represent the primary energy source in aquatic food webs (Arts et al. 2009) and also provide critical fatty acid (FA) constituents that are required for normal growth and development (Arts 1999). In this capacity, dietary FA have the potential to provide insight toward the specific nutrient and energy resources exploited by

individual consumers (Dalsgaard et al. 2003). Specifically, essential fatty acids (EFA) cannot be synthesized by many animal species and must therefore be obtained from the diet in sufficient quantities to ensure optimal growth and development (Olsen 1999).

Central to their use as dietary tracers is the consideration that EFAs are also minimally modified during their transfer from primary production to higher trophic level consumers (Parrish et al. 2000; Dalsgaard and St. John 2004). Due to these characteristics, FAs have been applied as natural diet biomarkers for a variety of applications including understanding diet composition (Kharlamenko et al. 2001; Bradshaw et al. 2003), changes in foraging strategies (Hebert et al. 2009), investigating bottom-up primary production dynamics (Richoux and Froneman 2008; Koussoroplis et al. 2011), and impacts of non-indigenous species introduction on nutrient transfer (Nordin et al. 2008). Fatty acid biomarkers have been established/identified as characteristic biomarkers of bacterial (Richoux and Froneman 2008), diatom, dinoflagellate (Parrish et al. 2000), macroalgal (Johns et al .1979; Hanson et al. 2010) and vascular plant production sources in a range of aquatic and terrestrial ecosystems (Wannigama et al. 1981; Alfaro et al. 2006; Richoux and Froneman 2008). Such specificity of individual FAs to primary production sources provides the potential to trace the origin of organic matter in a system and to potentially resolve the differential contributions of the range of autochthonous and allochthonous production sources in dynamic systems such as tidal rivers. For example, aquatic primary production is typically defined by greater proportions of ω3 polyunsaturated FA (PUFA) including eicosapentaenoic acid (EPA; 20:5ω3), docosapentaenoic acid (DPA; 22:5ω3), and docosahexaenoic acid (DHA; 22:6ω3). In contrast, terrestrial resources are commonly

characterized by increased contributions of ω6 PUFA such as linoleic acid (LIN; 18:2ω6), arachidonic acid (ARA; 20:4ω6) and γ-linolenic acid (18:3ω6; Smith et al. 2005; Koussoroplis et al. 2008).

Using the stable isotopes of carbon ($\delta^{13}C$), it was recently demonstrated that high flow disturbance events may alter the general resource pathways exploited by primary and secondary consumer species in an estuarine food web (Olin et al. *unpublished data*). Indeed, Wai et al. (2008) demonstrated a shift in resource pathways of estuarine invertebrates to a higher dependence on decomposing marine algae and terrestrial detritus subsidies after an extreme tropical storm disturbance event. The authors further went on to track these allochthonous trophic subsidies to higher order consumers, the bamboo shark *Chiloscyllium plagiosum* (Wai et al. 2011). Given the influence of freshwater flow in estuarine systems, the primary objective of the current study was to use FA biomarkers to determine the main trophic pathways and relative importance of different energy sources to estuarine consumers. To accomplish this objective, we compared seasonal FA biomarker composition of estuarine nekton conspecifics from contrasting tidal rivers; one that experiences regulated freshwater flow that often results in high flows, and one that experiences more natural riverine flows. We hypothesized that the contribution of allochthonous carbon sources (i.e., terrestrially-derived) would be more important during the wet season than the dry season and would be especially evident during extreme high flow. We expected that these differences will be manifest in reductions of ratios of ω3/ω6 indicating a greater contribution of terrestrial organic matter to production as opposed to marine-based organic matter.

MATERIALS AND METHODS

Study sites, species and sample collection

We measured the concentrations of fatty acids in the total lipid fractions of muscle tissue in estuarine consumers collected from the Caloosahatchee and Myakka estuaries of southwest Florida. The Caloosahatchee River (26°30' N, 81°54' W) is part of a cross-Florida canal system that passes through Lake Okeechobee and connects the intracoastal waterways of Florida's east and west coasts. The Caloosahatchee River has been substantially altered over the past 100 yrs through the construction of an artificial link to Lake Okeechobee, extensive canal systems, three locks to permit boat passage, and dams to regulate water flow (Doering and Chamberlain 1998). These alterations to the Rivers' hydrology have greatly changed the freshwater flow in this system, resulting in large fluctuations in timing and quantity of discharge to the estuarine portion of the river (Flaig and Capece 1998; Barnes 2005). During periods of low freshwater discharge (i.e., during winter/spring months), salt water regularly intrudes to S-79, the most downstream water control structure, often exceeding 10‰ (see Fig. 2.1). High freshwater discharge (i.e., during summer/fall months) can cause salinity to drop below 5‰ at the mouth of the River and the transition between the two states can be rapid, sometimes occurring in less than a week (Doering et al. 2002). The Myakka River (82°12' W, 26°57' N) was chosen for comparison with the Caloosahatchee, as it is proximately located (< 100 km; Fig. 2.1) and therefore is accessible to fishes of Charlotte Harbor and experiences similar temperature and weather patterns. More importantly, the Myakka estuary has not been greatly modified by water control structure and experiences

relatively natural seasonal flow periods (for more detailed description of the estuaries, see Olin et al. *in review*).

Species were collected following the dry (May and June) and wet (September and October) seasons of 2008 from the Caloosahatchee and Myakka estuaries, using shallow water (< 10 m) longlines (800 m), seines (21.3 m with 3.2-mm stretch mesh, center bag), and trawls (6.1-m 3with 8-mm stretch mesh, 3.2-mm stretch mesh liner). Longlines were set for periods from 30 min to 2 h, with most set for approximately 1.5 h. The seine was deployed from a boat in a shallow arc parallel to shore and hauled directly along the shoreline. The two ends of the seine were pulled together, sampling an area of ~68 m^2. The trawl was towed for 5–7 minutes at 0.6 $m \cdot s^{-1}$, providing a tow length of ~180 m. Trawl width averaged ~4 m, providing an approximate area of 720 m^2 sampled by a typical tow.

For comparative purposes with previous studies that used stable isotopes to assess altered high-flow affects on estuarine nekton consumers (Olin et al. *unpublished data*), we selected six species representing different trophic guilds for FA analysis. These species included (1) secondary consumers, blue crab *Callinectes sapidus*, pinfish *Lagodon rhomboides* and Atlantic spadefish *Chaetodipterus faber*; (2) tertiary consumers, hardhead catfish *Ariopsis felis* and gafftopsail catfish *Bagre marinus* and; (3) a piscivore bull shark *Carcharhinus leucas*. Upon collection all species were measured; carapace width for crabs and standard length for fishes (to the nearest mm). Crabs were dissected prior to drying and only soft tissue was retained for analyses. White muscle tissue was excised from the dorsal area anterior to the first dorsal fin from all fishes. Muscle samples for fatty acid analysis were stored in a liquid nitrogen (LN_2) dewar in the

field and then stored frozen in a cryogenic freezer upon return to the laboratory (-80ºC). Only individuals of *C. leucas* with healed umbilical scars (c.≥ 1 year old) were included in this study to eliminate any potential influence from maternal resources (Olin et al. 2011; Belicka et al. unpublished data).

Lipid and fatty acid analysis

Fatty acid methyl esters (FAME) were obtained in a three-step process: extraction, derivatization, and quantification on a gas chromatograph (GC). Briefly, muscle tissues were sub-sampled and ~15–20 mg samples were extracted 3 times by grinding freeze dried tissue in (2:1 vol:vol) chloroform:methanol (Bligh and Dyer 1959) and centrifuged at 4,000 r.p.m. for 5 min to remove non-lipid material. A synthetic lipid (cholestane) was added to all samples as an internal standard to provide an estimate of extraction efficiency (Sigurgisladottir et al. 1992). From a final volume of 2 ml, duplicate, 200 µL aliquots were dispensed into pre-weighed vessels which were dried and re-weighed on a Sartorious M5 electron balance with 1 µg precision to provide a quantitative measure of total lipid content. The remaining extract (1.6 ml) was then transferred into a 5 ml Shimadzu vial (Sigma-Aldrich Canada Ltd, Oakville, CA) and evaporated to dryness using nitrogen gas and stored at -80°C until derivatization. The fatty acid extracts were re-suspended in 1.5 ml toluene prior to derivatization. Two milliliters of H_2SO_4/methanol (1%) were added to the vial before overnight methylation (16 h) in a water bath at 50°C. The extract was then evaporated to dryness under nitrogen, and re-dissolved in 2 ml hexane and transferred to a 2 ml glass GC vial and stored in a -

80°C cryogenic freezer prior to GC analysis. A 250 µl portion of the resulting extracts was used for FAME analysis.

FAME were analyzed using a Agilent 6890 Series GC System which was configured as follows: splitless injection; column = Supelco (SP-2560 column) 100 m X 0.25 mm ID X 0.20 µm film thickness; oven = 140°C (hold for 5 min) to 240°C at 4°C min-1, hold for 15 min; carrier gas = helium, 1.2 mL min-1; detector = FID at 260°C; injector = 260°C; total run time = 45 min per sample. A 37-component FAME standard (Supelco no. 47885-U) was used to identify and quantify (four-point calibration curves) individual FAME in the samples, i.e., by comparing their retention times to those of the FAME standard. Results are reported as µg FAME · mg dry weight tissue^{-1} and are presented as weight percent or proportion of total fatty acids. Each fatty acid was described using the shorthand nomenclature of *X:AωB*, where *X* represents the number of carbon atoms, *A* the number of double bonds, and *B* the position of the double bond nearest the terminal methyl group.

Data analysis

Given the substantial seasonal flow from the Caloosahatchee River (ranging between ~ 10 and 1,278 m^3s^{-1}; South Florida Water Management District 2008) and the observed depletion in ^{13}C in estuarine primary and secondary consumers (Olin et al. *unpublished data*), we anticipate that carbon utilization of consumers in the downstream estuary would vary according to season (wet *vs*. dry) and this variation would be evident in both low and high trophic level species. Simply finding significant differences in levels of a given FA between two groups however, does not indicate whether this difference is

biologically meaningful (Budge et al. 2006). Because the main focus of this analysis was to assess whether a seasonal shift in the contribution of primary production sources to a consumers' diet occurred with the onset of the wet season in both the Caloosahatchee and Myakka estuaries, a subset of known FA biomarkers was used to characterize the potential differences (Table 6.1). Our statistical assessment focused primarily on FA biomarkers that have been established previously in the literature as useful indicators of specific primary production sources that are characteristic of estuarine ecosystems. As lipid content can vary within and among species, relative proportions of FA biomarkers were calculated (% of total fatty acids) as a method for standardization across species, and FA proportional data rather than FA concentration data was used for all statistical comparisons.

Principal component analysis (PCA), an unconstrained ordination maximizing variation displayed on successive orthogonal axes, was performed on proportional FA data using correlation matrices of the 10 biomarkers (Table 6.1), to examine changes in FA composition of species within trophic guilds, for each estuary separately (*rda* function in the *vegan* package; Oksanen et al. 2011). Our initial PCA included species from all trophic guilds. However, the results of the PCA were highly skewed on account of the seasonal FA biomarker differences in the piscivore, *C. leucas*. We therefore chose to conduct further analyses on each trophic guild, instead of the complete dataset. The PCA analysis was performed with FAs as dependent variables and species within each trophic guild as independent variables. Results of the PCAs were graphically displayed as mean species FA biomarker values with ellipses representing one standard deviation placed around the mean of each season-species group, for secondary and tertiary

consumers respectively. To evaluate differences between season and among species on the principal components in the secondary and tertiary consumers, a two-way analysis of variance (ANOVA) of factor scores for each estuary was performed. To meet assumptions of normality and equal variances factor scores were transformed (log(x+10)). For the piscivore, ANOVA was used on transformed FA biomarker data to distinguish seasonal trends in each estuary. Ratios of ω-3 to ω-6 PUFAs were transformed similarly and the results were used in one-way ANOVA to determine the differences between season for each species in the Caloosahatchee and Myakka estuaries. All statistical analyses were performed in R 2.13.0 (R Development Core Team 2011) with a criterion for significance of $P < 0.05$ used for all comparisons.

RESULTS

Fatty acids of consumers

Saturated fatty acids (SFA) constituted ~32-45% of the total fatty acids of consumers (see Table 6S.1 and 6S.2 *Supplemental Material*) and were predominantly represented by palmitic acid-16:0. Monounsaturated fatty acids (MUFA) constituted ~40% of the total fatty acids in *Carcharhinus leucas* sampled from the Caloosahatchee (Table 6S.1), but only ~14-24% in all other consumers, including *C. leucas* sampled from the Myakka (Table 6S.1 and 6S.2). Oleic acid-18:1ω9 (brown alga biomarker), and to a lesser extent palmitoleic acid-16:1ω7 (diatom biomarker), constituted the highest proportion of MUFAs. All consumers exhibited high levels of polyunsaturated fatty acids (PUFA > 35%; Table 6S.1 and 6S.2) with the exception of *C. leucas* sampled from the Caloosahatchee (Table 6S.1). Most dominant, docosahexaenoic acid-22:6ω3 (DHA),

which is specific to dinoflagellates, constituted a high proportion in the secondary and tertiary consumers, except for *Callinectes sapidus,* where eicosapentaenoic acid-20:5ω3 (EPA), a diatom biomarker, was in high proportion (Table 6S.1 and 6S.2). Although PUFA were generally high in all consumers, the proportions of particular PUFA varied substantially among them. For example, the ω-3/ω-6 ratios varied considerably and ranged from 1.7 to 5.8 in the Caloosahatchee and 1.6 to 7.6 in the Myakka.

Variation in FA biomarkers of consumers in each trophic guild

The variation in FA biomarker composition of species (Table 6. 2) within each trophic guild was examined first using principal component analysis (PCA), using a correlation matrix that included the 10 FA biomarkers for each PCA. For secondary consumers from the Caloosahatchee, the first principal component was positively related to six biomarkers, yet largely determined by 16:1ω7 (diatom marker) and the combined 18:2ω6 + 18:3ω3 biomarkers representative of seagrass (Fig. 6.1A, Table 6.3). The second principal component was separated by DHA (positively) and by LCSFA and bacteria-Σ15Σ17 biomarkers (negatively; Fig. 6.1A, Table 6.3). The variance explained by these first two principal components was 39% and 22%. There were significant species and species x season interaction differences for PC1 and species differences for PC2 (Table 6.4). These differences were primarily driven by decreased proportions of LCSFA and bacteria, and increased proportions of 18:1ω9 biomarkers in *Lagodon rhomboides,* with the onset of the wet season (Fig. 6.1A). For the secondary consumers of the Myakka the first two principal components derived from the PCA accounted for 44% and 18% of the variation in FA biomarker composition in these consumers. The first

principal component was negatively related to three FA biomarkers, primarily DHA (Fig. 6.1B, Table 6.3). The second principal component was positively associated with seagrass and α-linolenic-18:3ω3 (ALA) (Fig. 6.1B, Table 6.3). Significant species differences were identified by the two-way ANOVA for PC1, driven by high proportions of 18:1ω9, 16:1ω7 and EPA in *Callinectes sapidus* (Fig. 6.1B, Table 6.4).

For tertiary consumers from the Caloosahatchee, the first principal component was positively related to six biomarkers, yet largely determined by seagrass and ALA, and negatively related to DHA, EPA and arachidonic acid (ARA) (Fig. 6.2A, Table 6.3). The second principal component was separated by seagrass, ALA and linoleic-18:2ω6 (LIN) (positively) and by LCSFA and Σ15Σ17 (negatively; Fig. 6.1A, Table 6.3). The variance explained by these first two principal components was 28% and 25%. The significant seasonal differences for PC2 (Table 6.4) resulted from the shift from biomarkers of LCSFA to seagrass in *Bagre marinus* (Fig. 6.2A). For the tertiary consumers of the Myakka the first principal component was negatively related to seagrass, LCSFA, ALA and LIN (Fig. 6.2B, Table 6.3), whereas the second principal component was positively associated with DHA and negatively associated with 16:1ω7 and 18:1ω9 (Fig. 6.1B, Table 6.3). No significant seasonal or species differences were identified for tertiary consumers of the Myakka (Table 6.4).

Significant differences were identified from the one-way ANOVAs of biomarker proportions for *C. leucas* sampled from the Caloosahatchee estuary (Fig. 6.3A). Although, the FA biomarkers of *C. leucas* were dominated by 18:1ω9, the significant increase in DHA and decrease in 16:1ω7 suggests a shift from diatom to dinoflagellate production source with the onset of the wet season. In the Myakka, the fatty acid

biomarkers of *C. leucas* were dominated by DHA and 18:1ω9 and did not exhibit a shift in production source with the wet season (Fig. 6.3B).

Ratio of ω3/ω6 PUFA

To assess the possible influence of terrestrial production sources on nekton species in the Caloosahatchee and Myakka estuaries, we examined the proportion of ω-3 with respect to ω-6 in the consumers. Variability among consumers' values was greater following the wet season in the Caloosahatchee and greater following the dry season on the Myakka. Overall seasonal ω-3/ω-6 ratios for consumers of the Caloosahatchee were similar between seasons (~3; Fig. 6.4A). There were no significant seasonal ω-3/ω-6 ratio differences in the consumers of the Caloosahatchee (Fig. 6.4A). In contrast, in the Myakka there was a general trend of decreasing ω-3/ω-6 ratios (by ~1) in consumers with the onset of the wet season, with the exception of *C. leucas* (Fig. 6.4B), suggesting increase use of terrestrial sources. This trend was significant for *Lagodon rhomboides* ($F_{1,10}$ = 4.713, P = 0.044), *Ariopsis felis* ($F_{1,4}$ = 11.241, P = 0.028) and *Bagre marinus* ($F_{1,9}$ = 4.951, P = 0.039).

DISCUSSION

Fatty acid biomarkers indicated that the relative importance of particular production sources to estuarine consumers shifted with season in the Caloosahatchee suggesting that high flow affects multiple components of the food web, including high trophic level species. In general, a FA signature consistent with dinoflagellate phytoplankton species (Alfaro et al. 2006) constituted the highest proportion of the

consumers' diets, regardless of season or estuary sampled. Species-specific seasonal shifts in dominant production sources, however, were evident in consumers from the Caloosahatchee River, which receives freshwater inputs in the wet season. In contrast, seasonal shifts in dominant production sources were not observed in the consumers of the Myakka River which experiences a more natural flow regime throughout an annual period. These results support the contention that altered-high flow can influence the available production sources in estuarine systems. Moreover, consumers collected from both estuaries showed a greater influence from terrestrially derived allochthonous carbon following the wet season based on the ω3/ω6 ratios. This supports the hypothesis that terrestrially-derived carbon contributes to secondary production in estuaries during the wet season.

Our analysis of FA biomarkers indicates that the majority of species utilize phytoplankton, namely dinoflagellates (22:6ω3-DHA) and to a lesser extent brown algae (18:1ω9-oleic acid) as their predominant primary production source, despite being characterized as benthic or pelagic feeders. These biomarkers for dinoflagellates and brown algae have been previously reported as major production resources for estuarine consumers (Alfaro et al. 2006). Similar patterns of fatty acid profiles in species that are unrelated or occupy different trophic levels may be associated with one of two mechanisms related to horizontal or vertical food web interactions (Czesny et al. 2011). Organisms can either share common food resources (horizontal) or one group constitutes prey for the other group (vertical; Czesney et al. 2011). Moreover, changes in FA profiles in an organism may reflect a combination of factors; (1) physiological or accumulation changes to the FA profile by the organism itself, (2) changes in FA profiles of prey

resources, or (3) a change in prey resources (Dalsgaard et al. 2003). As such, determining the specific trophic links and exact mechanisms by which these FA biomarkers are acquired would require direct sampling of carbon sources in the system and specific prey items of each predator. Regardless, the results of this study demonstrate that FA biomarkers are reliable tracers for characterizing the basal nutrient and resource pathways utilized by estuarine consumers in systems with varying flow regimes (Kharlamenko et al. 2001; Alfaro et al. 2006; Hanson et al. 2010).

Both *C. leucas* and *B. marinus* collected from the Caloosahatchee River exhibited seasonal differences in the dominant FA biomarkers of their tissues. However, these shifts were not toward a similar dominant resource. Specifically, FA proportions indicated that dinoflagellate production contributes to a greater extent to *C. leucas* relative to *B. marinus* for which seagrass type FA resources were identified as important dietary components. These shifts may be indicative of dietary changes rather than seasonal fluctuations with regards to the available FA pools. Although we cannot specify as to whether these shifts represent a direct dietary change of the consumers, we can indicate that nutrient and resource utilization pathways do change for these species. However, it is unknown as to whether this occurs at the predator (consumption) or prey (production) level. It could be argued that body-size driven ontogenetic diet shifts can account for the shifts in dominant FA biomarkers in these species. However, consumer $\delta^{13}C$ stable isotope signatures from these estuaries were not shown to have any significant relationships with body size (Olin et al. *unpublished data*). Although relationships between body size and fatty acid biomarkers cannot be ruled out owing to limited sample sizes for some species in the current study, body size is not anticipated have a strong

influence on the fatty acids biomarker profiles here. The lengths of individuals collected for each species were not vastly different and larval and young juvenile individuals were also not included in this study. Larval and young-of-year individuals commonly represent life history stages when critical ontogenetic diets shifts and maternal/in-utero mechanisms are known to influence biochemical tracer signatures (Czesney et al. 2011; Olin et al. 2011).

Consumers sampled from the Myakka River showed a greater consumption of allochthonous carbon following the wet season relative to conspecifics sampled following the dry season based on ω3/ω6 ratios. This finding supports our hypothesis of increased dependence on terrestrial subsidies by higher order consumers following the wet season. It is well-established that estuaries are dependent on riverine inflows that provide floodplain detritus and nutrients that facilitate high levels of primary and secondary production (Chanton and Lewis 2002). That a similar shift toward allochthonous carbon dependence does not exist in the Caloosahatchee is surprising. Previous studies have demonstrated the increased dependence on terrestrially-derived sources following the wet season in estuaries, particularly in studies tracking major storm events such as monsoons (Wai et al. 2008; 2011; Abrantes and Sheaves 2010). The lack of significant decline in the ω3/ω6 ratios in the Caloosahatchee could result from the magnitude of the flow being so great that it provides large amounts of allochthonous materials that are used by consumers throughout the year. Allochthonous fluxes of carbon to ecosystems are often large (Pace et al. 2004) and therefore would be expected to be greater in the Caloosahatchee as the drainage basin is nearly 2.5 times greater than that of the Myakka.

Mangrove contributions were identified as more important in the dry season relative to the wet season in both estuaries, based on higher proportions of long-chain saturated fatty acids (LCSFA) in the consumers' tissues. These results support the findings of Meziane and Tsuchiya (2000) whereby mangrove contributions to the diets of estuarine consumer species are greater when flow regimes are low. Dependence on mangroves declined in all consumers in both estuaries with the onset of the wet season, with the exception of *C. sapidus* that showed an increased proportion of mangrove in their tissues. Although mangrove forests are often considered to be highly productive, a number of studies have challenged the paradigm that mangroves provide a major source of nutrients to estuarine communities (Loneragan et al. 1997; Kieckbusch et al. 2004; Heithaus et al. 2011). Based on stable isotopes, Heithaus et al. (2011) found that mangroves did not contribute greatly to the carbon supply in a mangrove estuary in Australia. Kieckbush et al. (2004) made a similar conclusion for a tropical lagoon in the Bahamas. In contrast, Alfaro et al. (2006) demonstrated that mangrove detritus dominates the suspended organic matter (SOM) fraction in estuarine waters and also concluded that grazing and filter feeding species have high reliance on these materials based on their LCSFA biomarker profiles. However, Heithaus et al. (2011) and Kieckbusch et al. (2004) quantified carbon stable isotope signatures in leaves as opposed to SOM as the primary mangrove component in consumers' diets. Mangrove leaves are difficult to digest and are generally broken down through bacterial action in the sediments which could alter the stable isotope values (Hall et al. 2006). Therefore, the decrease in mangrove contribution may be a result of the flushing of the SOM from estuaries with high flow or a shift in bacterial composition and abundance. In any event, the greater proportions of LCSFA

observed in species sampled during the dry season suggest a greater availability of mangrove derived resources in the system at this time.

Conclusions

Seasonal variation in nutrient and resource availability at the base of aquatic food webs is tied to nutrient fluxes and physical conditions which in turn affect food resources for primary, secondary and higher trophic level consumers. Consequently, quantifying the temporal variability in such basal resources of an aquatic food web remains challenging. Characterizing the seasonal presence and abundances of the various primary production and organic matter sources in estuaries is critical to resolve the specific trophic responses of consumers to extreme flow events. The application of FA biomarkers provides a context to begin to understand seasonal resource and nutrient dynamics with relatively minimal sampling effort. However, sampling production sources is extremely important for comparing and resolving seasonal composition and dynamics of FA biomarkers across season, conspecifics and estuaries. Such studies provide valuable information for understanding shifts in carbon pathways and the responses of estuarine nekton species to high freshwater flow events. While quantitative estimates of diet using fatty acid profiles were not achieved here, our findings provide a general baseline to assess food web relationships influenced by extreme flow events. The results of the current study using FA biomarkers clearly distinguish seasonal dynamics in high trophic level species, a result not attained using stable isotopes (Olin et al. *submitted*). Under this consideration, FA profiles quantified in estuarine consumers may

provide greater resolution with respect toward characterizing the specific production resources impacted by anthropogenically altered flow events.

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Table 6.1 Fatty acids and fatty acid ratios used as biomarkers for potential estuarine organic matter sources compiled from published literature.

Source	FA Biomarker	Common Name	References
Diatoms	20:5ω3; 16:1ω7	Eicosapentaenoic acid (EPA); Palmitoleic acid	Parrish et al. 2000; Budge & Parrish 1998; Richoux & Froneman 2008
Dinoflagellates	22:6ω3	Docosahexaenoic acid (DHA)	Parrish et al. 2000; Napolitano et al. 1997
Bacteria	Σ15 + Σ17	Pentadecanoic acid; Heptadecanoic acid	Volkman et al. 1980; Budge & Parrish 1998; Richoux & Froneman 2008
Vascular plants (e.g., mangrove)	LCSFA 20:0-24:0	Arachidic acid; Heneicosanoic acid; Behenic acid; Tricosanoic acid; Lignoceric acid	Wai et al. 2011
Seagrass	18:2ω6 + 18:3ω3	Linoleic acid (LIN); α-Linolenic acid (ALA)	Kharlamenko et al. 2001; Hanson et al. 2010
Macroalgae (e.g., *Sargassum* sp. & red algae)	20:4ω6	Arachidonic acid (ARA)	Wai et al. 2011; Turner & Rooker 2006; Hanson et al. 2010
Brown algae (e.g., *Dictyota* sp.)	18:1ω9	Oleic acid	Johns et al. 1979; Hanson et al. 2010

Biomarkers for zooplankton (20:1ω9 and 22:1ω9, eicosenoic acids) were not included in the analyses as they have been shown to be relatively uninformative in estuarine systems (Alfaro et al. 2006; Richoux & Froneman 2008).

Table 6.2 Length, lipid content (% dry weight) and fatty acid values (*n* = number of individuals; % mean ± SE total fatty acids) of selected biomarkers of estuarine consumers sampled from the Caloosahatchee and Myakka estuaries during dry and wet seasons. For *n* < 3, all values are presented.

CALOOSAHATCHEE												
	Callinectes sapidus		*Lagodon rhomboides*		*Chaetodipterus faber*		*Ariopsis felis*		*Bagre marinus*		*Carcharhinus leucas*	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Length (cm)	19.0 ± 1.7	9.8 ± 0.1	12.0 ± 1.9	9.3 ± 0.6	12.1 ± 1.2	17.9 ± 0.9	30.1 ± 0.8	24.8 ± 2.1	39.6 ± 1.8	24.4 ± 4.6	94.3 ± 2.9	102.1 ± 12.6
Lipid content	4.95 ± 1.90	6.45 ± 0.63	6.6 ± 1.5	6.57 ± 1.48	4.59 ± 0.84	4.66 ± 1.27	6.42 ± 0.94	4.30 ± 2.40	6.51 ± 1.44	3.91 ± 0.89	7.66 ± 0.43	5.23 ± 0.64
n	3	3	4	5	4	6	4	16	5	5	3	3
C16:1n7	3.88 ± 1.96	4.01 ± 0.80	1.49 ± 0.32	3.45 ± 1.36	1.59 ± 0.40	1.80 ± 0.19	1.43 ± 0.44	1.77 ± 0.75	1.91 ± 1.30	2.12 ± 1.06	12.49 ± 0.24	4.84 ± 2.56
C18:1n9	15.36 ± 2.77	12.87 ± 4.16	6.92 ± 1.69	14.24 ± 3.14	9.05 ± 0.67	12.95 ± 2.69	12.09 ± 7.59	12.75 ± 3.47	9.21 ± 2.63	12.69 ± 2.30	27.17 ± 1.84	26.30 ± 2.64
C18:2n6	2.44 ± 1.18	1.14 ± 1.27	0.45 ± 0.13	0.64 ± 0.29	0.60 ± 0.22	0.39 ± 0.37	0.63 ± 0.22	1.06 ± 0.77	0.86 ± 0.49	1.92 ± 1.52	1.45 ± 0.65	0.73 ± 0.23
C18:3n3	2.52 ± 1.68	0.98 ± 0.85	0.52 ± 0.38	1.70 ± 2.09	0.91 ± 0.66	0.49 ± 0.39	1.81 ± 2.52	1.14 ± 1.80	0.38 ± 0.86	2.12 ± 2.16	0.81 ± 0.16	0.54 ± 0.03
C20:4n6	6.34 ± 0.42	9.75 ± 4.01	8.95 ± 2.17	5.78 ± 1.67	14.15 ± 1.09	10.84 ± 3.78	8.53 ± 2.10	11.54 ± 3.69	8.22 ± 1.95	10.05 ± 3.09	0.30 ± 0.26	5.31 ± 3.54
C20:5n3	15.45 ± 1.18	17.86 ± 0.66	8.61 ± 2.56	6.15 ± 1.07	6.10 ± 0.89	6.53 ± 1.31	7.30 ± 1.20	6.88 ± 2.28	5.03 ± 1.20	4.67 ± 1.69	0.82 ± 0.22	2.02 ± 0.53
C22:6n3	13.03 ± 0.70	13.85 ± 3.02	17.37 ± 6.12	14.97 ± 1.61	18.36 ± 1.22	16.00 ± 4.59	14.84 ± 3.12	15.92 ± 4.65	18.30 ±5.73	22.15 ± 4.70	2.29 ± 0.93	12.12 ± 2.98
Seagrass	4.96 ± 2.66	2.52 ± 1.36	0.97 ± 0.44	2.35 ± 2.17	1.51 ± 0.62	0.88 ± 0.64	2.45 ± 2.67	2.20 ± 1.82	1.24 ± 0.93	4.04 ± 3.23	2.26 ± 0.75	1.27 ± 0.24
Bacteria	3.57 ± 0.72	4.11 ± 1.31	6.32 ± 6.12	3.33 ± 1.42	2.65 ± 0.35	3.02 ± 0.69	5.98 ± 4.99	3.14 ± 1.64	5.67 ± 3.18	2.42 ± 2.06	3.28 ± 2.03	1.19 ± 1.03
LCSFA	1.05 ± 0.17	2.65 ± 3.32	6.38 ± 3.31	1.53 ± 1.01	0.99 ± 1.27	0.68 ± 0.92	4.04 ± 6.92	0.83 ± 2.01	4.11 ± 4.32	0.51 ± 0.78	6.76 ± 0.90	5.19 ± 4.79
MYAKKA												
Length (cm)	12.2 ± 1.1	13.1	9.7 ± 0.4	11.7 ± 1.1	10.5 ± 4.0	7.0, 20.0	25.7 ± 1.1	31.1 ± 0.7	42.2 ± 3.8	40.2 ± 1.3	102.5 ± 3.0	91.6 ± 13.8
Lipid content	4.17 ± 1.28	2.12	5.43 ± 1.80	6.78 ± 2.53	6.05 ± 1.34	6.34 ± 1.62	5.38 ± 3.35	5.55 ± 2.12	4.85 ± 1.17	5.11 ± 0.99	6.47 ± 2.01	5.55 ± 2.92
n	4	1	8	4	3	2	3	3	6	5	3	3
C16:1n7	5.92 ± 2.47	3.38	1.70 ± 0.81	2.71 ± 1.20	1.55 ± 0.33	1.36; 2.08	2.02 ± 1.03	1.59 ± 0.45	2.47 ± 1.97	4.37 ± 2.59	2.88 ± 0.45	3.33 ± 0.97
C18:1n9	15.87 ± 1.66	15.48	8.84 ± 1.60	11.98 ± 4.67	10.35 ± 1.29	7.79; 11.00	15.71 ± 3.31	14.75 ± 3.92	12.14 ±3.29	12.42 ± 0.94	22.52 ± 3.03	17.34 ± 4.66
C18:2n6	1.76 ± 0.73	3.20	0.76 ± 0.44	1.14 ± 0.42	0.71 ± 0.75	0.37; 1.01	0.97 ± 1.10	0.41 ± 0.08	0.56 ± 0.49	0.88 ± 0.31	1.23 ± 1.21	0.93 ± 0.72
C18:3n3	2.70 ± 2.47	3.09	1.39 ± 1.17	2.44 ± 3.00	1.80 ± 1.59	1.82; 0.79	2.05 ± 3.26	1.36 ± 2.36	1.90 ± 1.53	0.25 ± 0.27	1.02 ± 1.25	0.23 ± 0.40
C20:4n6	6.01 ± 0.94	9.60	7.83 ± 2.41	8.63 ± 1.08	9.37 ± 0.47	13.25; 15.05	10.82 ± 2.51	14.06 ± 0.80	7.42 ± 1.91	9.84 ± 2.68	6.48 ± 1.41	4.24 ± 1.27

C20:5n3	15.49 ± 5.11	14.27	7.74 ± 1.34	6.27 ± 0.76	6.01 ± 0.42	5.19; 8.45	8.04 ± 2.28	6.98 ± 1.56	6.34 ± 1.44	5.51 ± 1.05	1.80 ± 0.36	1.99 ± 1.06
C22:6n3	10.25 ± 1.97	13.01	28.04 ± 6.49	21.30 ± 6.23	16.51 ± 1.03	24.93; 13.17	16.36 ± 2.29	15.30 ± 2.12	20.19 ±6.44	17.96 ± 4.23	16.12 ± 4.00	12.12 ± 4.55
Seagrass	4.46 ± 2.72	6.29	2.15 ± 1.34	3.58 ± 2.72	2.51 ± 1.03	1.80; 2.18	3.02 ± 4.38	1.78 ± 2.44	2.46 ± 1.67	1.08 ± 0.39	2.25 ± 2.34	1.16 ± 0.71
Bacteria	3.14 ± 0.57	5.68	2.22 ± 0.24	2.35 ± 0.22	3.61 ± 1.22	2.82; 4.24	3.85 ± 3.11	3.69 ± 0.98	4.53 ± 2.88	3.32 ± 1.22	1.62 ± 1.84	3.21 ± 1.52
LCSFA	1.30 ± 0.57	1.86	2.70 ± 3.45	0.90 ± 0.70	2.10 ± 0.35	0.00; 0.00	2.24 ± 1.52	0.57 ± 1.00	2.17 ± 2.32	0.31 ± 0.69	2.31 ± 0.63	1.49 ± 2.58

Table 6.3 Loadings for the first two principal components (PC loadings) of the PCA of FA biomarkers of secondary (Fig. 1A, B) and tertiary consumers (Fig. 1C, D) from the Caloosahatchee and Myakka estuaries.

	CALOOSAHATCHEE				MYAKKA			
	Secondary Consumers PC Loadings		Tertiary Consumers PC Loadings		Secondary Consumers PC Loadings		Tertiary Consumers PC Loadings	
	1	2	1	2	1	2	1	2
16:1ω7	**1.105**	-0.009	0.089	-0.011	**0.984**	-0.377	0.157	**-0.842**
18:1ω9	0.641	0.240	-0.686	0.424	**1.009**	-0.504	0.158	**-0.801**
18:2ω6	**0.980**	0.021	0.362	**0.791**	**0.935**	-0.029	**-0.820**	-0.284
18:3ω3	**1.004**	-0.056	**0.769**	**0.752**	0.456	**1.058**	**-0.990**	0.316
20:4ω6	-0.460	0.485	**-0.829**	0.161	-0.268	0.139	0.549	0.128
20:5ω3	**0.709**	-0.118	**-0.822**	-0.318	**0.830**	-0.403	0.327	0.690
22:6ω3	-0.503	**0.726**	**-0.800**	0.354	**-1.031**	-0.083	0.180	**0.875**
LCSFA (20:0-24:0)	-0.287	**-1.139**	0.697	**-0.749**	-0.256	0.251	**-0.838**	-0.115
Bacteria (Σ15 + Σ17)	-0.231	**-1.165**	0.633	**-1.060**	0.602	0.147	0.197	0.005
Seagrass (18:2ω6 + 18:3ω3)	**1.136**	-0.028	**0.802**	**0.966**	**0.751**	**0.913**	**-1.064**	0.199
Eigenvalue (%)	39	22	28	25	41	18	32	22

The strongest contributions to principal components are bolded.

Table 6.4 Results of two-way ANOVAs performed on transformed factor scores from PCA used to test the effect of (1) trophic guild, (2) season (dry vs. wet) and (3) interaction on FA biomarkers profiles (α = 0.05; statistical significance highlighted in bold).

		CALOOSAHATCHEE					MYAKKA				
Secondary Consumers	**PC1**	df	SS	MS	*F*	*P*	df	SS	MS	*F*	*P*
	Species	2	0.015	0.007	21.033	**0.000**	2	0.020	0.010	35.227	**0.000**
	Season	1	0.001	0.001	1.8162	0.194	1	0.001	0.001	1.946	0.183
	Species x season	2	0.005	0.003	7.512	**0.004**	2	0.001	0.000	1.294	0.303
	Error	19	0.007	0.000			15	0.004	0.000		
	PC2										
	Species	2	0.008	0.004	3.335	**0.047**	2	0.006	0.003	2.632	0.105
	Season	1	0.001	0.001	0.721	0.406	1	0.003	0.003	3.164	0.096
	Species x season	2	0.004	0.002	1.680	0.213	2	0.001	0.000	0.472	0.633
	Error	19	0.023	0.001			15	0.016	0.001		
Tertiary Consumers	**PC1**										
	Species	1	0.001	0.001	1.132	0.297	1	0.000	0.000	0.000	0.988
	Season	1	0.001	0.001	1.123	0.299	1	0.004	0.004	2.151	0.166
	Species x season	1	0.000	0.000	0.209	0.652	1	0.000	0.000	0.035	0.854
	Error	26	0.029	0.001			13	0.024	0.002		
	PC2										
	Species	1	0.000	0.000	0.156	0.696	1	0.000	0.000	0.008	0.932
	Season	1	0.009	0.009	9.582	**0.005**	1	0.001	0.001	0.558	0.468
	Species x season	1	0.002	0.002	1.751	0.197	1	0.001	0.001	0.358	0.560
	Error	26	0.023	0.001			13	0.022	0.002		

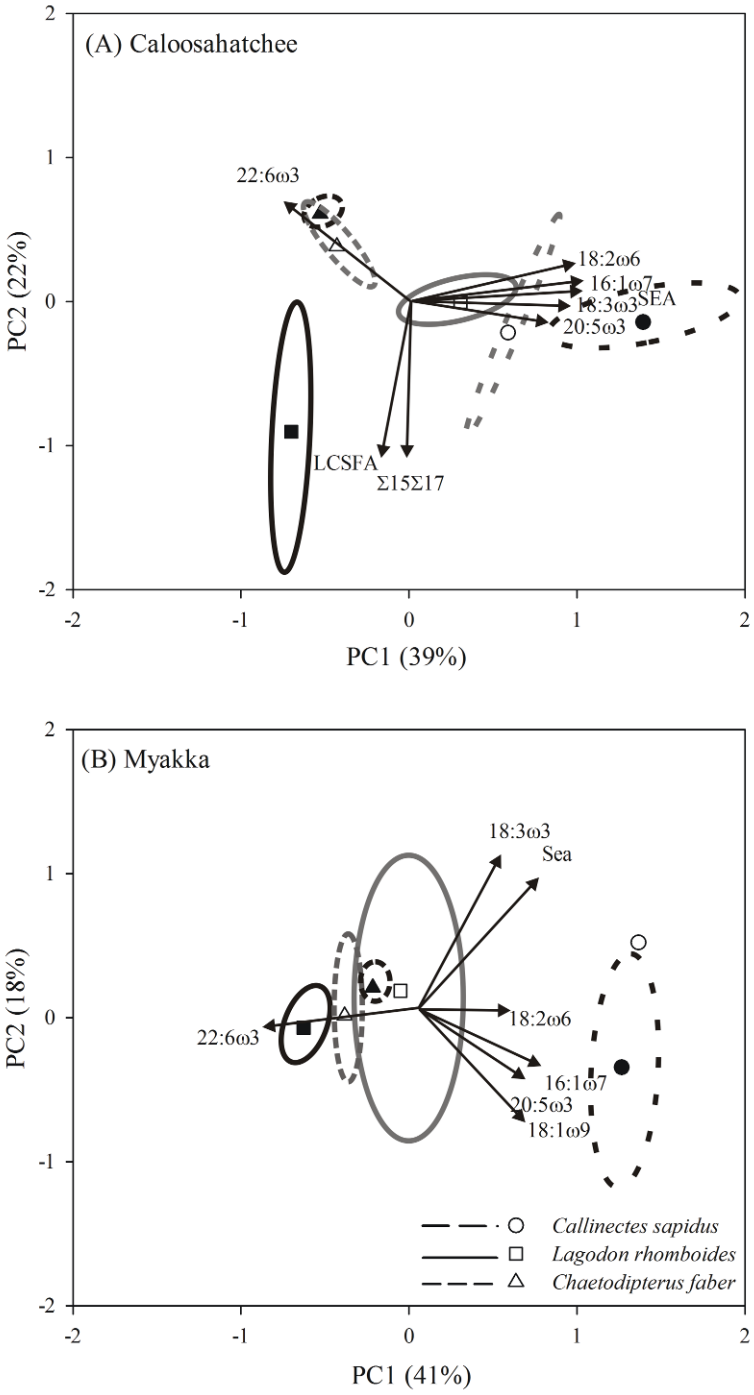

Figure 6.1 Principal component analyses of the secondary consumers depicting seasonal differences using FA. Ellipses are one standard deviation around the mean of each consumer's biomarker profile given the season (closed symbols and black lines represent dry season; open symbols and gray lines represent wet season). Only biomarkers with the strongest contribution to principal components are depicted (Table 6.3).

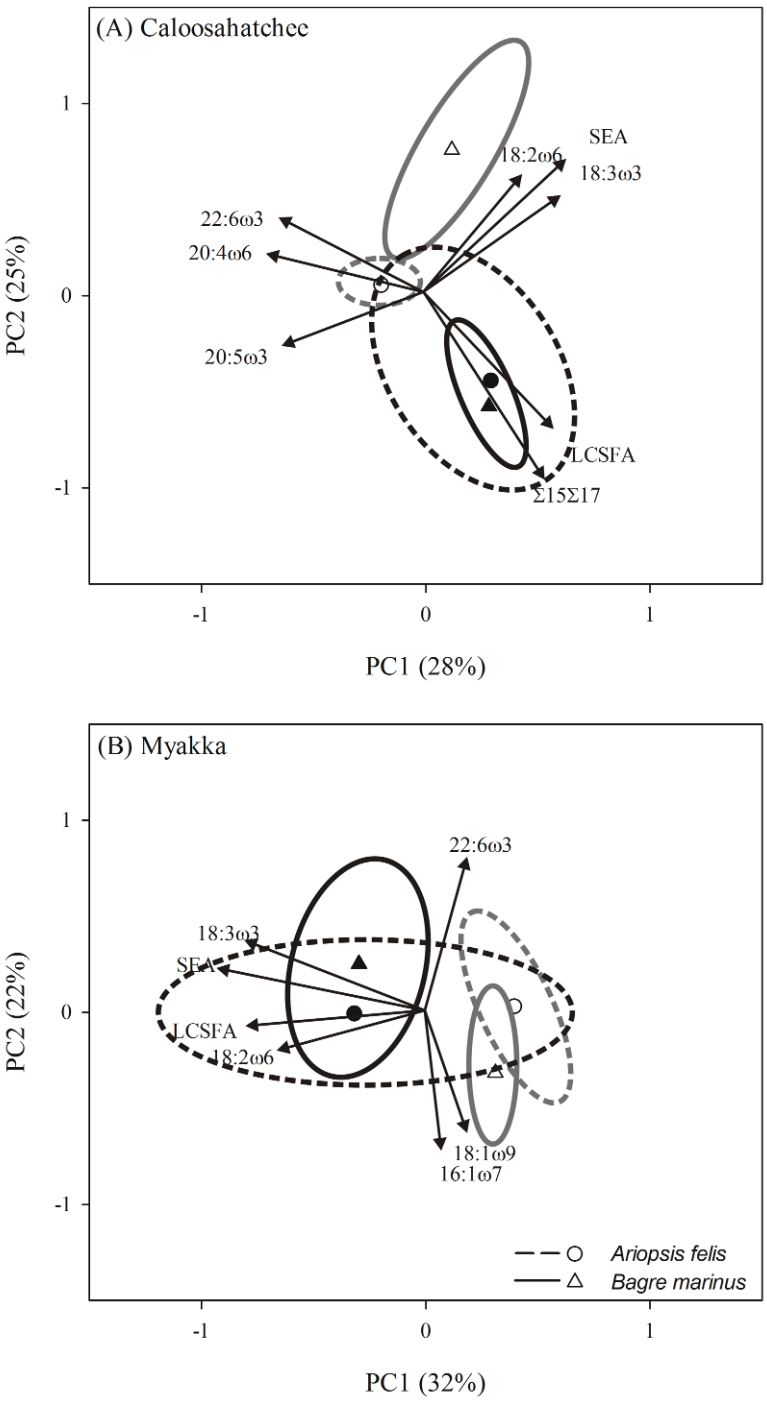

Figure 6.2 Principal component analyses of the tertiary consumers depicting seasonal differences using FA biomarkers. Ellipses are one standard deviation around the mean of each consumer's biomarker profile given the season (closed symbols and black lines represent dry season; open symbols and gray lines represent wet season). Biomarkers with the strongest contribution to principal components are depicted (Table 6.3).

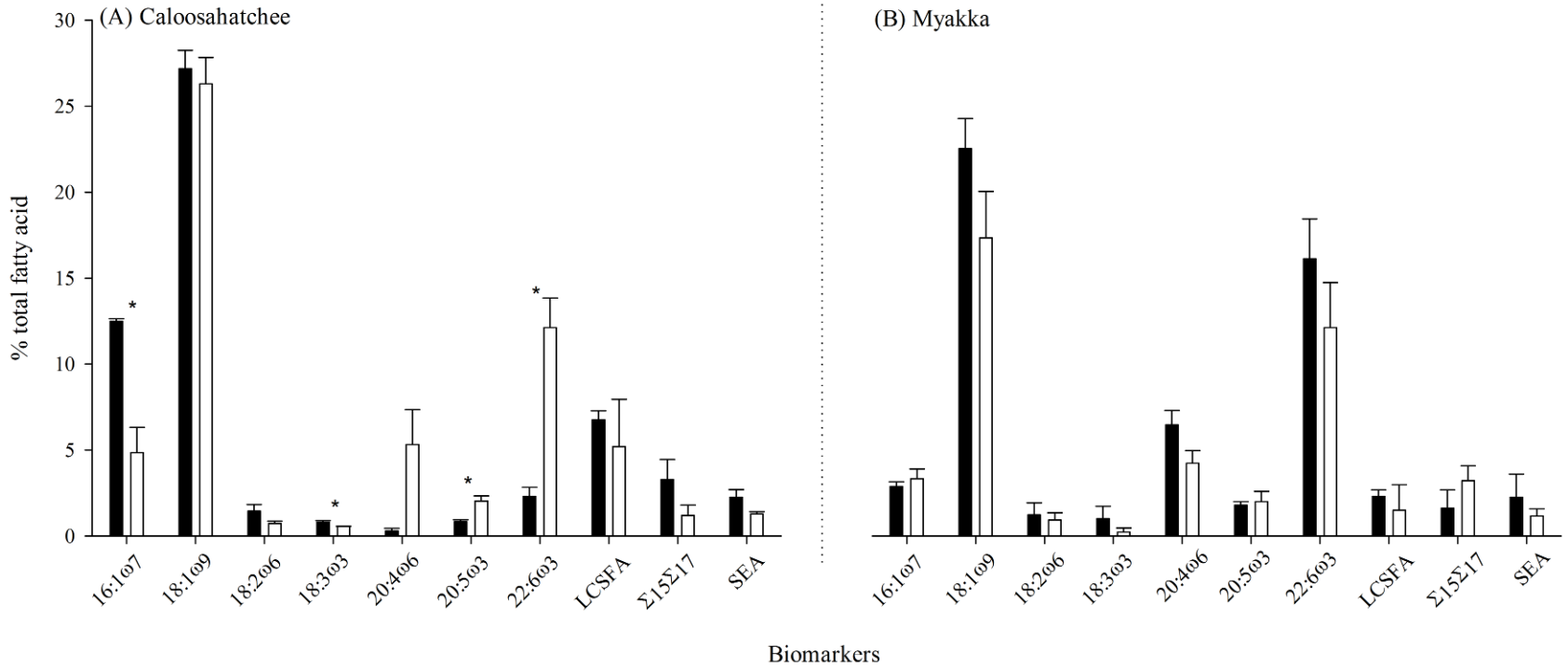

Figure 6.3 Seasonal mean ± SE % FA biomarkers of total lipids of *Carcharhinus leucas* (black bars represents dry season; white bars represents wet season). Significant differences between seasons are indicated by asterisks ($P < 0.05$).

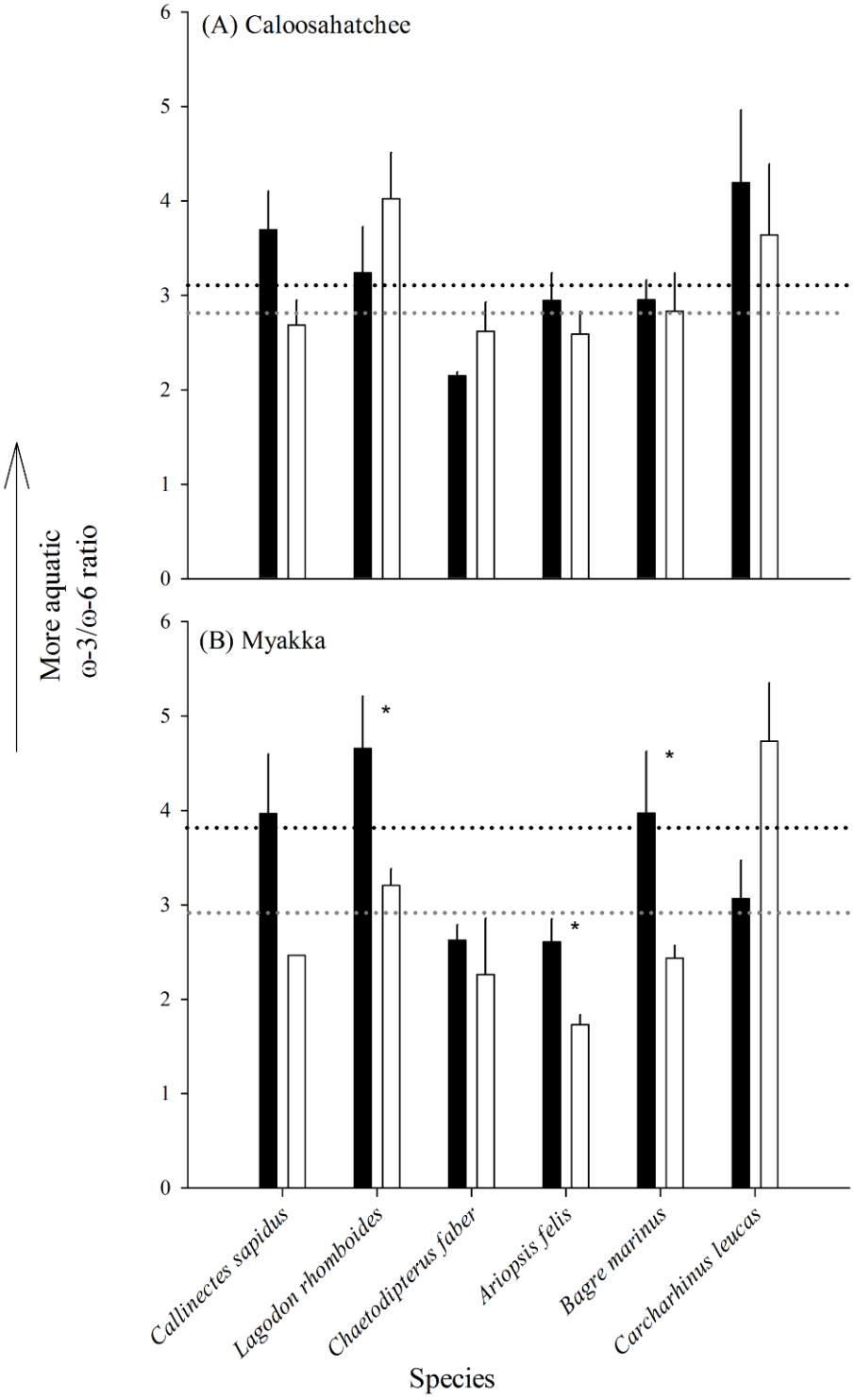

Figure 6.4 Ratio of ω3/ω6 FA (mean ± SE) in consumer species sampled following dry (black) and wet (white) season of the (A) Caloosahatchee and (B) Myakka estuaries. Dotted lines represent overall mean of ratios for each season (black represents dry; gray represents wet). Asterisk indicates significant one-way ANOVA at $\alpha = 0.05$.

SUPPLEMENTAL MATERIAL

Table 6S.1 Fatty acid composition of macro-invertebrate and fish consumers sampled from the Caloosahatchee estuary (mean % proportion ± SE of the total fatty acids) in 2008.

	Callinectes sapidus	*Lagodon rhomboides*	*Chaetodipterus faber*	*Ariopsis felis*	*Bagre marinus*	*Carcharhinus leucas*
n	6	9	10	20	10	6
Saturated fat acids (%)						
12:0	0.4 ± 0.4	0.4 ± 0.2	1.4 ± 0.4	1.2 ± 0.4	0.1 ± 0.1	0.7 ± 0.6
14:0	1.3 ± 0.6	2.9 ± 1.0	1.6 ± 0.3	2.0 ± 0.5	1.3 ± 0.5	3.0 ± 1.2
15:0	1.1 ± 0.4	1.9 ± 0.3	1.1 ± 0.1	1.5 ± 0.2	1.2 ± 0.4	1.1 ± 0.6
16:0	15.2 ± 0.7	22.4 ± 1.7	21.8 ± 0.8	16.7 ± 0.8	17.6 ± 0.7	20.9 ± 1.5
17:0	2.7 ± 0.6	2.7 ± 0.7	1.7 ± 0.1	2.2 ± 0.4	2.8 ± 0.6	1.2 ± 0.3
18:0	10.3 ± 0.5	10.2 ± 0.7	9.7 ± 0.4	11.5 ± 0.5	12.2 ± 0.6	10.4 ± 0.5
20:0	1.0 ± 0.3	1.5 ± 0.6	0.3 ± 0.1	0.8 ± 0. 5	1.2 ± 0.5	0.0 ± 0.0
21:0	0.3 ± 0.3	0.8 ± 0.5	0.1 ± 0.1	0.2 ± 0.2	0.4 ± 0.3	0.0 ± 0.0
22:0	0.5 ± 0.3	0.9 ± 0.4	0.4 ± 0.2	0.2 ± 0.2	0.3 ± 0.3	6.0 ± 1.3
24:0	0.2 ± 0.2	0.5 ± 0.3	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.0 ± 0.0
Subtotal	32.9 ± 1.8	44.4 ± 3.0	38.2 ± 1.1	36.6 ± 1.5	37.8 ± 3.0	43.2 ± 1.4
Monounsaturated fatty acids (%)						
14:1ω5	0.7 ± 0.4	0.7 ± 0.4	0.4 ± 0.3	0.7 ± 0.3	0.6 ± 0.4	0.0 ± 0.0
16:1ω7	3.9 ± 0.6	2.6 ± 0.5	1.7 ± 0.1	1.7 ± 0.2	2.0 ± 0.4	8.7 ± 1.8
17:1	0.0 ± 0.0	0.1 ± 0.1	0.7 ± 0.5	0.9 ± 0.3	3.5 ± 1.5	4.2 ± 1.9
18:1ω9	14.1 ± 1.3	10.6 ± 1.7	10.8 ± 0.7	12.4 ± 0.9	11.0 ± 0.8	26.7 ± 0.8
20:1ω9	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.7 ± 0.0
22:1ω9	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.4 ± 0.3	0.0 ± 0.0
24:1ω9	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
Subtotal	19.5 ± 1.7	15.3 ± 2.0	14.7 ± 1.5	16.9 ± 1.0	17.7 ± 1.5	40.3 ± 3.9
Polyunsaturated fatty acids (%)						
18:2ω6	1.8 ± 0.5	0.6 ± 0.1	0.5 ± 0.1	1.0 ± 0.2	1.4 ± 0.4	1.1 ± 0.2
18:3ω6	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
18:3ω3	2.0 ± 0.5	1.2 ± 0.6	0.7 ± 0.2	1.3 ± 0.4	1.3 ± 0.6	0.7 ± 0.1
20:3ω6	0.2 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	0.2 ± 0.2
20:3ω3	0.1 ± 0.1	0.7 ± 0.3	0.1 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.0 ± 0.0
20:4ω6	8.8 ± 1.2	7.2 ± 0.9	12.2 ± 1.1	10.9 ± 0.8	9.1 ± 0.8	2.8 ± 1.5
20:5ω3	16.7 ± 0.6	7.2 ± 0.8	6.4 ± 0.4	7.0 ± 0.5	4.9 ± 0.4	1.4 ± 0.3
22:5ω3	3.9 ± 0.4	5.0 ± 0.4	7.8 ± 0.9	7.6 ± 1.6	4.9 ± 0.3	4.2 ± 0.8
22:6ω3	13.4 ± 0.8	16.1 ± 1.5	16.9 ± 1.2	15.7 ± 1.0	20.2 ± 1.7	7.2 ± 2.3
Subtotal	49.4 ± 2.1	40.2 ± 2.9	47.1 ± 2.4	46.5 ± 1.3	44.5 ± 2.8	17.6 ± 4.8
Sum ω-3	36.0 ± 1.2	30.1 ± 2.3	31.9 ± 1.4	31.9 ± 1.2	31.6 ± 2.0	13.4 ± 3.3
Sum ω-6	11.9 ± 1.3	8.7 ± 0.9	13.9 ± 1.2	13.0 ± 0.8	11.32 ± 1.0	4.1 ± 1.5

Table 6S.2 Fatty acid composition of macro-invertebrate and fish consumers sampled from the Myakka estuary (mean % proportion ± SE of the total fatty acids) in 2008.

	Callinectes sapidus	*Lagodon rhomboides*	*Chaetodipterus faber*	*Ariopsis felis*	*Bagre marinus*	*Carcharhinus leucas*
n	5	12	5	6	11	6
Saturated fat acids (%)						
12:0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	1.0 ± 0.5	1.3 ± 0.7
14:0	1.1 ± 0.3	0.9 ± 0.2	1.7 ± 0.2	0.7 ± 0.4	1.7 ± 0.2	1.5 ± 0.7
15:0	1.8 ± 0.4	0.9 ± 0.1	1.6 ± 0.2	1.8 ± 0.8	2.5 ± 0.7	1.3 ± 0.4
16:0	16.3 ± 1.0	19.6 ± 1.2	21.3 ± 0.6	15.7 ± 1.2	18.6 ± 0.9	19.3 ± 1.1
17:0	1.8 ± 0.2	1.4 ± 0.1	2.0 ± 0.2	1.9 ± 0.3	1.5 ± 0.2	1.2 ± 0.5
18:0	9.9 ± 0.8	8.2 ± 0.2	10.7 ± 0.5	13.3 ± 0.9	10.3 ± 1.0	12.9 ± 0.4
20:0	1.1 ± 0.2	1.4 ± 0.9	0.5 ± 0.2	1.1 ± 0.6	1.1 ± 0.6	0.2 ± 0.2
21:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:0	0.3 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	1.50 ± 0.7
24:0	0.2 ± 0.0	0.2 ± 0.1	0.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2
Subtotal	32.3 ± 1.1	33.0 ± 1.0	38.9 ± 1.4	34.9 ± 1.8	36.9 ± 1.50	39.2 ± 2.0
Monounsaturated fatty acids (%)						
14:1ω5	1.3 ± 0.6	0.9± 0.5	1.1 ± 0.7	1.0 ± 0.7	0.8 ± 0.3	0.0 ± 0.0
16:1ω7	5.4 ± 1.1	2.0 ± 0.3	1.6 ± 0.2	1.8 ± 0.3	3.1 ± 0.7	3.1 ± 0.3
17:1	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.7	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0
18:1ω9	15.8 ± 0.6	9.9 ± 0.9	10.0 ± 0.7	15.2 ± 1.3	12.3 ± 0.7	19.9 ± 1.8
20:1ω9	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	1.2 ± 0.3
22:1ω9	0.2 ± 0.2	0.7 ± 0.4	0.3 ± 0.2	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
24:1ω9	0.5 ± 0.2	0.7 ± 0.1	0.6 ± 0.3	0.5 ± 0.2	0.4 ± 0.1	0.2 ± 0.2
Subtotal	23.9 ± 2.2	14.8 ± 1.0	14.8 ± 1.6	19.3 ± 1.8	17.8 ± 1.3	24.8 ± 1.8
Polyunsaturated fatty acids (%)						
18:2ω6	2.1 ± 0.4	0.9 ± 0.1	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.1	1.1 ± 0.4
18:3ω6	0.3 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.0 ± 0.0
18:3ω3	2.8 ± 1.0	1.7 ± 0.6	1.6 ± 0.5	1.7 ± 1.1	1.1 ± 0.4	0.6 ± 0.4
20:3ω6	0.2 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.8 ± 0.4	0.9 ± 0.1	0.9 ± 0.1
20:3ω3	0.8 ± 0.5	0.6 ± 0.2	0.4 ± 0.2	0.8 ± 0.5	0.6 ± 0.3	0.2 ± 0.2
20:4ω6	6.7 ± 0.8	8.1 ± 0.6	11.3 ± 1.2	12.4 ± 1.0	8.9 ± 0.7	5.4 ± 0.7
20:5ω3	15.2 ± 2.0	7.3 ± 0.4	6.3 ± 0.6	7.5 ± 0.8	6.0 ± 0.4	1.9 ± 0.3
22:5ω3	2.3 ± 0.4	4.7 ± 0.2	5.9 ± 0.3	4.3 ± 0.2	5.8 ± 0.3	10.6 ± 3.0
22:6ω3	10.8 ± 0.9	25.8 ± 2.1	17.5 ± 2.0	15.8 ± 0.8	19.5 ± 1.6	14.1 ± 1.8
Subtotal	43.7 ± 2.6	52.2± 1.6	46.4 ± 2.0	45.8 ± 2.1	45.3 ± 2.3	36.0 ± 2.1
Sum ω-3	32.0 ± 2.6	40.1 ± 1.8	31.8 ± 1.9	30.1 ± 1.8	33.0 ± 2.36	27.4 ± 2.1
Sum ω-6	9.3 ± 1.1	10.2 ± 0.6	13.2 ± 1.2	14.3 ± 1.1	10.8 ± 0.8	7.3 ± 0.6

CHAPTER 7

GENERAL DISCUSSION

Disturbances are natural events occurring in nearly all ecosystems and are important mechanisms shaping community structure and food web dynamics (Pickett and White 1985). However, extreme disturbance, where the frequency and severity of the disturbance is too great can disrupt natural community complexity and food web function (sensu Connell 1978). It has been argued that maintenance of a community is dependent on temporal and spatial variability in the structure of the community, as well as the ability of the species to rapidly respond to such variation (McCann and Rooney 2009). Consequently, understanding how communities respond to and persist in anthropogenic-altered environments has become one of the most fundamental objectives in ecology, particularly as human modifications to the landscape increase. This type of evaluation holds particular importance for estuarine ecosystems, as there are few estuaries worldwide that remain unaffected by upstream manipulation of their freshwater flow (Dynesius and Nilsson 1994; Nilsson et al. 2005). Predicting the response of estuarine ecosystems to changing environmental condition is however challenging, as it necessitates understanding interactions among several trophic levels and multiple nutrient sources (marine, freshwater and terrestrial) (Rush et al. 2010).

Collectively this dissertation provides important data regarding the effects of human-altered freshwater flow on estuarine nekton communities in tidal rivers, and in so doing has provided important findings regarding the application of stable isotopes to estuarine fishes and large vertebrates. In particular, we document how altered high-flow reduces seasonal-variability of nekton

community, through loss of density, diversity and richness of nekton species, highlighting the implications for food web simplification with this type of disturbance (Chapter 2). We further go on to demonstrate, through application of stable isotopes and fatty acids that altered high-flow shifts the carbon resources available to both lower (i.e., primary and secondary consumers; Chapter 5) and higher (i.e., tertiary consumers and piscivores; Chapter 6) trophic levels towards more terrestrially-derived resources and reduces the inter-species variability in carbon resource use (Chapter 5). Collectively, these chapters provide insight on the role that altered freshwater flow plays in shaping estuarine nekton community structure and food web dynamics, in both space and time.

In addition to demonstrating the ecological consequences of altered high-flow to estuarine communities, we improved our knowledge and hence the applicability of stable isotopes for understanding isotope dynamics of estuarine fishes (Chapter 4) and high trophic level fish species, with unique life history strategies (Chapter 3). These two chapters highlight some of the limitations of stable isotope analyses that need to be considered and addressed prior to conducting diet composition and/or food web analyses using these tracers. Moreover, fatty acids emerged as an informative tool, by providing a unique perspective for assessing production sources used by estuarine species (Chapter 6), offering a compliment to, and even advantages over application of stable isotopes for assessing trophic relationships and production sources of estuaries species.

CONTRIBUTIONS OF THE DISSERTATION

Community ecology

Loss of seasonal variability in community metrics of the Caloosahatchee was demonstrated in Chapter 2. Freshwater flow in the Caloosahatchee during the time of this study was not being managed for minimizing prolonged or excessive high flow, and therefore represents unadulterated release from Lake Okeechobee. As such, this chapter provides unique empirical evidence that high-flow affects the structural complexity of the different components of the estuarine nekton community, i.e., small-bodied and large-bodied species, a result not demonstrated in the Myakka. Further, by categorizing species into ecological and trophic guilds, this chapter contributes an original assessment of community response to altered high-flow disturbance, by demonstrating which component of the community is most affected. In this context, we demonstrated a shift in diversity and richness of ecological guilds, from more marine to freshwater dominated, as well as in trophic guilds, from higher to lower trophic level concomitant with altered-flow. The use of ecological and trophic guild to document the community response provides a new framework by which flow managers can assess overall effects on a community and develop management strategies to maximize community composition and diversity as opposed to using species-specific response to inform overall community management. Based on this analysis and in light of the prediction of increases in large storm events (Easterling et al. 2000), we can expect estuaries to become less seasonally-diverse and more simplified, being dominated by seasonally-tolerant species.

Food web ecology

Freshwater flow is necessary for proper estuarine functioning, as it provides nutrients and sediments, fueling primary and secondary productivity. Chapter 5 highlights shifts in stable isotopes of estuarine consumers, predominately lower trophic level species, faced with altered flow. The significant depletion in ^{13}C and enrichment in ^{15}N alludes to changes in resource use (i.e., production sources) and changes in resource availability with high-flow. The fact that this trend is observed in the majority of primary and secondary consumers is a important result, as it suggests a homogenization of carbon sources in estuaries with extreme high flows. Although this shift was not evident in the tissues of higher trophic level species, the results do demonstrate that altered high flow does impact estuarine food webs. Because significant changes were only documented in lower trophic levels, when using stable isotopes to track seasonal variability, focus should be at the base of the food web, e.g., primary and secondary consumers, rather than across multiple trophic levels. We argue that the lag associated with transfer of stable isotopes from lower to higher trophic levels explains that lack of significant isotopic changes in the higher trophic levels.

The novel application of fatty acid biomarker in Chapter 6 to track altered flow events in estuaries demonstrated that resource use of high trophic level species is indeed influenced by altered high-flow; a result not identified using stable isotopes of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$. This chapter provides a compliment to Chapter 5 and further demonstrates that terrestrially-derived allochthonous

resources are used by species across a broad range of trophic levels and because of the magnitude flow are utilized differently between the two estuaries. It is often difficult to sample seasonally all of the production and organic matter sources in food web, particularly in estuarine environments, where sources are derived from autochthonous and allochthonous sources. This unique application of fatty acid biomarkers may indeed prove an alternative to sampling each production source seasonally. Regardless, the use of fatty acids to track flow-related responses greatly improved the resolution by which we can observe a response and thereby provided a greater understanding of how high-flow manifests in estuarine food webs.

Biochemical tracers

Despite the prevalence of stable isotope analyses in ecological studies of diet and food webs, there are still a number of factors that can complicate interpretations of stable isotope data and studies have recommended establishing species-specific criteria for accurate isotopic assessment of an organism (Sweeting et al. 2007). Size and season-based changes in diet are common and often explain variation in stable isotope composition between species and among individuals in a population. However, a caveat of stable isotope analysis is that changes in the diet are not instantly manifest in the isotopic composition of a consumer's tissues and a consumer's tissues may reflect a combination of effects apart from diet (Vander Zanden et al. 2000).

In light of the documented declines in many shark populations, raising concerns over ecosystem effects (Heithaus et al. 2008), understanding the trophic role of young sharks, assumed to be top predators within coastal habitats is important (Cortés 1999). Chapter 3 provides an original and important contribution to understanding the application of stable isotopes in organisms that are provisioned, through a placental connection, with maternal resources. The results of this chapter indicate that retention of the maternal isotopic signal by neonate and young-of-year sharks is dependent on species-specific life history and tissue characteristics. This chapter highlights the use of a unique characteristic of sharks, the umbilical scar, to determine the time when the tissues of young-of-year/juvenile sharks represent their own diet as opposed to their maternal provisions. These findings are especially relevant, as misinterpretation of feeding strategies, specifically overestimation of trophic position and incorrect assignment of dominant carbon sources to the diet would occur without these considerations. This chapter not only identifies the inability of using stable isotopes to characterize the diet of this young age-class, but takes the first step in attempting to quantify the change and provide guidance for future research addressing these age classes. As such, this chapter provides a significant contribution not only to the application of stable isotopes in young individuals of a species but to the study of sharks, and placental species in general.

As I have advocated throughout this dissertation, estuaries are highly complex. This complexity makes characterizing feeding relationships of fishes in these systems especially challenging, particularly when considering that estuaries

are used by a range of life history stages of individual species, many of which exhibit ontogenetic diet shift with size. While body-size based shifts are often cited as drivers of isotopic dynamics, in Chapter 4 I found no evidence for body-size based-isotopic relationships in estuarine fishes. Our results are consistent with previous observations that body size is not an important determinant of isotopic enrichment in estuarine fishes (Wilson et al. 2009). As such this chapter contributes to the broader understanding of stable isotope dynamics is relation to size and the relative importance of this factor in affecting stable isotopes dynamics in estuarine fishes. Outside of larval and young-of-year of fishes that show clear size based-isotopic shifts (Mittelbach and Persson 1998), estuarine fishes analyzed here do not appear to be influenced by size-based-isotopic relationships. Whether this result is a consequence of the fact the species analyzed here are predominantly secondary and tertiary consumers (Scharf et al. 2000), or that despite diverse diets throughout their lives, they likely select prey of relatively similar trophic level (Deudero et al. 2004), remains to be seen. However what we have show is that when including a species in food web analyses of estuarine ecosystems, sampling the entire size ranges of each consumer is perhaps less important, than it would be in a pelagic system that exhibits clear size-based structuring (Jennings et al. 2008).

FUTURE DIRECTIONS

Whether altered high-flow disturbance results in negative or positive effects on overall persistence of estuaries, remains to be seen. However,

analogous to high flow events, nekton communities have been shown to return to pre-storm conditions within a short period of time (e.g., 6-12 months), indicating relatively short effects on biota and community structure, and high ecosystem resiliency to hurricane pulses (Piazza and La Peyre 2009). Understanding the range of community change and resiliency that is experienced by a system in response to disturbance provides insight into ecosystem function that can guide management and potentially restoration of estuarine ecosystems. With this in mind, this dissertation provides a number of avenues for future research.

We can extend the results of the community analysis by sampling over broader temporal scales, and in so doing address annual variability in flow dynamics. This dissertation initially set out to compare annual variation in flow regimes, however, in order to answer the primary questions regarding effect of flow on community structure and food web interactions, using multiple years with variable flow regimes became complicated. Testing temporal related hypotheses, particularly with the knowledge of which years were classified as high flow and those as drought, would be an avenue by which we can monitor how an estuarine system contends with such extremes. Droughts, similar to floods have been shown to produce distinct changes in community structure (Baptista et al. 2010). These environmental fluctuations influence the economic productivity of commercial and recreational fisheries by modifying the availability of fisheries resources (Gillson et al. 2011). Thus understanding these dynamics collectively could aid in providing information to managers.

The stable isotope and fatty acid results presented in this dissertation collectively suggest that altered high-flow shifts carbon flow. Sampling primary production and organic matter sources during the low and high flow season would confirm the conclusions of this dissertation and would allow for implicit conclusions regarding specific dietary changes of these consumers. In addition, sampling of primary consumers over seasonally relevant timescale would enhance our understanding and conclusions regarding seasonal changes to trophic structure in these estuaries, as it would allow for calculation of food chain length. Food chain length is an important characteristic of ecological communities, based on the ultimate trophic position in the food web and may be strongly influenced by disturbance. Shifts in food chain length can alter ecosystem function and modify trophic interactions (Walters and Post 2008) and can provide a top-down perspective of disturbance.

The application of biochemical tracers to answer questions regarding food web structure and mechanisms regulating that structure are widely used. The different responses of conspecifics identified from stable isotopes and fatty acids techniques, highlight the differing conclusions that can be drawn from these tracers regarding effects of flow on estuarine consumers. Muscle tissue was the main tissue used for the analyses presented here. However, current literature suggests that liver, skin and blood have faster turnover rates relative to muscle (Hobson and Clark 1992; MacNeil et al. 2005) and therefore have the potential to track species response to altered flow across multiple timescales.

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