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The Economically Important Nitrogen Pathways of Southwest Florida

by

Elon M. Malkin

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
College of Marine Science
University of South Florida

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ABSTRACT

The global phenomenon of burgeoning coastal population growth has led to coastal watershed landscape transformation and ecosystem degradation, prompting policy-makers to set limits on freshwater withdrawals and labile nutrient loads. Important components of Florida's economies lie in the state's expansive coastal zone; the organisms driving the billion-dollar recreational fishing industry are rooted in coastal habitats, while the agriculture and real-estate industries sprawl throughout numerous coastal watersheds. This study aimed to identify the connections between anthropogenic land use and essential juvenile fish nursery habitats within the coastal zone, which is the first critical step for sustaining the ecology and related economies of the region.

The need for this study arises from the fact that these economies are interconnected through nitrogen, and therefore nitrogen management can influence their prosperity or collapse. Juvenile fish nursery habitats are located in waters that receive nitrogen from adjacent landscapes. Runoff delivers nitrogen derived from human nitrogen use and processing within the watersheds to the juvenile fish nursery habitats. Ecosystem managers must understand that although copious amounts of nitrogen applied to land may ultimately support nursery habitat foodwebs, overwhelming nitrogen loads may also create algal blooms that decay and cause lethal hypoxic events leading to ecosystem degradation. This study aims to pinpoint the specific nitrogen sources that support primary production and ultimately fish production in watersheds dominated by agricultural landscapes and residential neighborhoods

Stable isotopes are versatile tools used to identify these connections. The nitrogen and carbon compounds that make up the moieties of an ecosystem inherently carry information on major nitrogen sources, trophic structure as well as the crucial information concerning dominant nitrogen removal and transformative processes that occur within sediments. Specifically in this study, the stable isotopes of carbon and nitrogen of dissolved inorganic nitrogen, primary producers, and fish were used to identify 1) the connections between urban and agricultural landscapes and the nutrients that percolate through the foodweb, 2) the primary producers that support fish biomass, 3) the origins of sedimentary organic matter that can provide new nitrogen via recycling, and 4) the heterogeneous function of fish nursery habitats in polluted systems. This study was conducted during the region’s wet and dry seasons and in over thirty watersheds that differ from each other in terms of size and anthropogenic influence.

In agricultural watersheds, nitrogen derived from row crops and tree crops ultimately supported fish production during the wet season. Convective afternoon thunderstorms coupled with runoff delivered nitrogen from the landscape to receiving waters. These nutrients supported phytoplankton which deposited into the sediments and supported benthic foodwebs. During the dry season, nitrogen derived from row crops and nitrogen transformation in the sediments ultimately supported fish production. In this case, irrigation water used for agriculture delivered nitrogen from lands covered with row crops to the nursery habitats in receiving waters.

The dry season was characterized by the nitrogen transformation process known as dissimilatory nitrogen reduction to ammonium (DNRA), where biologically available nitrate is converted to biologically available ammonium. Phytoplankton deposits, most likely delivered during the wet season, were recycled through the slow burning DNRA processes, which provided nitrogen for the benthic microalgae that dominated in the dry season. These organisms in turn supported benthic communities which ultimately supported dry season fish production.

In small urban watersheds, nitrogen derived from septic tanks, lawn irrigation, leaky sewage pipes, and atmospheric deposition ultimately supported fish production via phytoplankton, but unlike the nitrogen sources in agricultural watersheds, these sources (with the exception of atmospheric deposition) were seasonally consistent because a mechanisms to deliver nitrogen derived from septic tanks, lawn fertilizer, and leaky sewage pipes were, at least to some extent, available during both seasons.

In polluted, tidal, fish-nursery habitats, the specific mechanism that allowed nursery habitats to decrease the ratio of mortality over growth rates of juvenile fish was not consistent among systems. These mechanisms were likely dependent on physical-chemical parameters and stream geomorphology. If the geomorphology or physical-chemical characteristics of nursery habitats are not adequate to set up an efficient nitrogen transfer process to fish, these habitats become more of a haven from predators rather than a source of food for fish.

This study has several implications for management. Managers must first recognize that microalgae are dominant supporters of tidal nursery foodwebs. Managers must define the relationship between nitrogen loads and fish abundance. If this relationship is unknown, the results of increasing nitrogen loads on fish production will remain uncertain; foodwebs in nursery habitats may collapse due to eutrophication, or fish abundance may increase due to increases in food supply. Connectivity factors derived from stable isotope mechanistic mass-balance models can be used as measurable targets for groups of watersheds. The use of wetlands as nitrogen remediation tools may not be effective at removing nitrogen; nitrogen transformation processes such as DNRA likely outweigh removal processes in wetland soils.

CHAPTER 1:

INTRODUCTION TO FISH NURSERY HABITATS AND THE NITROGEN CYCLE

Anthropogenic Influences on Coastal Habitat Services

Coastal zone habitats provide manifold benefits to society, despite covering only 7% of the ocean's surface. In a coastal temperate setting, over forty types of ecosystem resources were identified and classified as providing provisional, regulatory, and cultural services (Ronnback et al. 2007). These habitats are especially abundant in Florida, the state with the longest coastline in the United States. Encompassing ecological, economical, and cultural dimensions, the most valuable resource provided by the coasts of Southwest Florida is the juvenile fish nursery habitat. These diverse aquatic ecosystems support the region's billion dollar recreational and commercial fishing industries, tourism, as well as a fishing culture, cherished by both the local population and vacationers alike.

Juvenile fish nursery habitats include small water bodies, which not only provide structural refuge from predation, but also provide the primary production that ultimately supports the growth and survival of juvenile fish. While in nursery habitats, economically and ecologically important species such as juvenile snook, red drum, hogchoker, striped mullet, and blue crab obtain biomass by consuming small resident fishes (e.g., rainwater killifish, mosquito fish, and sheepshead minnow), benthic invertebrates (e.g., small crustaceans, worms and insect larvae) and in some cases, by directly consuming microalgae or plants (Peters and McMichael 1987, Killam et al. 1992, Poulakis et al. 2002). Because these habitats are typically situated in water bodies adjacent to the burgeoning coastal population, the services provided by these critical resources can undoubtedly be impacted by land use and human activity.

Fundamental Need for this Study: Impacts on Florida's Major Economies

This study focuses on the nitrogen pathways that originate from land and percolate through juvenile nursery food webs. Two major Floridian land-based economies, agriculture and real-estate development, may pose a significant threat to the economically essential organisms that utilize these nursery habitats. Nitrogen derived from agricultural operations and urban land use directly influences the trophic systems that operate in these nursery habitats; runoff typically delivers unutilized nitrogen from developed land into adjacent water bodies, which in turn is taken up by primary producers and transferred through the food web. Although copious amounts of nitrogen applied to land may ultimately support nursery habitat food webs, overwhelming nitrogen loads may also create algal blooms that decay and cause lethal hypoxic events leading to ecosystem degradation.

The oxymoronic term “nutrient pollution” has been applied to scenarios where non-point source nutrients derived from anthropogenic land use invade aquatic environments and cause hypoxia (Smith et al. 1997), seagrass declines (Greening and Janicki 2006), and fish kills (Clarholm et al. 1988). Oftentimes, nutrient pollution is associated with estuarine systems that have been over-utilized and under-protected with adverse economic consequences including the degradation of habitats essential for fish production (Braun, 2007); Chatterjee (2009) has concluded that eutrophication of freshwater regions alone accounts for over a four billion dollar annual loss to the United States.

Although nutrients have been widely perceived by ecosystem managers and ecologists as damaging to ecosystems, they are a key building block for fish production.

Cushing (1975) coined the term “agricultural model” that relates fish biomass to the amount of nutrients supporting the food chain. This theory has been confirmed by positive relationships between primary production and fish landings observed by Nixon (1982). Further evidence of this phenomenon was provided by Josefson and Rasmussen (2000) who found a positive curvilinear relationship between nitrogen load and benthic microfauna up to a critical point at which the relationship either leveled-off or reversed. These results support the models proposed by Caddy (1993) which relate responses of fish yield to nutrient loads in a variety of semi-enclosed seas. Caddy (1993) proposed that the shapes of the fish catch to biomass curves would be dependent on the benthic or pelagic nature of trophic pathways leading to a particular fish catch. Especially notable were the benthic foodwebs, described as extremely susceptible to hypoxia under high nutrient loads. In a recent study, Oczkowski and Nixon (2008) defined the nutrient enrichment scale associated with the Caddy (1993) models in the tropical lagoons of the River Nile in Egypt and concluded that anthropogenic nitrogen initially increased higher trophic level productivity to a critical point after which an exponential decline in fish landings occurred. Oczkowski et al. (2008) also reported that half of hard clam biomass (*Mercenaria mercenaria*) in Narragansett Bay is supported by anthropogenic nutrient sources.

Sources of Nitrogen Inputs and Land Use

The heterogeneous nitrogen inputs to nursery habitats are directly related to watershed land use, which has changed during Florida’s history. Upland forests and open rangeland have been replaced with modern agriculture and urbanization. In the civil war period, cattle roamed freely over wide spans of rangeland, wetlands and upland forests. Landscapes in Southwest Florida changed dramatically in the late 19th century as North Florida citrus growers established groves in the region below the frost-free line in the Caloosahatchee valley. As the region became more developed in the first half of the twentieth century, more land was cleared and soils were exposed for increased row crop production. The second half of the twentieth century to the present is considered the era of urban development; there area of urban landscape is now 100,000 times larger than the area of urban landscape at the end of the civil war. Furthermore with an increase in property values, the modern real estate industry is engulfing lands formerly occupied by either agriculture or upland forests. Using stable nitrogen and carbon isotopes and a variety of sediment cores, Peebles et al. (2009) identified the general historical land use trend in the Old Tampa Bay that influenced trophic ecology. Old Tampa Bay, an urbanized estuary with mostly residential land use is known to have an agricultural past. Figure 1.1 derived from Peebles et al. (2009) indicates that in pre-development times (early 1900's), Old Tampa Bay was supported by nitrogen fixers and marine dissolved inorganic carbon. In the 1940's, the nitrogen sources shifted to rangeland and pastureland, and in modern times, residential fertilizer and terrestrially derived carbon supported the autotrophs that ultimately provided the basis of the foodwebs and that deposited into the sediments.

The Delivery of Nitrogen to Receiving Waters

Water transports nitrogen from land to tidal nurseries, but the specific mechanism of delivery can be directly affected by the above mentioned agricultural and urban economic sectors. Especially in Southwest Florida, there is a growing emphasis on using rivers to support agricultural water demands rather than groundwater. Traditionally, agricultural operations withdraw large amounts of groundwater for irrigation, which has often been shown to cause salt intrusion into drinking water sources. On the other hand, groundwater irrigation processes provide a mechanism of water and nutrient delivery, especially during Southwest Florida's dry season (March through May) where

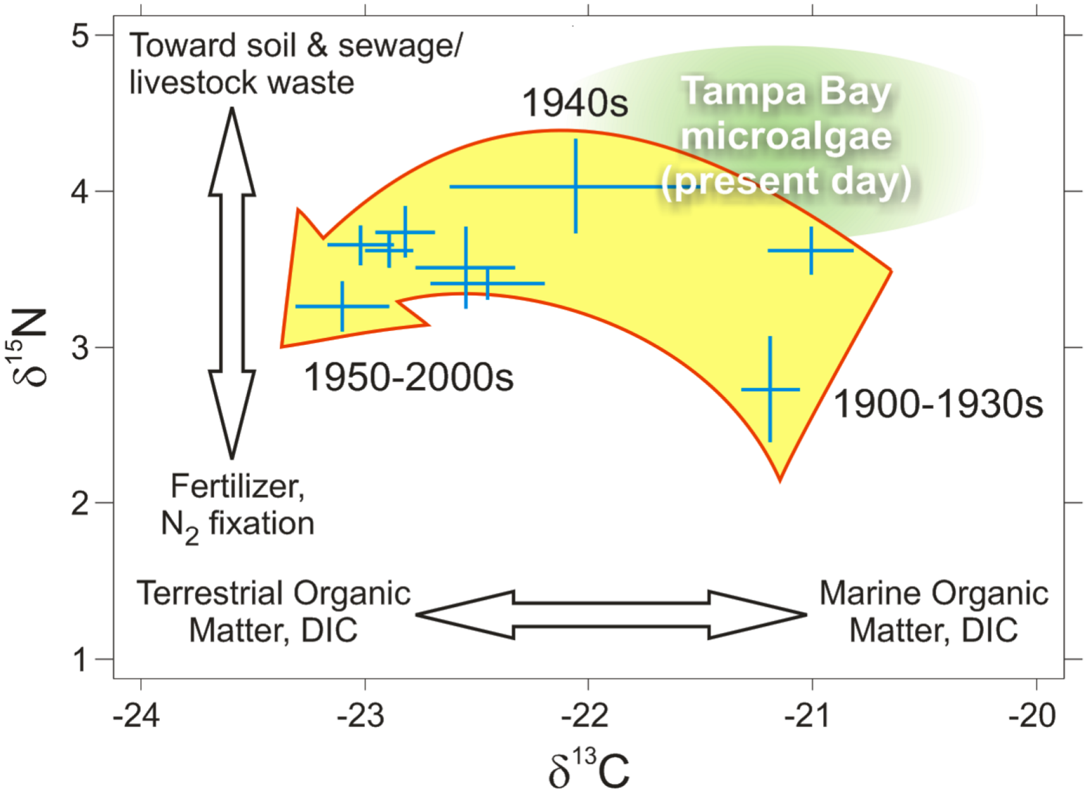


Figure 1.1: Isotopic Record of Historical Land Use in Tampa Bay. Sediment record from Old Tampa Bay indicates an estuary supported by nitrogen fixation, followed by pastureland, and finally residential fertilizer (Figure from Peebles et al. 2009).

agricultural groundwater withdrawals provide river baseline flow. Furthermore, the Southwest Florida Water Management District (SWFMD) highly limits residential lawn water use during the dry season (March through May), and current policy supports reclaimed water utilization for golf course and public park maintenance. Recently passed laws also limit fertilizer application during the wet season (late June through September).

Dynamics of Flow and Light on Determining Nitrogen Pathways

Water is not only the major delivery medium of nitrogen into food webs, but also has far reaching effects on controlling where nitrogen is most effectively transferred to fish production within each coastal fish nursery. The dynamics of water residence time, light attenuation and stream geomorphology determine the locations of maximum chlorophyll concentrations and locations of maximum benthic microalgae survivability. Since microalgal organisms have the potential to support the basis of fish nursery food webs, the location of “benthic hot spots” (zones of high fish productivity) are directly influenced by the dynamics that control the locations of maximum planktonic and benthic microalgae. The mechanisms underlying these interacting processes are reviewed in detail below.

Residence Time and Zones of High Phytoplankton Density

The generalized response of dissolved and particulate material to variation in freshwater inflow is illustrated in Figure 1.2. Residence time, the measure of the time spent by any given water mass in any given location (Sheldon and Alber 2006), dictates local depositional characteristics as well as the feeding communities present. When residence time is long, phytoplankton is more likely to deposit and is therefore more likely to support deposit feeding invertebrates, contingent on suitable oxygen availability. These zones of high deposition can be viewed as benthic hotspots of prey availability (Vetter 1994) that form in largely predictable geomorphic locations but are nevertheless subject to the temporal and spatial instability of their source in the water column above (Roman et al. 2005). During periods of low flow, maximum phytoplankton densities occur toward the upper reaches of the estuary. These upper ends are often filled with sediment and shallow topographic slopes. As water encounters these shallow slopes, residence time decreases and local depositional conditions occur. In contrast during periods of high flow, maximum phytoplankton densities occur toward the mouth estuaries. As water flows from the narrow portions of the estuary into expanding volumes downstream, residence time greatly increases and deposition ensues.

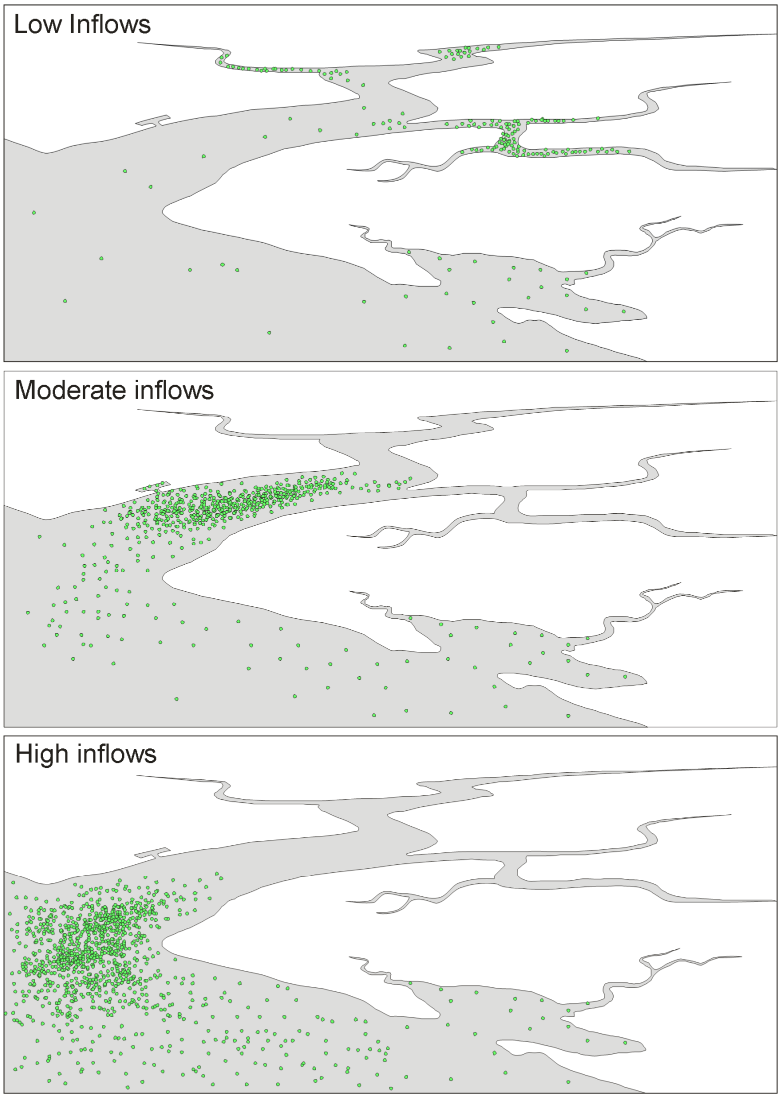


Figure 1.2: Effect of Freshwater Flow on Phytoplankton Densities. Freshwater Inflow along with stream geomorphology controls water residence time, a major control of phytoplankton density and deposition (Illustration by E. Peebles from Tampa Bay 2008)

Light Attenuation and Benthic Microalgae Survivability

Along with nitrogen, water also delivers colored dissolved organic matter (CDOM), the principal light attenuating material in Southwest Florida (Morel and Prieur 1977, Keith et al. 2002, Branco and Kremer 2005, 2006). CDOM commonly consists of tannic acid, humic acid, and fulvic acid. Tannic acids are leached by vascular plants, while humic and fulvic acids are produced in soils during the decomposition of plant material. In the oceanic environment and to a lesser extent in tidal habitats, CDOM also contains materials derived from microalgae.

CDOM has the potential to shade tidal nursery habitats on its journey from watersheds through estuaries and to the ocean. However, once CDOM reaches the ocean, it becomes diluted and may precipitate (or “salt out”); CDOM solubility diminishes due to the increasing ionic strength of the ambient water, and various CDOM molecules drop out of solution.

Benthic shading by CDOM reduces photosynthesis and the amount of oxygen evolving from benthic microalgae (BMA). Release of nitrogen sequestered by BMA is likely to occur as conditions become unfavorable for BMA production (Tobias et al. 2003). CDOM clearly plays a critical role in controlling BMA within Southwest Florida’s tidal tributaries and creeks. During wet periods, CDOM concentrations increase which causes BMA production to become inhibited. Light absorption by phytoplankton blooms and their associated algal-derived CDOM molecules (Keith et al. 2002) further limit BMA production.

Potential controls of light attenuation on the dominant autotrophs and the potential nursery communities are depicted in Figure 1.3.

During dry periods when CDOM leaching is low, phytoplankton blooms are commonly absent and there is virtually no light attenuation in the water column. Light reaches the benthos, and benthic microalgae survivability is high enough to support a benthic microalgal community (BMAC) that consists of benthic microalgae and the associated benthic invertebrates that support fish production (left of Figure 1.3).

In cases where water residence time is high and there is sufficient runoff to provide nutrients (middle of Figure 1.3), a phytoplankton bloom occurs which restricts benthic microalgae and associated BMAC to the shallows where light attenuation remains adequate for growth. In contrast, during periods of high CDOM leaching,

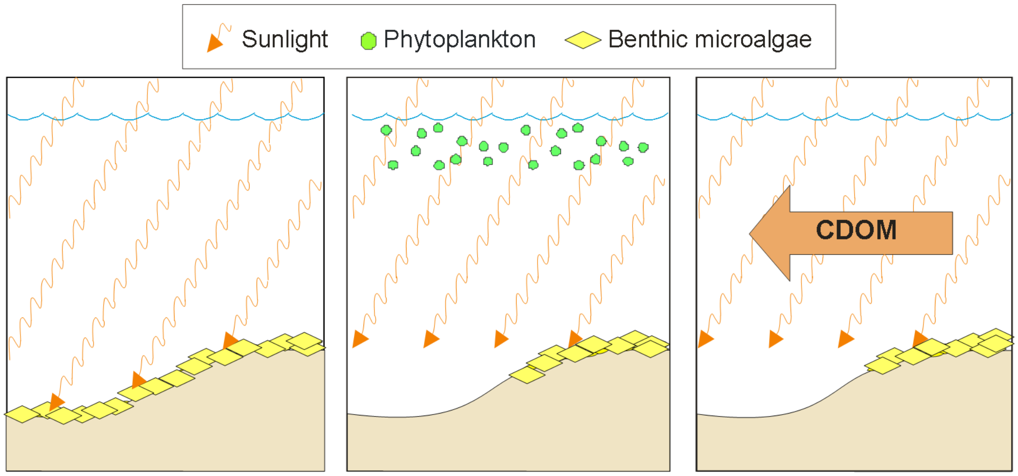


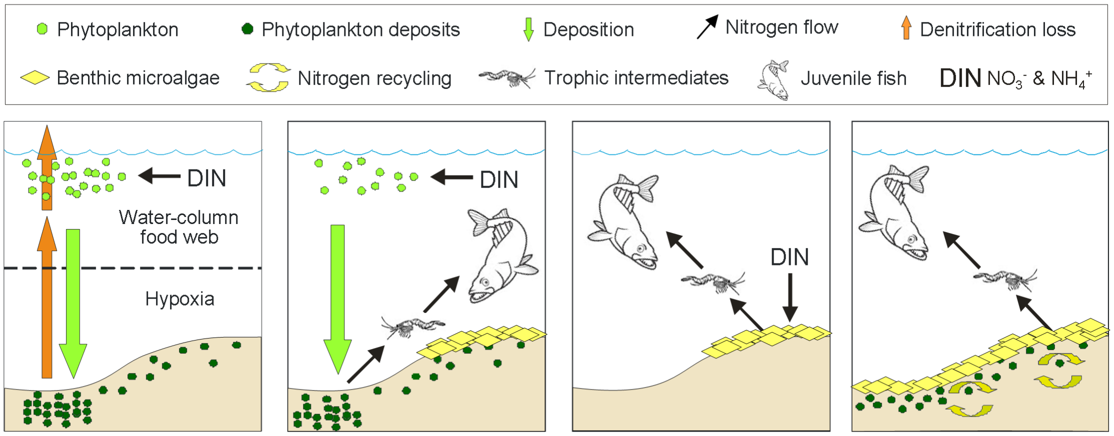
Figure 1.3: Light Controls on Dominant Microalgae. Left: Phytoplankton bloom is absent, BMAC occurs at greater depths, and hypoxia is unlikely. Middle: Phytoplankton bloom is present, BMAC is restricted to shallows, and hypoxia more likely at depth. Right: High CDOM, so BMAC restricted to shallows, and there is no phytoplankton bloom, and hypoxia is less likely (Illustration by E. Peebles from Tampa Bay 2008).

phytoplankton becomes light-limited, and BMA and associated BMAC is restricted to the shallows (right of Figure 1.3).

A Universe of Potential Nitrogen Pathways

By systematically considering the dynamics described above, a series of generalized nitrogen pathways can be predicted simply based on different scenarios of water flow, light attenuation, and geomorphology. It should be noted that BMA is assumed to occupy shallow regions under the conditions of all scenarios because light would not be a limiting factor unless these autotrophs are situated under a mangrove canopy or other shade producing structure. It is also assumed that microalgae support these systems rather than vascular plants as in Hollander and Peebles (2004). Four resulting potential nitrogen pathways (Figure 1.4) can be considered.

In cases where nitrogen loadings are extreme, residence time is long, and there is sufficient light in the water column, nitrogen is incorporated into phytoplankton and can



Scenario 1: **PBWC** Requires water-column nutrients; causes benthic hypoxia. Some nitrogen removed via denitrification

Scenario 2: **PBBC** Requires water-column nutrients, a depositional environment and benthic normoxia

Scenario 3: **BMAC** Scenario requires benthic normoxia and nitrogen in water column. Assumes CDOM shading at depth

Scenario 4: **BMAC** Scenario dependent on benthic normoxia and nitrogen from benthic recycling

Figure 1.4: Four Hypothesized Nitrogen Pathway Scenarios. Predicted nitrogen pathways under different scenarios of light, depth, and residence time (modified from Tampa Bay 2008).

support food webs in the water column, referred to here as a phytoplankton based water column community (PBWC). Note in this case, there is no BMAC community because the overwhelming nitrogen loads results in the oxygen-consuming carbon remineralization of phytoplankton deposits and benthic hypoxia. Released nitrogen would then undergo complex sedimentary biogeochemical recycling or removal via denitrification (depicted in Figure 1.4: Scenario 1).

If, on the other-hand, sediments remain normoxic, the copious amount of nitrogen incorporated into phytoplankton deposits can be transferred to benthic fauna and eventually to fish (depicted in Figure 1.4: Scenario 2), a beneficial pathway referred to here as a phytoplankton based benthic community (PBBC).

If nitrogen concentrations are relatively high, but water residence time is too short, phytoplankton will not develop to high enough densities in the water column and will not deposit. It would be more likely that dissolved inorganic nitrogen (DIN) from the water column would be directly incorporated into benthic microalgae, which in turn could support a BMAC in the shallows with minimal light attenuation (depicted in Figure 1.4:

Scenario 3). In this case, it is assumed that the flowing water also contains CDOM which shades BMA at depth.

In contrast, during very dry periods when water column nutrient concentrations and light attenuation are at a minimum, it is likely that nitrogen recycling in sediments would support a BMAC (depicted in Figure 1.4: Scenario 4). Chapter 5 examines the recycling that occurs in these sediments in greater detail.

In the scenarios depicted above, as nutrient loading increases to dangerous levels, scenario 1 will become more prevalent leading to the presence of more demersal species compared to benthic species. This is in accordance with Caddy's (1993) theory about the use of benthic to demersal ratios as an indicator of ecosystem health.

Considering the aforementioned physical, chemical, and biological principals, a universe of nitrogen pathways can be present in tidal nursery habitats for the wet season, the dry season, and inter-seasonal transitions. The flow regimes that characterize each season, coupled with the effects of stream morphology and nutrient chemistry, produces a specific type of benthic or water column community that supports fish production along the estuarine continuum.

During dry or low inflow periods (Figure 1.5), phytoplankton blooms would only accumulate upstream in the freshwater fed portions of the estuary, and consequently a PBBC or a PBWC occurs depending on oxygen conditions. Since unpolluted tidal creeks have no freshwater source, nitrogen that supports BMA and consequently BMAC in these habitats must either come from the tight nutrient recycling of senescent BMA or from the low concentration of nutrients in the water column.

In the middle to downstream portions of the estuary a BMAC dominates. Nutrients in the water column are removed upstream by the PBBC, and therefore will not be available to support phytoplankton at this portion of the estuary. Furthermore, low flow regimes do not provide adequate CDOM concentrations to shade benthic microalgal

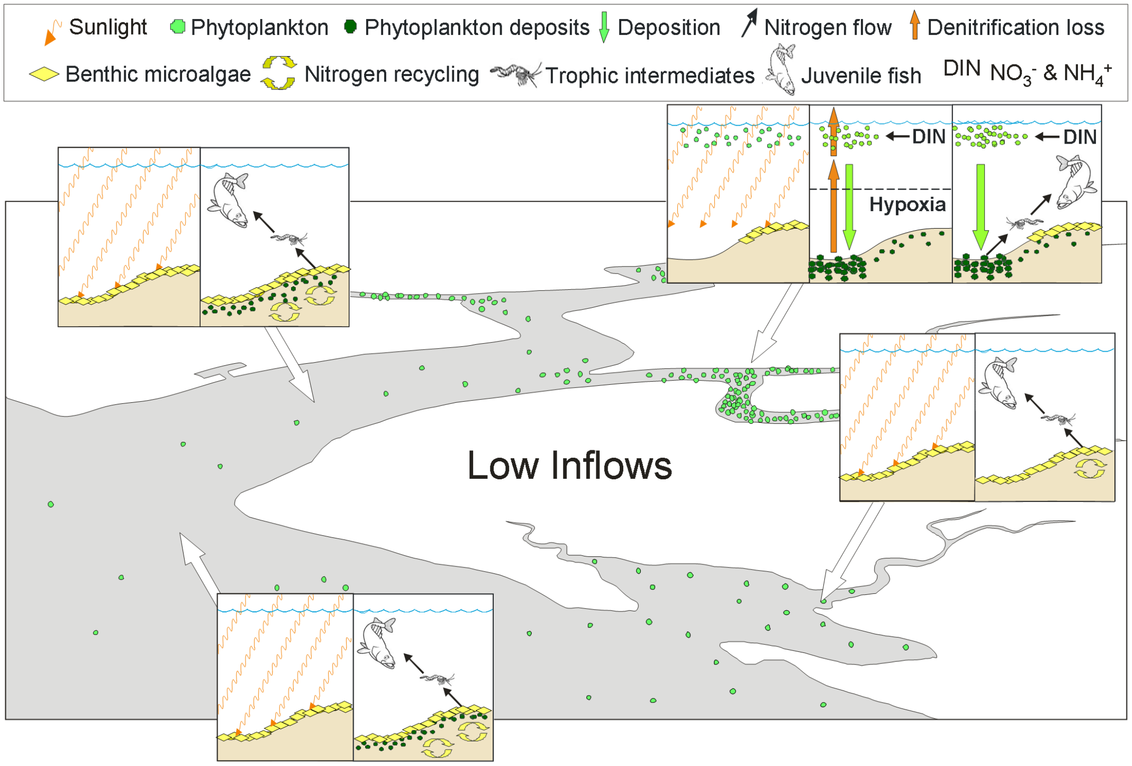


Figure 1.5: Hypothesized Nitrogen Pathways during Low Inflows (Adapted from Tampa Bay 2008).

production. As a result, BMA is the dominant autotroph, and the nitrogen driving the system is likely derived from the benthic recycling of senescent phytoplankton deposits (Figure 1.5).

During interseasonal moderate inflow periods (Figure 1.6), residence time is longest in the middle reaches of the estuary, and upstream nutrients support the numerous phytoplankton that deposit and sustain a PBBC depending on sufficient oxygen concentrations. In the regions upstream of the PBBC, CDOM shades the deep sediments, while in the shallows BMA receive nitrogen from the water column or the sediments and support a BMAC. Water-column nitrogen is present in the upstream region because it has not yet been taken up by middle reach PBBC. Towards the mouth of the estuary, CDOM salts out, light reaches the benthos; and in-situ nutrient recycling would cause BMA to dominate which in turn supports a BMAC. Similar to the low-flow

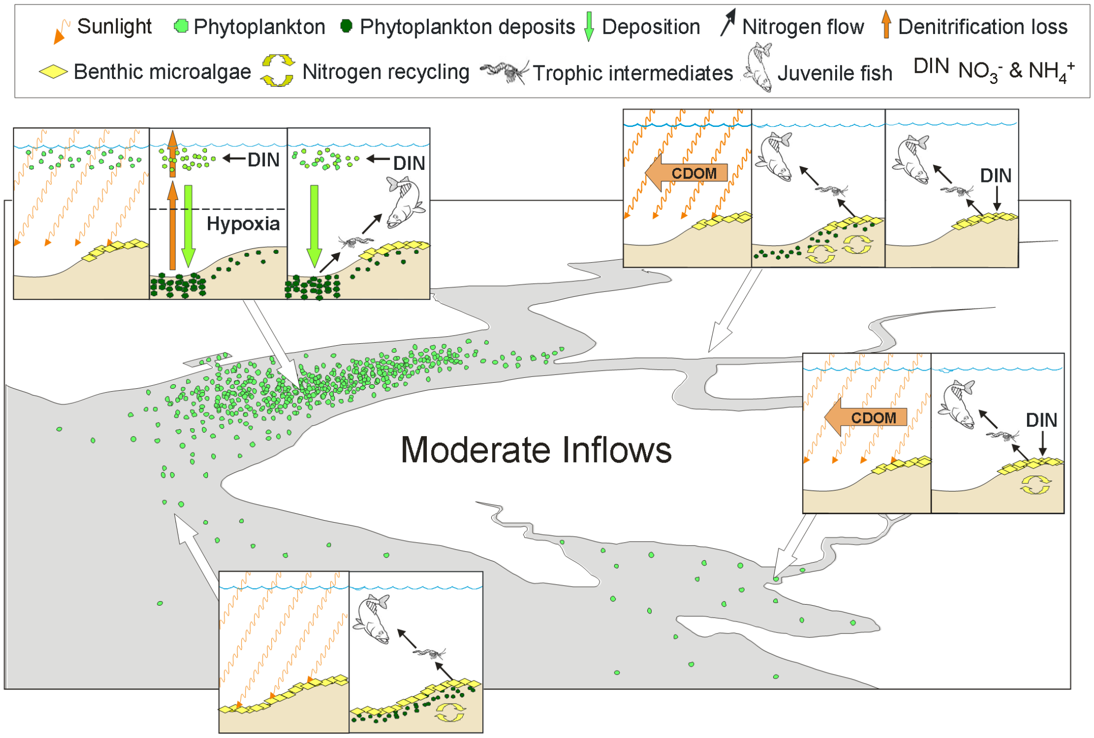


Figure 1.6: Hypothesized Nitrogen Pathways during Moderate Inflows (Adapted from Tampa Bay 2008).

scenario, nitrogen in these portions is likely derived from the benthic recycling of senescent phytoplankton deposits derived from the PBBC present during periods of high water flow and plentiful nitrogen delivery. In tidal creeks with no freshwater nutrient source, CDOM derived from mangroves and marsh grasses limit the BMAC to shallow regions only.

During periods of high inflow (Figure 1.7), phytoplankton concentrations are highest at the mouth of the estuary, and upstream nutrients support dense phytoplankton blooms that deposit and maintain a PBBC. However, if the bacterial breakdown of phytoplankton deposits consumes oxygen to the point of causing benthic hypoxia, no PBBC will occur. The middle and upper portions of the estuary have BMAC communities which are restricted to the shallows because CDOM concentrations are high and light levels are low. The nitrogen that supports benthic microalgae may be

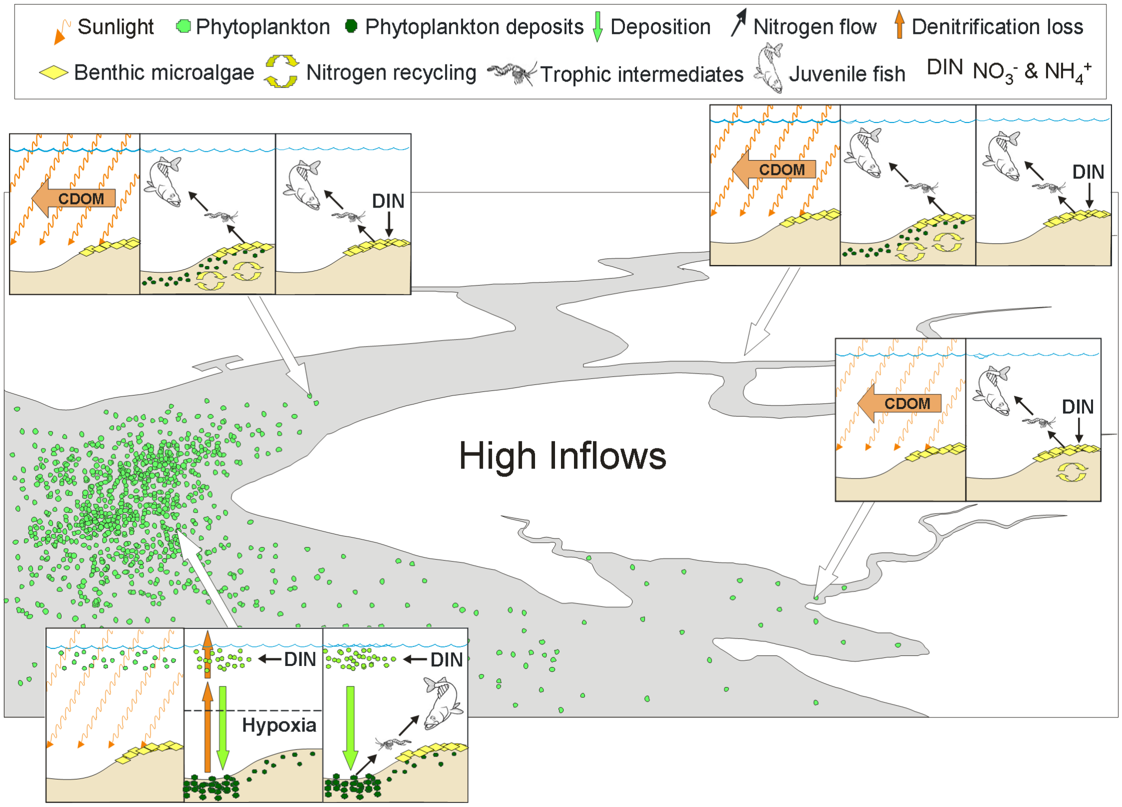


Figure 1.7: Hypothesized Nitrogen Pathways during High Inflows (Adapted from Tampa Bay 2008).

derived directly from the water column, since the phytoplankton maximum is downstream, but nitrogen may also be derived from the recycling of older algal deposits in the sediments. Conditions in the tidal creeks are similar to those depicted under the moderate inflow scenario.

General Objectives of Study

This study explores this universe of nitrogen pathways from the perspectives of both trophic ecology and biogeochemistry. The first principles and hypotheses described above will be built upon by addressing specific questions with the goal of synthesizing a complete picture of nitrogen transfer in juvenile fish nursery habitats. A framework for how this study builds upon these existing principles in shown in Figure 1.8.

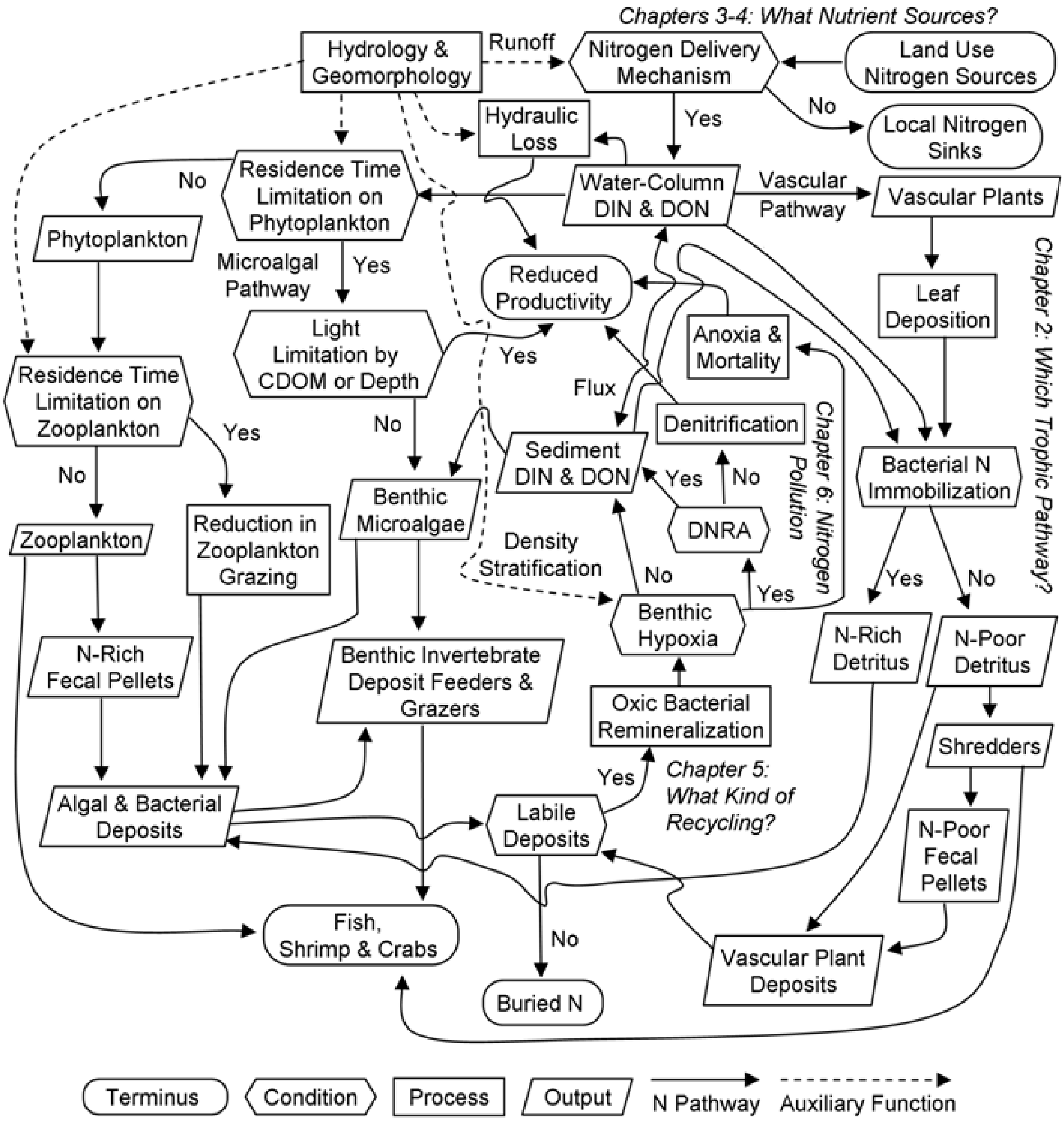


Figure 1.8: Exploring the Fundamental Pathways of a Larger Framework. This study aims to fine-tune and build upon the above framework in terms of 1) determining if trophic pathways are predominantly microalgal or vascular-plant based, 2) identifying land use based nutrient sources that drive the system, 3) examining types of dominant recycling, and 5) exploring nitrogen pollution scenarios.

The fundamental components of the framework depicted in Figure 1.8 were conceptualized from information provided by a larger project entitled the *Tampa Bay Tidal Tributary Habitat Initiative* (Tampa Bay 2008). This vast research project was designed to improve fisheries management in Tampa Bay by establishing the importance of tidal tributary habitats to fish production. A variety of individual research tasks made up this larger research project, and each task generated copious amounts of data. These datasets included 1) benthic and nekton community data, 2) fish gut-content data, 3) a suite of water quality data consisting of nutrient concentrations, colored dissolved organic matter (CDOM) concentrations, and 4) measurements of the absolute abundances of fish, phytoplankton and benthic microalgae. The manifold datasets and conclusions provided by the *Tampa Bay Tidal Tributary Habitat Initiative* (Tampa Bay 2008) were used in this dissertation to construct the hypothetical framework in Figure 1.8 and to aid in the interpretation of stable isotopic data, which oftentimes requires supplementary data to establish sound conclusions (Kendall 1998).

The hypothetical framework in Figure 1.8 provides a model that explains the relationships between the questions and conclusions contained in this dissertation. The goal of each individual chapter is to fine-tune and build upon the model presented in Figure 1.8.The alternative trophic pathway that stems from vascular plants is considered in Chapter 2, which will either confirm or reject the notion that microalgal-based food webs dominate in these systems. Chapters 3 through 4 explore the specific watershed sources of nitrogen that influence tidal nurseries surrounded by agricultural or urban landscapes. Chapter 5 investigates the nutrient recycling hypothesized to occur during periods of low-flow and low water column nitrogen concentrations. Lastly, Chapter 6 compares an impacted tidal tributary with a polluted tidal creek in terms of effectiveness in transporting nitrogen from land to fish. Chapter 7 provides a summary of the conclusions found in this dissertation as well as synthesis based on all theses conclusions. A summary of how the findings and methods used in this dissertation can be applied to ecosystem management is also considered in Chapter 7.

Application of Stable Isotopes to Ecosystem Studies

The major tools used in this study are naturally occurring stable isotopes. These versatile natural tracers will be applied to identify trophic structures, sediment biogeochemical processes, and potential nitrogen sources in these ecosystems. A review of how stable isotopes can be applied to investigate these phenomena is presented below.

Stable Isotope Terminology and Measurement

Stable isotope abundances are typically presented as δ values in per mil (‰) notation relative to a standard: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$ where X is ^{13}C, ^{15}N, or ^{34}S and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$. The δ values are a measure of the abundance of heavy and light isotopes in a sample relative to international references determined by the National Bureau of Standards (NBS). Increasing δ values indicate an increase in the heavy isotope component (enrichment), while decreasing δ values indicate an increase in the light isotope component (depletion). Standard reference materials are carbon in Pee Dee Belemnite limestone (PDB), nitrogen gas in the atmosphere (Air) and sulfur from the Canyon Diablo meteorite (CDT).

Using Stable isotopes to Identify Trophic Pathways

The capability to isotopically trace nutrient cycling and trophic dynamics within an ecosystem arises from two biochemical phenomena that regulate the flow and transfer of chemical species through biological systems (Peterson and Fry 1987). First, the carbon and nitrogen isotopic composition of primary producers reflect both assimilated nutrient sources and the carbon metabolic pathway used. Second, these tracers can identify the primary producers that support biomass at higher trophic levels because stable isotopes change predictably during biomass transfer from key primary producers upward through the food web to fish (Lajtha and Michener 1994; Peterson and Fry 1987). This systematic increase per trophic level is 3 to 4 times greater in nitrogen than carbon, and therefore nitrogen isotope compositions provide a more sensitive indicator of trophic position. The tendency of higher trophic levels to have higher $\delta^{15}N$ and $\delta^{13}C$ levels stems from the fact that metabolic waste products such as ammonia and urea tend to be depleted in ^{15}N and that byproducts of respiration tend to be depleted in ^{13}C. Since unmetabolized materials are passed up the food web, higher level consumers become enriched in ^{15}N and ^{13}C, and consequently have higher $\delta^{15}N$ and $\delta^{13}C$ values (Peterson and Fry 1997).

The principal mechanism that transfers nitrogen from primary producers to fish has traditionally been viewed as a food web based on vascular plant detritus. In this type of pathway, vascular plant detritus is shredded by invertebrates, colonized by bacteria, consumed by detritivores, and then passed to higher trophic levels (Odum and Heald 1975). Nitrogen enters this pathway either as the original nitrogen compounds in vascular plants or as nitrogen assimilated from the surrounding aquatic environment by bacterial colonies (Mann 1988). The application of stable isotope analysis to trophic studies has led to an improved understanding of trophic ecology that was once largely based on difficult-to-identify materials in diet analyses. While some studies using carbon, nitrogen, and sulfur isotopes have identified vascular plants as supporters of fish production (Wainright et al. 2000; Chanton and Lewis, 2002; Wissel and Fry 2005), numerous additional studies have attributed the bulk of fish production to microalgal production (Galvan et al. 2008; Choy et al. 2008). In Southwest Florida, which is characterized by small watersheds, distinct wet and dry seasons, varying land use, and a multi-billion dollar recreational fishing industry, microalgae also appear to ultimately support fish production (Hollander and Peebles 2004).

The nutrient pathways that support microalgal food webs are relatively straightforward. Microalgal production depends on nutrients that originate from either the watershed or from in-situ recycling. Once nutrients are assimilated, these primary producers transfer their distinctive isotopic composition to organisms that occupy the higher levels of the foodweb. Since different land-use types produce distinct isotopic signatures (Kendal 1998), a number of studies have succeeded in attributing the isotopic composition of various consumers to differential patterns of land-use (Anderson and Cabana 2005; Wigand et al. 2007).

By isolating organic matter derived from primary producers, detritus, bacteria colonies, trophic intermediates (zooplankton, hyperbenthos), and fish, detrital vascular plant trophic pathways and algal trophic pathways can be distinguished. This is possible because the primary producers that support these pathways utilize distinct carbon, nitrogen, and sulfur sources, which are reflected in the stable isotopic composition of these organisms. The $\delta^{13}C$ values of vascular plants and algae are offset from one another due to the different sources of carbon used in photosynthesis. While both groups make use of the C3 photosynthetic pathway, and therefore imprint an identical enzymatic fractionation on their respective carbon sources, the former utilize atmospheric CO_2 and the latter use water-column dissolved inorganic carbon (DIC). Water-column DIC is characteristically enriched in the heavy isotope relative to atmospheric CO_2. This signature is carried forward through photosynthesis and can therefore be used a diagnostic tracer for identifying sources of primary production.

The $\delta^{15}N$ values of vascular plants are generally lower than the $\delta^{15}N$ of aquatic autotrophs. Emergent plants such as marsh grasses and upland plants such as trees and shrubs often show a greater dependence on rainwater N or bacterially fixed N_2 (Kendall 1998). Water-column microalgae often utilize nitrate derived from watershed runoff that has land-use-related $\delta^{15}N$ values. It should be noted that if vascular plants deliver nitrogen-depauperate (high C:N) detritus, then bacterial colonies will take up N from the water column (Mann 1988), as do phytoplankton. In this case, $\delta^{15}N$ should not be used as the exclusive tracer.

The $\delta^{34}S$ differences between vascular plants and microaglae are due to differences in the $\delta^{34}S$ of sulfate in marine and freshwater ecosystems. Microalgae in estuarine waters often derive sulfur from marine sulfate, which has a heavy isotopic composition due to oceanic bacterial sulfate reduction, whereas vascular plants utilize sulfates that originate from rainwater with lighter $\delta^{34}S$ values (Peterson et al. 1985). If no marine end-member is present, $\delta^{34}S$ values may not distinguish between these two pools unless one group of primary producers utilizes a portion of sulfur that undergoes unique biogeochemical processing.

Stable isotopes can also be used to determine if fish biomass is derived from benthic or pelagic pathways. Water-column microalgae and benthic microalgae often have distinct isotopic C, N, and S compositions. Benthic microalgae, due to their small size and their position at the sediment-water interface, exist in a boundary-layer microenvironment where diffusive, rather than turbulent, forces dominate the supply of CO_2. This leads to a condition of local carbon depletion, forcing these organisms to discriminate less in their uptake of carbon isotopes (Trudeau and Rasmussen 2003). Other possible mechanisms behind the increased $\delta^{13}C$ values of benthic microaglae may include light limitation, growth rates and nutrient availability (Hill et al. 2008). These mechanisms typically reduce the proportion of light isotopes available to benthic microalgae, and since there is a strong preference for the uptake of light isotopes when nutrients are available, an isotopic distinction between benthic and pelagic microalgae is often apparent. In shallow systems, this may not be the case.

Sedimentary biogeochemical processes can also create distinctions between the isotopic composition of benthic and pelagic primary producers (Peterson et al. 1985). In general, these processes produce light products and heavy reactants. Since benthic primary producers are more likely to use compounds that undergo sedimentary processing, an isotopic distinction between these primary producers and those in the water column often becomes apparent. In terms of nitrogen, denitrification produces light, biologically unavailable N_2 while the residual heavy bioavailable NO_3^- that remains behind can be utilized by benthic microalgae, leading to an enrichment of their nitrogen isotopic composition. In terms of sulfur, benthic microalgae may also utilize the sulfate that remains or hydrogen sulfide that is produced by bacterial sulfate reduction, creating an additional distinction.

Since isotopic distinctions can be made between vascular plants and microalgae as well between benthic and pelagic autotrophs, the specific pathways that support fish production can be identified by interpreting the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of fish, invertebrates, detritus, and variety of pelagic and benthic primary producers. Since these distinct isotopic signatures are predictably passed up the food web, any member of the ecosystem can provide a clue about the dominant pathway present.

Using Stable Isotopes to Identify Nitrogen Sources

A variety of potential nitrogen sources are available to aquatic ecosystems and these are generally associated with specific types of watershed land use. Because nitrogen is processed uniquely on different types of land-use, often times each type of land-use exports nitrogen with a specific isotopic composition. Dissolved inorganic nitrogen (DIN) derived from each land use type supports primary production downstream and also passes along its distinctive isotopic character in the form of its stable isotopic composition of nitrogen (Kendall 1998). As a result, these tracers are appropriate for determining the specific land-use types that deliver nutrients to aquatic ecosystems because the isotopic composition of the actual organisms and the dissolved and particulate species have isotopic compositions that are derived from the surrounding watershed.

This phenomenon is responsible for the isotopic patterns observed in Figure 1.1 where Feather Sound nitrogen that was originally derived from nitrogen fixers became derived from pastureland and eventually from residential fertilizer. In pre-developed Florida, vast upland forests, brush land and open rangeland covered the face of most watersheds, and in these types of ecosystems, nitrogen oxides and ammonia deposited from the atmosphere can be considered the major nitrogen source.

Atmospheric reactions control the range of $\delta^{15}N$ values of nitrogen bearing molecules. Natural sources of ammonia and nitrogen oxides to the atmosphere include the volatilization of ammonia from fertilizer applied to soil, and incomplete denitrification products. Anthropogenic sources include chemical processing and combustion of fossil fuels in automobiles and power plants. Because atmospheric nitrogen sources are commonly the products of gas producing reactions, the wide $\delta^{15}N$ range of values of bioavailable nitrogen in the atmosphere are centered on low values (Kendall 1998).

In general, deposited NO_x has higher $\delta^{15}N$ values compared to NH_3. This is due to the equilibrium exchange reaction between gaseous NO_x and dissolved NO_3^-, which results in the enrichment of ^{15}N in the dissolved NO_3^- deposited during rainfall (Garten, 1992). Atmospheric nitrogen deposited to the natural vegetation on undeveloped land would be taken up by soils and plants, and nitrogen exported from these habitats would likely exhibit isotopic compositions comparable to the original isotopic composition of atmospherically deposited nitrogen.

Watersheds in the rural agricultural regions of Florida are often covered with cow pastures. Here, the dominant form of exported nitrogen is derived from cow manure and should be characterized by heavy $\delta^{15}N$ values (+10 to +20‰) due to the large amounts of $^{14}NH_3$ and $^{14}N_2$ released during ammonia volatilization and denitrification that occur after fresh manure is deposited. Identical $\delta^{15}N$-enriching processes may occur in agricultural land use types where Haber-Bosch fertilizer is applied, despite the fact that fresh fertilizer is derived from the atmospheric N_2 and has a nitrogen isotopic composition of 0‰.

Urbanized watersheds have various potential sources of nitrogen. For example, in Tampa Bay it has been reported that two-thirds of the nitrogen originates from non-point sources, one-fifth originates from atmospheric deposition, and only one-tenth originates from well defined point sources (Pribble et al. 2001). Potential nitrogen sources in this region as well as other urban landscapes may include septic systems (Valiela et al. 1990), lawn fertilizer use, leaky sewage pipes as well as nitrogen oxides derived from fossil fuels delivered via atmospheric deposition (Anisfeld et al. 2007, Gu et al. 2009). The isotopic composition of nitrogen derived from septic systems or sewage is similar to that of nitrogen derived from agriculture that results in high $\delta^{15}N$ values (+10 to +20‰). Nitrogen derived from fresh lawn fertilizer application has a light isotopic composition if the fertilizer was made using the Haber-Bosch process, a reaction that converts atmospheric dinitrogen into bioavailable forms of nitrogen. As described above, nitrogen derived from atmospheric deposition would have low $\delta^{15}N$ values.

Using Stable Isotopes to Identify Nitrogen Processes

A simplified summary of nitrogen cycle processes are displayed in Figure 1.9. Each of these processes and associated isotopic transformations are briefly reviewed below.

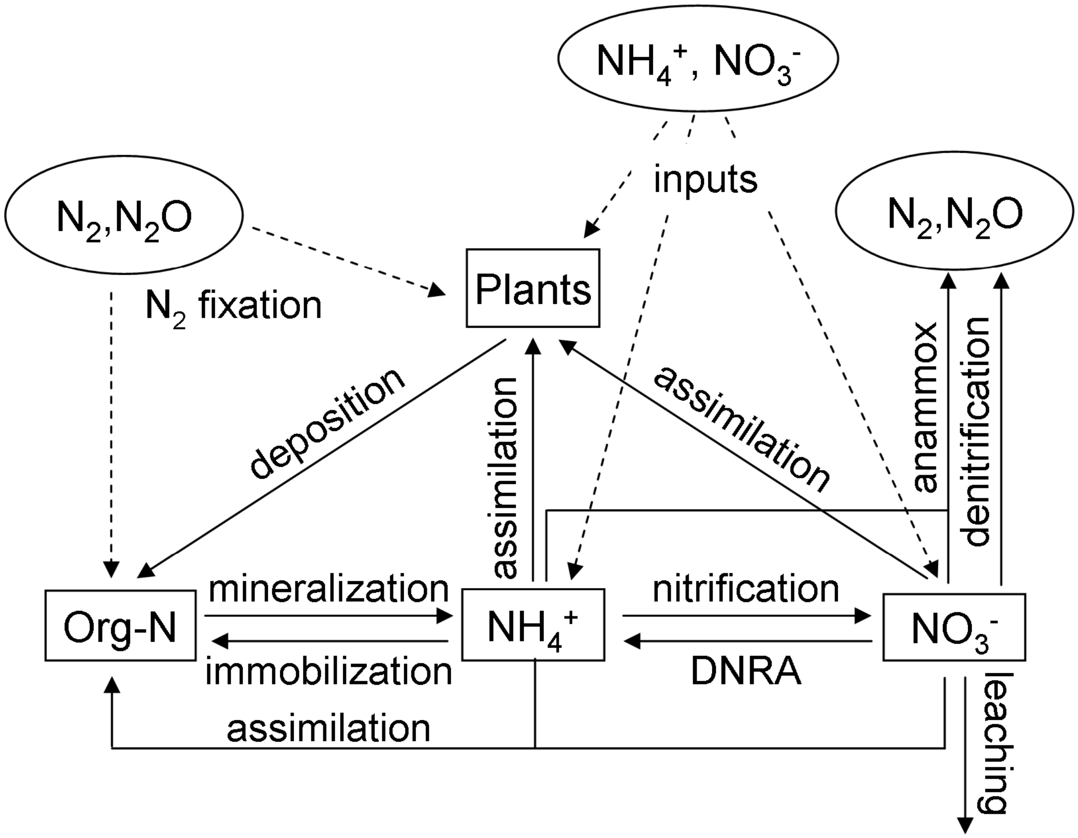


Figure 1.9: The Nitrogen Cycle Simplified. Solid arrows represent pathways, and squares represent different nitrogen pools. Dotted arrows represent source pathways while circles represent different nitrogen sources.

N-fixation refers to the process that converts atmospheric molecular nitrogen into bioavailable forms of nitrogen. Major N_2 fixers include blue-green algae and vascular plants such as legumes and alders that contain bacteria in nitrogen fixing root nodules (Kendall 1998). Micronutrients limit nitrogen fixation because the enzyme nitrogenase consists of an iron protein and a molybdenum-iron protein. Non-biological nitrogen fixation includes nitrogen fixed by lightening where N_2 reacts with oxygen in the atmosphere to produce NO_X, and Haber-Bosch fertilizer production where N_2 is converted into ammonium or urea. Because the isotope composition of nitrogen in the atmosphere is 0‰, any organism that fixes nitrogen or takes up recycled fixed nitrogen is generally characterized by an extremely light nitrogen isotope composition.

Assimilation refers to the uptake of bioavailable nitrogen bearing compounds such as ammonium and nitrate and the incorporation into living organic nitrogen biomass. Nitrate and other oxidized forms of nitrate are initially reduced before uptake and incorporation into organic matter. Enzymatic kinetic isotope fraction of nitrogen occurs during assimilation. In cases where autotrophs utilize an active transport mechanism, light isotopes of nitrogen come into contact with protein uptake sites on the cell membrane more often than their heavier counterparts. Similarly, in cases where autotrophs rely on simple diffusion for nitrogen uptake, the light isotopes of nitrogen diffuse more quickly across the cell membrane compared to their heavier counterparts. Furthermore, lighter molecules lower the activation energy of many of the enzymes that incorporate nitrogen into biomass. This lower activation stems from the fact that lighter isotopes occupy lower molecular vibrational energy levels and therefore form weaker bonds, which are easier for enzymes to break (Sharp 2007).

A wide range of isotopic fractionation has been reported for assimilation; however, in nitrogen limited environments, little or no fractionation is expected, since autotrophs cannot afford to exclusively uptake the lighter isotope. Immobilization is similar to assimilation, but specifically refers to bacterial uptake of ammonium for the breakdown and assimilation of nitrogen poor detritus. In this case, similar principles of fractionation should apply (Kendall 1998).

Mineralization refers to the release of ammonium from organic matter through bacterial respiration. Very small fractionation factors have been reported for nitrogen mineralization (less than 1‰), though there is a general consensus that light isotopes are preferentially mineralized (Kendall 1998).

Nitrification is the chemoautotrophic process that converts ammonium into nitrate in the presence of oxygen. This multistep oxidation process includes the oxidation of ammonium into nitrate by *Nitrosonomas* and the oxidation of nitrite into nitrate by

Nitrobacter. Since the slow oxidation of ammonium is generally a rate limiting step, the overall nitrification fractionation of this reaction is generally large (-12‰ to -29‰ from Heaton, 1986). The presence of light nitrate in aquatic ecosystems may be a fingerprint of nitrification.

Volatilization refers to the loss of gaseous ammonia from dissolved ammonium. The ammonia gas has a light isotopic composition compared to the residual ammonium pool, and this process involves both kinetic and isotopic fractionation. First an equilibrium fractionation is established between the ammonium and ammonia in solution. The second equilibrium fractionation is established between the ammonia in solution and the gaseous ammonia. A kinetic fraction also occurs from the diffusive loss of light ammonia molecules to the atmosphere (Heaton, 1986). These processes cause extreme fractionation between the original dissolved ammonium pool and the gaseous ammonia released to the atmosphere. Each of these steps can fractionate up to 20‰. This is the primary reason why nitrogen leached from fertilizer can have a very heavy isotopic composition, despite the fact that the original isotopic composition of the fertilizer may have been 0‰, indicative of a Haber-Bosch nitrogen source.

Denitrification is the dominant mechanism that removes nitrogen from aquatic ecosystems. In respiratory denitrification, heterotrophic bacteria use nitrate, which acts as the terminal electron acceptor for the oxidation of organic matter under anaerobic conditions. The nitrate is converted to unreactive N_2 or N_2O and is considered removed from the system. Common denitrifying bacteria include *Escherichia*, *Bacillus*, *Pseudomonas*, and *Micrococcus*. The facultative anaerobe *Thiobacillus denitrificans*, oxidizes sulfur and reduces iron but can switch to nitrate oxidation at low oxygen levels. The kinetic fractionation associated with denitrification is also extreme (up to 40‰). It is therefore oftentimes assumed that high $\delta^{15}N$ values of nitrate or primary producers are indicative of a system undergoing denitrification (Kendall 1998).

Recently, it has been suggested that transformative processes such as dissimilatory nitrate reduction to ammonium (DNRA) have been underestimated, and removal processes such as denitrification have been overestimated (Burgin and Hamilton 2007, Gardner and McCarthy 2009). Instead of removing nitrogen from the system as in denitrification, DNRA transforms nitrate into ammonium, another bioavailable form of nitrogen.

In fermentative DNRA, bacteria couple the oxidation of organic matter to nitrate reduction via fermentation reactions. Microbes that perform fermentative DNRA include species of *Clostridia*, *Desulfovibrio*, *Vibrio*, and *Pseudomonas* (Tiedje 1988; Megonigal et al. 2004). This process is thought to dominate in sediments with copious amounts of labile organic matter and low available nitrates; denitrification is though to dominate under carbon-limited conditions.

Chemoautotrophic DNRA couples the reduction of nitrate to the oxidation of reduced sulfur, specifically free sulfide and elemental sulfur (Otte et al. 1999). Nitrate reduced to ammonium is coupled to the oxidation of reduced sulfur to sulfate. This form of DNRA requires free sulfide. Since sulfide is known to inhibit the last reduction steps of denitrification, it is assumed that sediments rich in H_2S would benefit chemoautotrophic DNRA instead of denitrification.

Although the magnitudes of kinetic fractionation for these processes have not yet been defined, it can be assumed that both fermentative and chemoautotrophic DNRA would likely produce ^{14}N—enriched ammonium in the sediments because the products of enzymatic kinetic fractionations are nearly always lighter than the residual reactants.

Anaerobic ammonium oxidation (Anammox) is the chemoautotrophic process where ammonium is combined with nitrate to produce elemental nitrogen under aerobic conditions. Because Anammox requires suitable concentrations of both nitrate and ammonium and because it is inhibited by simple organic compounds (Jetten et al. 1998), it is not likely that this form of chemoautotrophy takes place in Southwest Florida's highly productive, nitrogen limited tidal nursery habitats, and therefore it is not considered a dominant process in this study.

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CHAPTER 2:

TROPHIC PATHWAYS IN TIDAL FISH NURSERY HABITATS

Introduction

Numerous estuarine-dependent fish and crustaceans utilize Southwest Florida's low-salinity tidal rivers, tributaries, and creeks. Most notable are the juveniles of estuarine-dependent species. These tidal aquatic habitats are known as tidal nurseries because they not only provide structural refuge from predation, but also provide the primary production that ultimately supports growth and survival.

While in nursery habitats, juvenile snook, red drum, hogchoker, striped mullet and blue crab directly obtain biomass by consuming small resident fishes (e.g., rainwater killifish, mosquito fish, and sheepshead minnow), benthic invertebrates (e.g., small crustaceans, worms and insect larvae) and in some cases, by directly consuming microalgae or plant detritus (Killam et al. 1992, Peters and McMichael 1987, Poulakis et al. 2002).

The occurrence of these species in tidal habitats is part of a habitat selection process that is mandated by the history requirements of these species, including dietary shifts that are necessary to permit continued growth (Marks and Conover 1993, Barry et al. 1996, Peebles 1996). Large proportions of Florida's juvenile snook, red drum (redfish), mullet, tarpon and blue crab populations are entirely dependent on tidal-creek and tidal-river habitats (Peters and McMichael 1987, McMichael et al. 1989, Killam et al. 1992, Poulakis et al. 2002). A diversity of other species, including important forage species such as menhaden and anchovies, are also dependent on tidal habitats during their juvenile stages (Friedland et al. 1996).

Potential Trophic Pathways: Microalgae versus Vascular Plants

The primary producers that support juvenile fish in tidal nursery habitats can vary. The principal mechanism that transfers nitrogen from primary producers to fish has traditionally been viewed as a food web based on vascular plant detritus. In this type of pathway, vascular plant detritus is shredded by invertebrates, colonized by bacteria, consumed by detritivores, and then passed to higher trophic levels (Odum and Heald 1975). Nitrogen enters this pathway either as the original nitrogen compounds in vascular plants or as nitrogen assimilated from the surrounding aquatic environment by bacterial colonies (Mann 1988). The vascular-plant-based pathway is generally considered to be less efficient at transferring biomass to higher trophic levels than microalgal-based pathways (Mann 1988, Moran and Legovic 1988). This inefficiency is attributed to large losses of organic matter to microbial respiration. Both pathways, however, have been clearly identified, often within the same estuary (e.g., Hughes and Sherr 1983).

The application of stable isotope analysis to trophic studies has led to an improved understanding of trophic ecology that was once largely based on difficult-to-identify material in diet analyses. While some studies using carbon, nitrogen, and sulfur isotopes have identified vascular plants as supporters of fish production (Wainright et al. 2000; Chanton and Lewis, 2002; Wissel and Fry 2005), numerous additional studies have attributed the bulk of fish production to microalgal production (Galvan et al. 2008; Choy et al. 2008).

In Southwest Florida, Hollander and Peebles (2004) investigated a series of tidal rivers that span a range from eutrophic to oligotrophic. Stable-isotope analyses successfully delineated watershed-specific and seasonal differences in the nutrient sources that support an array of primary producers and identified the primary producers that support fish production. One major finding of this study was the connection between anthropogenic land-use and the nitrogen isotopic composition of fish; heavy isotopic signatures of the urbanized Peace River managed to propagate throughout the entire trophic system, while the isotopic signatures of the mesotrophic Myakka trophic system showed lighter isotopic signatures. Key primary producers changed seasonally because of changes in hydrological connectivity and associated shadings due to high concentrations of Colored Dissolved Organic Matter (CDOM). Benthic microalgae were primarily responsible for production at higher trophic levels in all rivers during the dry season when CDOM, nutrients, and hydrological connectivity were low, whereas planktonic algae supported biomass during the wet season when CDOM, nutrients and hydrological connectivity were high.

Objectives

The current study will build on the findings of Hollander and Peebles (2004). A multiple-stable-isotope approach will be utilized to investigate ecosystem trophic structure. This will allow identification of the primary producers that support young fish and crustacean production within tidal nursery habitats in small sub-estuaries on the west coast of Florida. The investigative design takes advantage of the natural and anthropogenic heterogeneity that exists among the tidal estuaries in Southwest Florida, and attempts to isolate freshwater inflow effects by comparing a variety of sub-estuaries under both wet and dry conditions.

The objective of this research is to identify the primary producers that are ultimately responsible for biomass production within the tidal nursery habitats used by economically and ecologically important fish and crustaceans. Specifically, this study will identify if a vascular plant trophic pathway or a microalgae trophic pathway dominates in these systems, as found by Hollander and Peebles (2004).

Methods

Study Sites

Isotope samples that are analyzed in this study originate from two investigations. The first, “tidal tributaries study” (Tampa Bay 2008) targeted 3 tidal stream sub-estuaries. The second, Greenwood et al. (2009) targeted many smaller tidal stream sub-estuaries. Isotopic data from all sub-estuaries are in this project. Water body names, dominant land use type, and study of origin are summarized in Table 2.1. Figure 2.1 summarizes the watersheds studied along with those from Hollander and Peebles (2004). The term “sub-estuary” is used as a general term that refers to the different types of tidal-water bodies studied (tidal rivers, tidal creeks, and tidal tributaries)

Sample Collection

In the tidal tributaries project, fish samples were collected at 4-5 sites per sub-estuary with individual sub-estuary reaches ranging approximately from 1 to 4 km. Each sampling site was visited twice for sample collection during both seasons. Fish were collected monthly from March to June for dry season sample collection and from August through November for wet season sample collection. Greenwood et al. (2009) collected fish from approximately 3-4 sites along sub-estuary reaches ranging from 1 km to 3 km. Each sampling site was only visited once for sample collection during each season. Dry season sampling occurred in May 2007, while wet season sampling occurred in September 2007. Samples of fish tissues were obtained using shoreline seines. Where possible, fish samples were limited to tissues (primarily muscle) located posterior to the

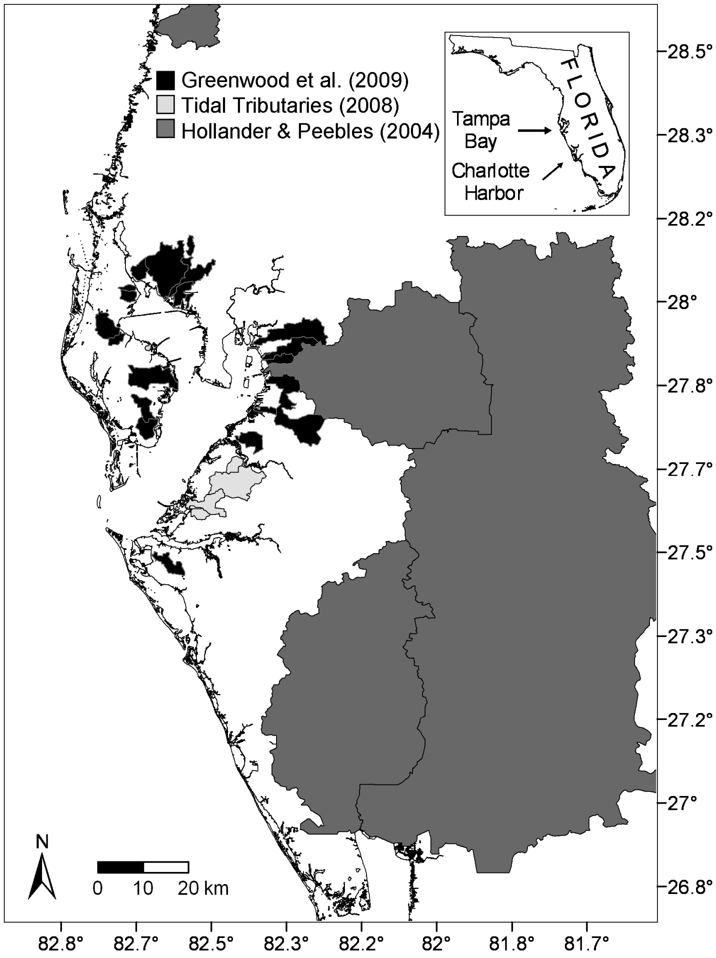

Figure 2.1: Map of Study Sites. Shaded areas indicate sample origin that includes the reports of Hollander and Peebles (2004), Tampa Bay (2008), and Greenwood et al. (2009).

peritoneal cavity. Smaller fish species were analyzed whole. All solid samples were dried at 50°C, powdered, and frozen until isotopic analysis.

Primary producer samples were collected within a given sub-estuary's tidally influenced reach during the same time periods as fish collections. In the tidal tributaries project, primary producer samples were collected from 3-4 sites, while in Greenwood et al. (2009), primary producer samples were collected from 1-2 sites. Leaves of dominant upstream trees and shrubs (*Taxodium distichum*, *Salix caroliniana*, *Ulmus americana*, *Baccharis halimifolia*, *Quercus laurifolia*, *Carya glabra*, *Myrica cerifera*, *Acer rubrum*, *Quercus virginiana*, *Sabal palmetto*, *Schinus terebinthifolius*, *Juniperus virginiana*,

Table 2.1:.Study Sites. Water body names, drainage basin names, dominant land use type, and study of origin are presented. *Mixed* indicates rural watershed land use consisting of low density residential, agricultural, and natural land cover, whereas *Residential* indicates watershed land use is predominantly urbanized with little or no agriculture and natural land cover.

Water Body	Drainage Basin	Land Use Group	Study
Alafia River	Alafia River	Mixed	Hollander and Peebles 2004
Myakka River	Myakka River	Mixed	Hollander and Peebles 2004
Peace Rive	Peace River	Mixed	Hollander and Peebles 2004
Weeki Wachee River	Weeki Wachee River	Mixed	Hollander and Peebles 2004
Frog Creek	Frog Creek	Mixed	Tidal Tributaries 2008
Curiosity Creek	Curiosity Creek	Mixed	Tidal Tributaries 2008
Grassy Creek	Feather Sound	Residential	Tidal Tributaries 2008
Allen Creek	Allen Creek	Residential	Greenwood et al. 2009
Booker Creek	Booker Creek	Residential	Greenwood et al. 2009
Bishop Creek	Bishop Creek	Residential	Greenwood et al. 2009
Channel A	Channel A	Residential	Greenwood et al. 2009
Dick Creek	Channel G	Residential	Greenwood et al. 2009
Delany Creek	Delany Creek	Residential	Greenwood et al. 2009
Long Branch Creek	Long Branch Creek	Residential	Greenwood et al. 2009
Moccasin Creek	Moccasin Creek	Residential	Greenwood et al. 2009
Mullet Creek	Mullet Creek	Residential	Greenwood et al. 2009
Peppermound Creek	Peppermound Creek	Residential	Greenwood et al. 2009
Palma Sola Bay	West Cedar Hammock	Residential	Greenwood et al. 2009
Rocky Creek	Channel G	Residential	Greenwood et al. 2009
Salt Creek	Salt Creek	Residential	Greenwood et al. 2009
Sweetwater Creek	Sweetwater Creek	Residential	Greenwood et al. 2009
Tinny Creek	Grand Bayou	Residential	Greenwood et al. 2009
Wood Creek	Wood Creek	Residential	Greenwood et al. 2009
Newman Branch	Newman Branch	Mixed	Greenwood et al. 2009
South Archie Creek	South Archie Creek	Mixed	Greenwood et al. 2009
Bullfrog Creek	Bullfrog Creek	Mixed	Greenwood et al. 2009
Double Branch Creek	Double Branch Creek	Mixed	Greenwood et al. 2009
Marsh Branch	Marsh Branch	Mixed	Greenwood et al. 2009
North Archie Creek	North Archie Creek	Mixed	Greenwood et al. 2009

Serenoa repens), emergent plants (*Cladium jamaicense*, *Typha spp.*, *Acrostichum danaeifolium*, *Eichhornia crassipes*, *Scirpus spp.*, *Juncus spp.*), and mangroves (*Rhizophora mangle*, *Avicennia germinans*) were collected from the banks of sub-estuary reaches.

Multiple methods were used to collect benthic microalgae (BMA). In the tidal tributaries project, BMA were grown in-situ on glass plates. The in-situ BMA plates were left near the lower limit of the intertidal zone for 1-2 weeks. After collection, plates were washed under a strong stream of tap water and examined microscopically to assure removal of non-plant material (sediment, polychaete and amphipod tubes, and extracellular mucilage). Areas of concentrated BMA growth were harvested by scraping with a glass microscope slide. Microscopy confirmed that these samples were dominated by benthic diatoms, but also included filamentous algae and occasionally small amounts of nonfilamentous green algae and cyanobacteria. Benthic filamentous algae samples were also collected from mats growing on the sediment surface. Greenwood et al. (2009) used both of the above methods to collect BMA, and additional BMA was obtained by scraping discarded bottles which had been collected from creek bottoms. All samples were investigated under the microscope to confirm the presence of primary producers and were rejected if a high number of barnacles were present.

In all studies, phytoplankton was sampled as particulate organic matter (POM). Samples were size fractionated into ranges of 0.8-2.7 and 2.7-30 μm. These fractions were obtained via a combination of Nitex mesh sieves and pressure filtration through pre-combusted glass fiber filters. Microscopic analyses indicated that POM samples were dominated by diatoms and amorphous detritus. All solid samples were dried at 50°C, powdered, and frozen until isotopic analysis.

Sample $\delta^{15}N$ and $\delta^{13}C$ values were measured using a Carlo Erba 2500 Series I elemental analyzer equipped with a zero-nitrogen blank auto-analyzer that was coupled to a Finnigan Mat Delta Plus XL stable-isotope mass spectrometer. All samples were run in duplicate using Spinach Leaves (NIST 1570a) as an internal reference material, and carbon and nitrogen isotope values are reported in conventional per mil notation (‰)

relative to the Pee Dee Belemnite limestone (PDB) and nitrogen gas in international atmospheric standards (Air).

Data Analysis

Stable isotopes were used to determine trophic pathways as these tools have the capability to trace nutrient cycling and the trophic dynamics within an ecosystem. This capability arises from two biochemical phenomena that regulate the flow and transfer of isotopic chemical species through biological systems (Peterson and Fry 1987). First, the carbon and nitrogen isotopic composition of primary producers reflect both assimilated nutrient sources and the carbon metabolic pathway used. Second, these tracers can identify the primary producers that support biomass at higher trophic because stable isotopes predictably change during biomass transfer from key primary producers upward through the food-chain to fish (Lajtha and Michener 1994, Peterson and Fry 1987). This systematic increase per trophic level is 3 to 4 times greater in nitrogen than carbon, and therefore nitrogen isotopic compositions provide a more sensitive indicator of trophic position.

Stable isotopes were used to determine the key primary producers that support juvenile fish production by examining conventional $\delta^{15}N$ vs. $\delta^{13}C$ plots for the isotope ratios of fish and autotrophic materials. Plots of $\delta^{15}N$ vs. $\delta^{13}C$ were constructed for each system in the tidal tributaries study for both wet (January to June) and dry seasons (July to December) for a total of 6 plots. Because of limited primary producer samples in the Greenwood et al. (2009) study, data were grouped based on season and land-use categories; seasonal $\delta^{15}N$ vs. $\delta^{13}C$ plots for each land use group were produced for a total of 4 plots. In all cases, POM samples with a carbon to nitrogen ratio greater than 10 (indicative of vascular plant material) were excluded from analysis.

Results and Discussion

Land Use-Specific Trophic Structure

Trophic plots for the data collected from the Greenwood et al. (2009) study are presented in Figures 2.2-2.5 below.

BMA and POM had overlapping isotopic ranges in all cases, so these two autotrophs were grouped as microalgae. During both wet and dry seasons, fish from both land use groups were predominantly supported by microalgae as either BMA or POM (Figures 2.2-2.5); the microalgal pathway is assumed to dominate in these systems.

Specifically, microalgae had wide ranges of carbon similar to the fish, and fish were typically offset from microalgae by about 1.5-2 trophic steps (offsets of ~6 ‰ $\delta^{15}N$), which agrees with the consumption of benthic invertebrates. In contrast,

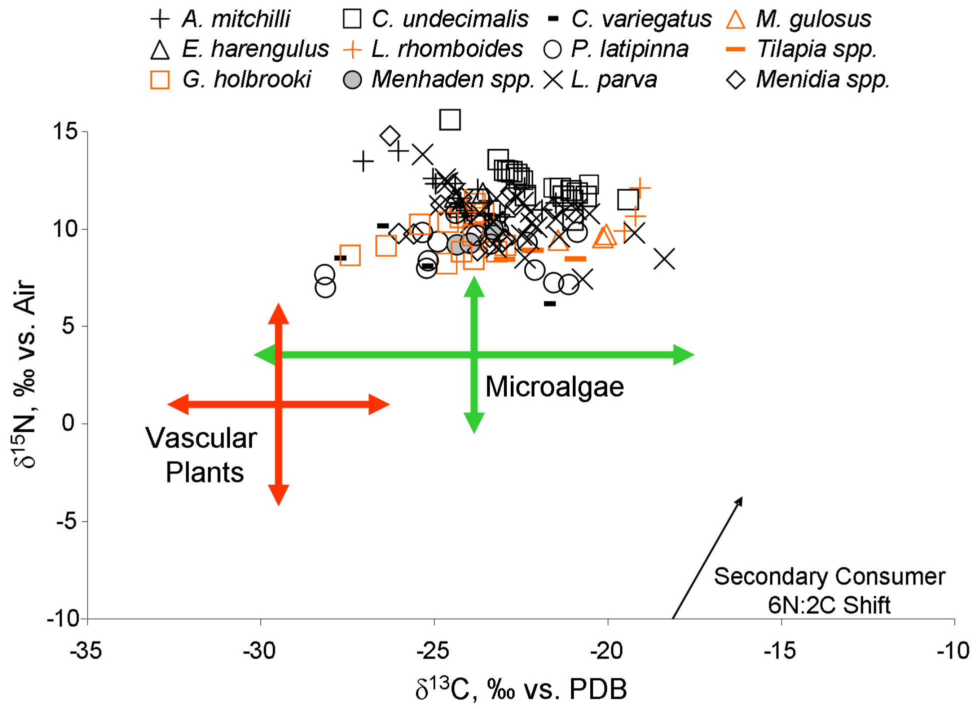

Figure 2.2: Trophic Plot of Nursery Fish in Residential Watersheds during the Dry Season. Ranges for microalgae and vascular plants depicted by arrows, and observations for each species are plotted as symbols.

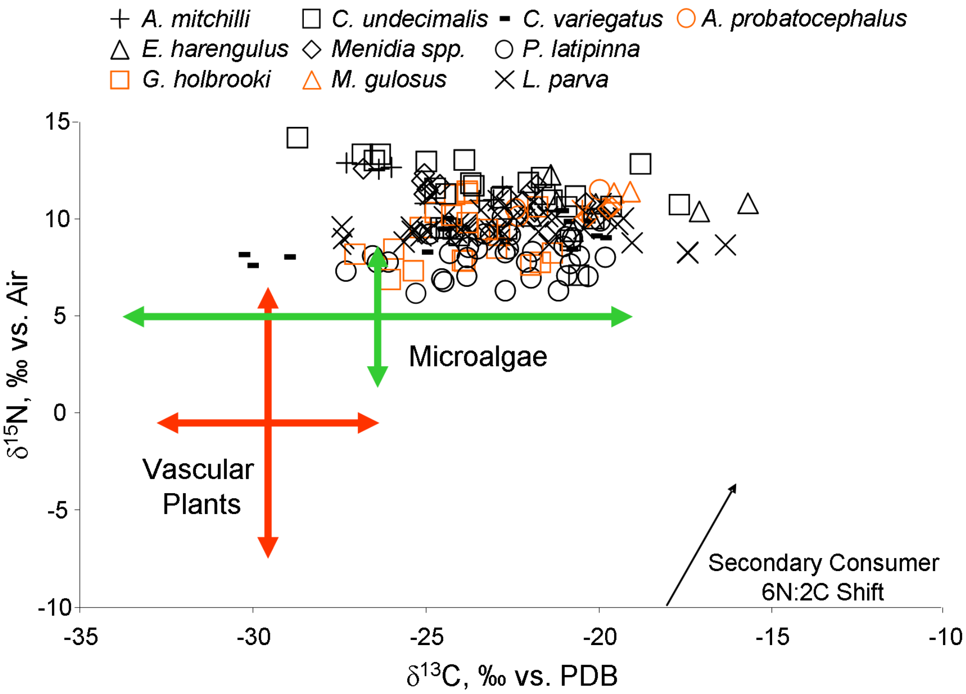

Figure 2.3: Trophic Plot of Nursery Fish in Residential Watersheds during the Wet Season. Ranges for microalgae and vascular plants depicted by arrows, and observations for each species are plotted as symbols.

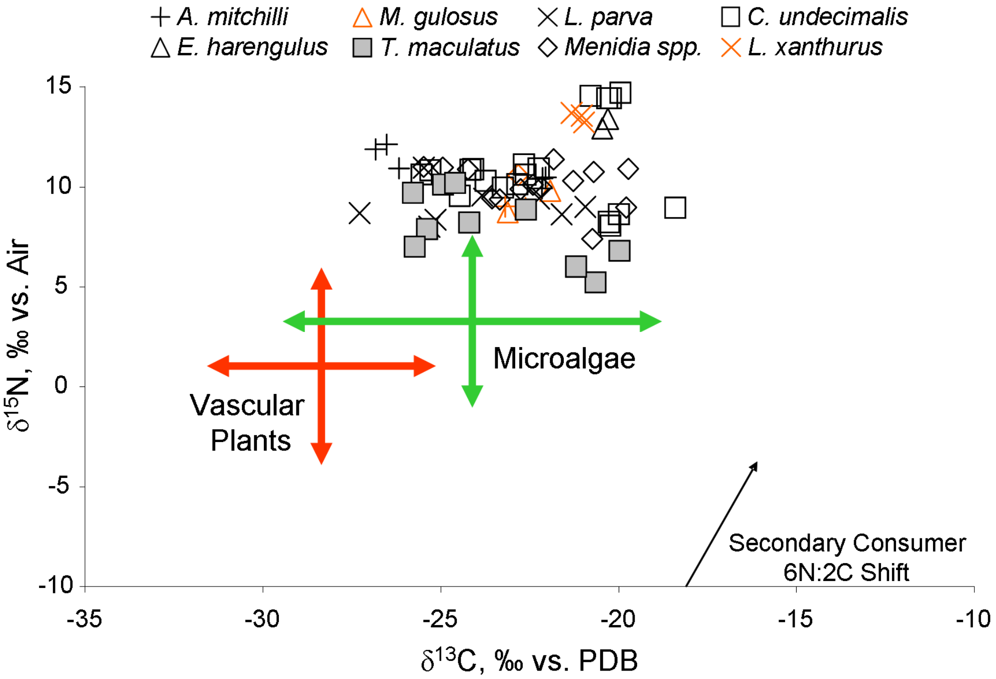

Figure 2.4: Trophic Plot of Nursery Fish in Rural Watersheds during the Dry Season. Ranges for microalgae and vascular plants depicted by arrows, and observations for each species are plotted as symbols.

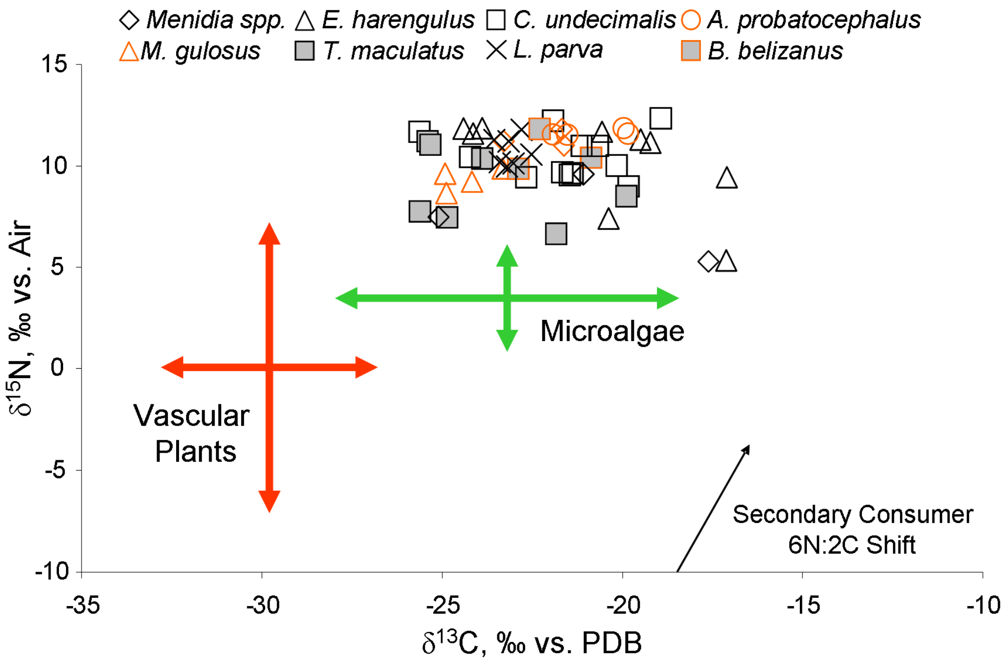

Figure 2.5: Trophic Plot of Nursery Fish in Rural Watersheds during the Wet Season. Ranges for microalgae and vascular plants depicted by arrows, and observations for each species are plotted as symbols.

upland plants emergent vegetation, and mangroves (grouped here as vascular plants) did not appear to be important in supporting the biomass of the fish community as a whole. These vascular plants had narrow ranges of carbon relative to the fish, and had wide ranges of δ^{15}N that did not match the trophic offsets expected for secondary consumers. In order for the vascular plants to serve as the trophic base, trophic offsets of 3-4 steps (offsets of ~9-12‰ δ^{15}N) would be required. It should be noted that although the POM reported here can possibly include particles of vascular plant detritus, samples with C:N >10 (indicative of vascular plant material) were excluded. Macroalgae and seagrass were not abundant autotrophs in these systems, and their stable isotope compositions are not shown. Seagrass was enriched in δ^{13}C (mean δ^{13}C of 10‰). Macroalgae had isotopic ranges which overlapped with microalgae. The vascular plant trophic pathway does not appear to occur in these systems. Even if bacteria were immobilizing nitrogen from the surrounding environment and incorporating it into bacteria-detritus complexes, it is unlikely the $\delta^{13}C$ value of those bacterial-detritus complexes would be significantly different from vascular plant material, since bacteria would most likely obtain their carbon from plant tissues. For the most part, fish had enriched $\delta^{13}C$ values compared to vascular plants and a much wider range. Thus, based on these results, bacterial-detritus pathway only minimally contributes to the biomass of fish. These results do not preclude the potential importance of emergent vegetation, mangroves, and riparian plants as structural habitat in these ecosystems (chapter 6).

Although microalgae seems to be the major nutrient source in this study, it should be noted that in systems with very low nitrogen inputs and in shaded areas, it is likely that vascular plant detritus indirectly supports fish biomass. Ammonium released from the bacterial breakdown of copious amounts of vascular plant detritus has the potential to support microalgae, which in turn can support benthic invertebrates and ultimately fish. Systems dominated by vascular plant detritus would also be occupied by shredding organisms which 1) fish can feed on directly and 2) have the capability to digest vascular plant material and deliver reusable nitrogen to the sediments via fecal pellets which can again be recycled to support benthic microalgae or phytoplankton.

Seasonality had a small but noticeable effect on the isotope data. During the dry season, fish, POM, and BMA had more scattered isotope values in accordance with variation in watershed-specific land use. These patterns are investigated in later chapters in great detail.

Watershed-Specific Trophic Structure

Trophic plots for the tidal tributaries study are presented in Figures 2.6-2.11 below. Ranges of carbon and nitrogen isotope values of different primary producers are depicted by arrows, whereas individual observations of different fish species are depicted by symbols.

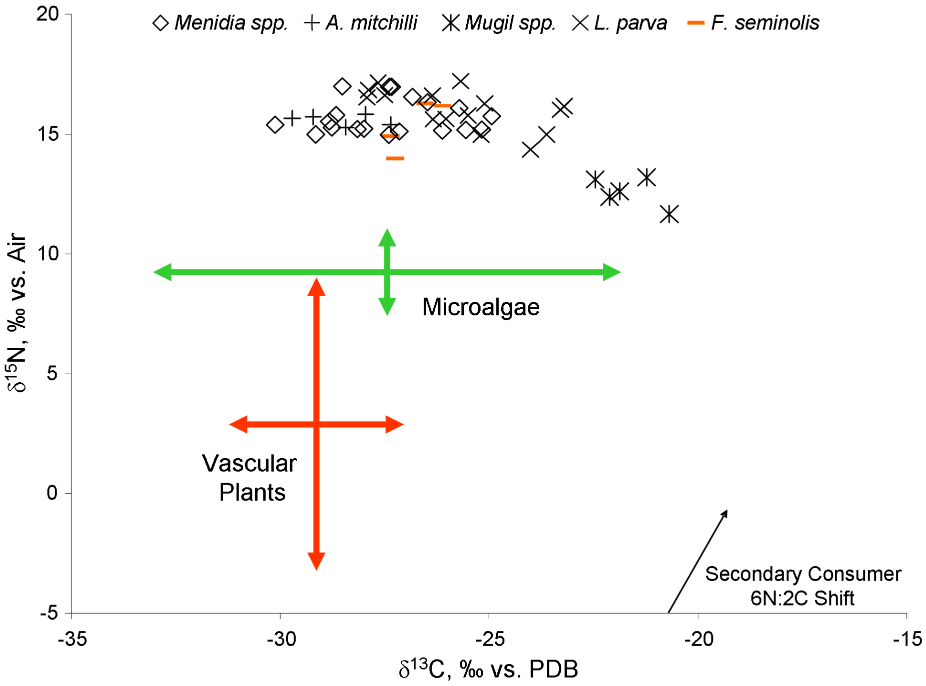

Figure 2.6: Trophic Plot of Nursery Fish in Curiosity Creek during the Dry Season. Ranges for microalgae and vascular plants depicted by arrows, and observations for each species are plotted as symbols.

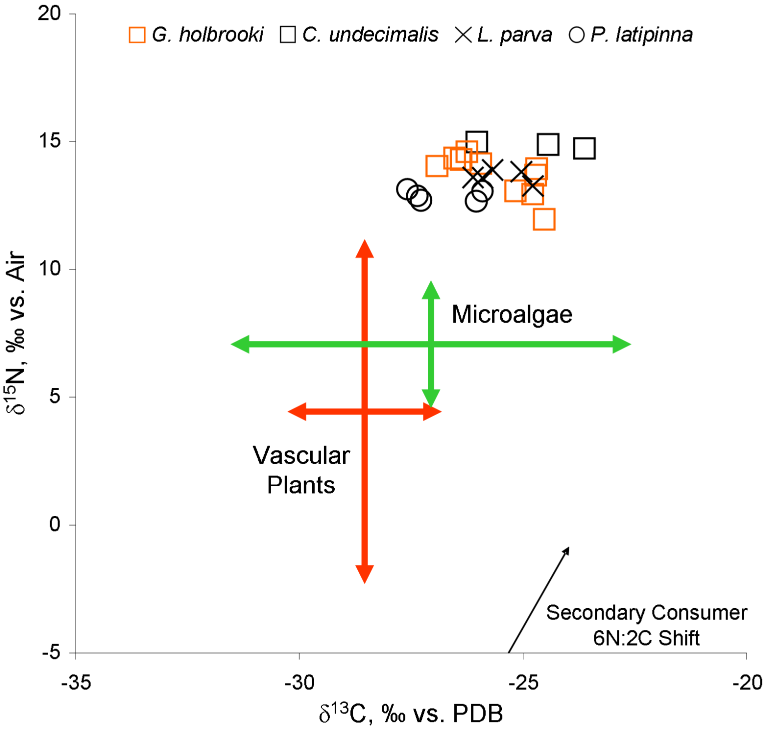

Figure 2.7: Trophic Plot of Nursery Fish in Curiosity Creek during the Wet Season. Ranges for microalgae and vascular plants depicted by arrows, and observations for each species are plotted as symbols.

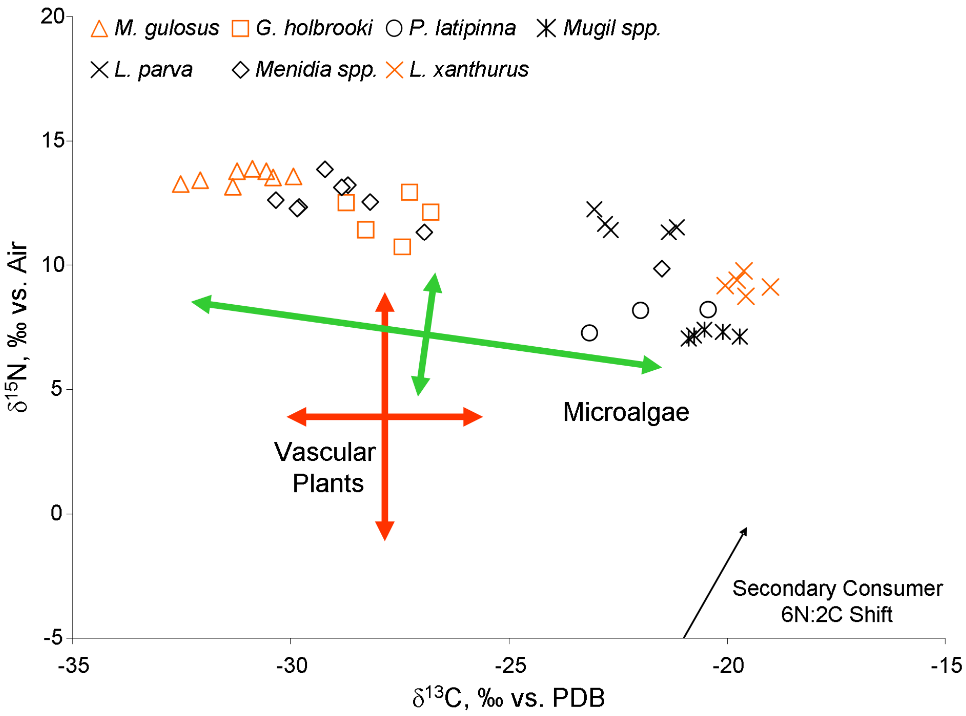

Figure 2.8: Trophic Plot of Nursery Fish in Frog Creek during the Dry Season. Ranges for microalgae and vascular plants depicted by arrows, and observations for each species are plotted as symbols.

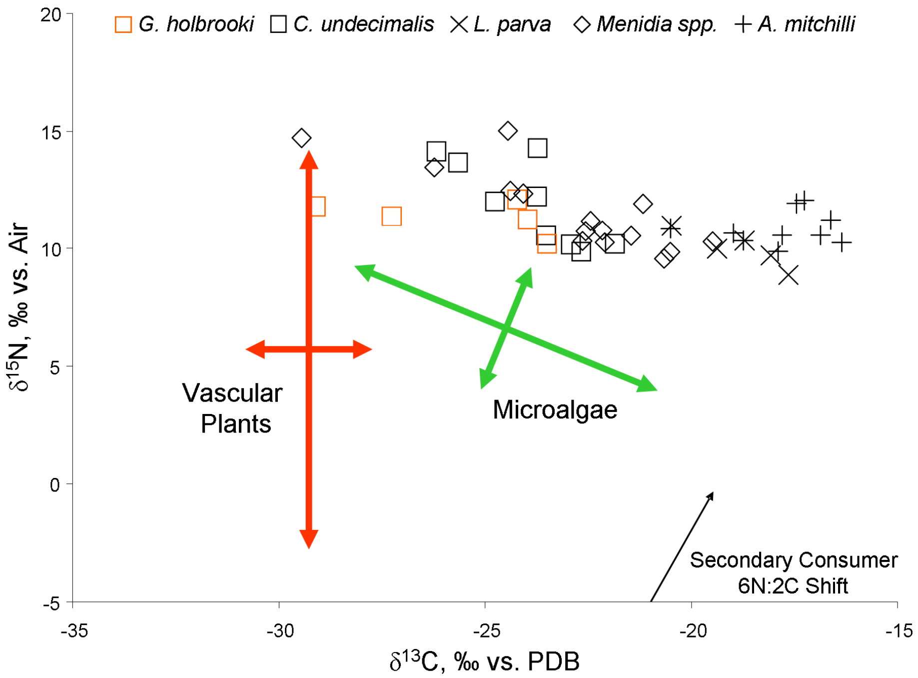

Figure 2.9: Trophic Plot of Nursery Fish in Frog Creek during the Wet Season. Ranges for microalgae and vascular plants depicted by arrows, and observations for each species are plotted as symbols.

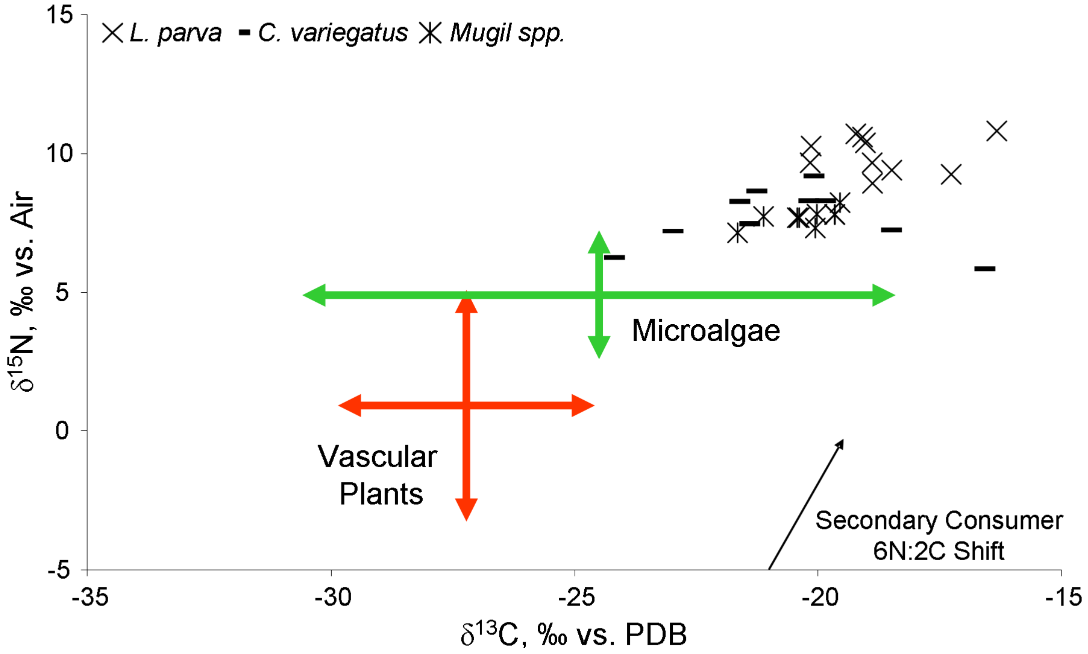

Figure 2.10: Trophic Plot of Nursery Fish in Grassy Creek during the Dry Season. Ranges for microalgae and vascular plants depicted by arrows, and observations for each species are plotted as symbols.

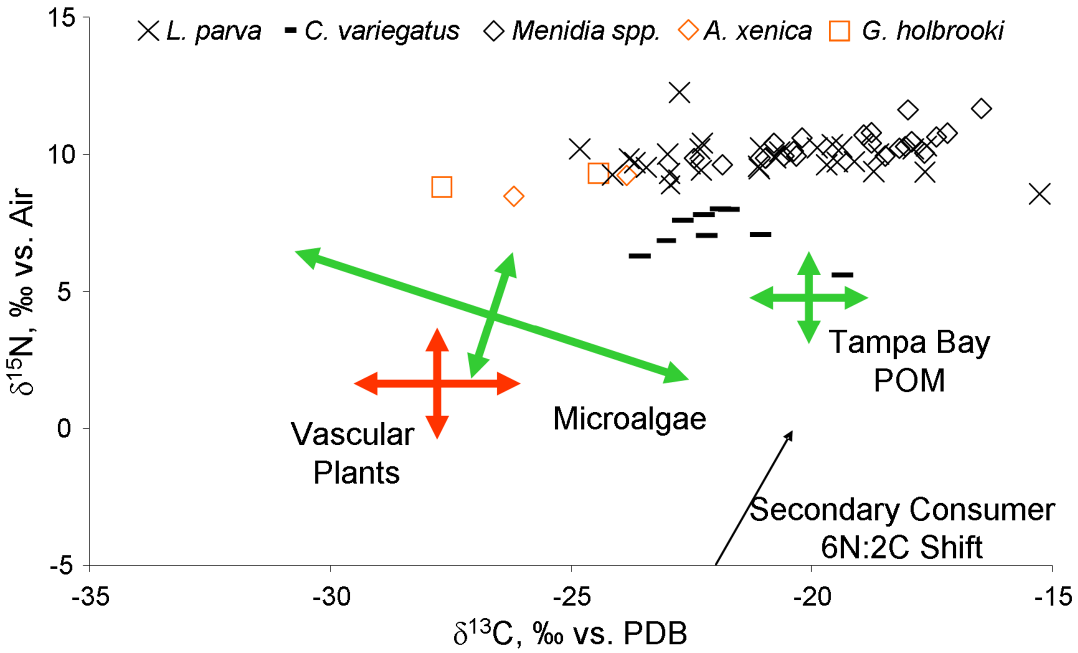

Figure 2.11: Trophic Plot of Nursery Fish in Grassy Creek during the Wet Season. Ranges for microalgae and vascular plants depicted by arrows, and observations for each species are plotted as symbols.

BMA and POM again had overlapping isotopic ranges, so these two autotrophs were grouped as microalgae. During both wet and dry seasons, each system's fish were predominantly supported by microalgae as either BMA or POM (Figs. 2.6-2.11); again, the microalgal pathway seems to dominate.

Vascular plants did not appear to be important in supporting the biomass of the fish community as a whole. The only exception may be Curiosity Creek during the wet season (Figure 2.7). This is not unexpected, since Curiosity Creek was the system with the lowest salinity and most likely to have a vascular plant trophic pathway, where biomass from recalcitrant plant material can be transferred to fish via shredder organisms. In general however, vascular plants had narrow ranges of carbon relative to the fish, and had wide ranges of $\delta^{15}N$ that did not match the trophic offsets expected for secondary consumers. Again, fish were typically offset from BMA and POM by about 1.5-2 trophic steps (offsets of ~4.5-6 ‰ $\delta^{15}N$), which agrees with the consumption of benthic invertebrates as in Greenwood et al. (2009).

Another interesting observation was that in Grassy Creek significant fish biomass appeared to be supported from outside the creek during the dry season. Phytoplankton values of Tampa Bay (indicated in Figure 2.11) appeared to support the majority of fish biomass. This phenomenon is investigated further in Chapter 6 with an in-depth comparison of Frog Creek and Grassy Creek.

POM and BMA are probably transferred to upper trophic level fish biomass through the benthic biological community, which consists of deposit feeders as well as a variety of other benthic invertebrates and some fish. Benthic invertebrates either feed directly on the benthic microalgae or on microalgal deposits, whereas fish derive biomass either directly from microalgal deposits as in the case of *Mugil spp.* or by consuming benthic invertebrates (gut study from Tampa Bay (2008)).

Although this study indicated that microalgal pathways dominate in these systems, the two isotopes of carbon and nitrogen did not provide enough resolution to determine whether the biomass of fish was derived from BMA or POM. Hypothetically, during the wet season, there is likely to be high amounts of CDOM in the water column which would likely shade benthic microalgal production. In fact, in Tampa Bay (2008), the ratio of benthic algae to phytoplankton dropped by at least an order of magnitude during the wet season.

Synthesis of Season-Specific Trophic Pathways in Tidal Fish Nursery Habitats

Based on the results of this study and the BMA and chlorophyll abundance data from Tampa Bay (2004), the following trophic pathway is assumed to occur during the wet season (Figure 2.12).

Daily afternoon showers provide hydrologic land-water connectivity that delivers DIN and CDOM to tidal fish nursery habitats. CDOM shades benthic microalgae, so water column phytoplankton become the dominant autotrophs (Tampa Bay 2008). In zones of high residence time, phytoplankton deposit into the sediments. Phytoplankton deposits are then consumed by benthic invertebrates which, in turn, are fed upon by fish. Further evidence of this pathway is presented in the next chapter where specific watershed land use types are related to the isotopic composition of fish.

During the dry-season, nitrogen loading is greatly reduced due to reduced rainfall. CDOM is not delivered to these systems in copious amounts, so it is assumed that a benthic microalgal pathway occurs (Figure 2.13). Reduced CDOM concentrations allow adequate light levels to support benthic microalgal production. These benthic autotrophs likely assimilate nitrogen remineralized in the sediments, since water column nutrient concentrations are generally low during this period. Live benthic microalgae as well as senescent BMA support benthic invertebrates which are, in turn, consumed by fish. It should be noted that in canopied habitats, BMA would likely be shaded, and

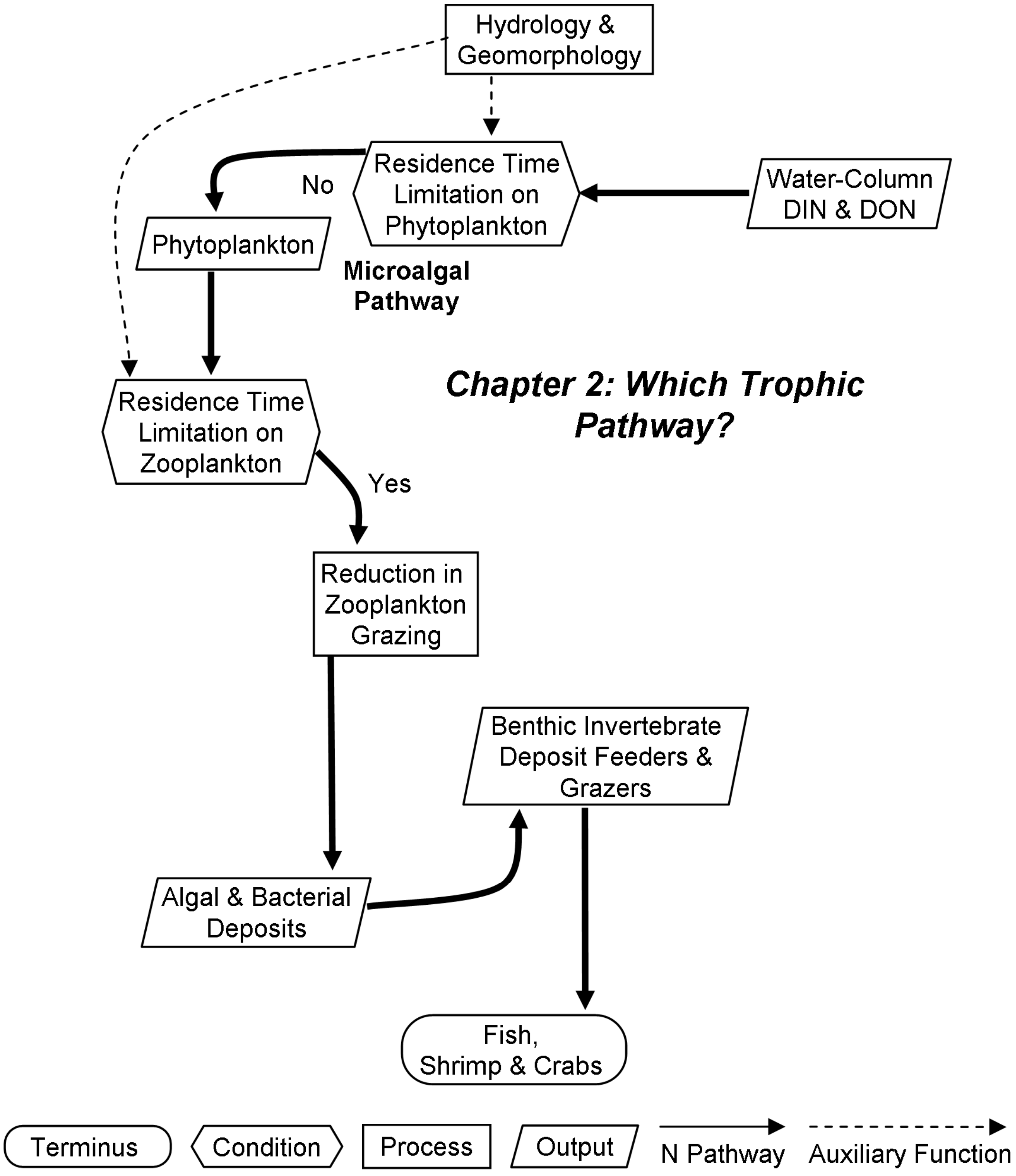

Figure 2.12: Wet Season Microalgae Trophic Pathway. Arrows indicate that phytoplankton deposits support a phytoplankton based benthic community during the wet season.

remineralized nitrogen may support some water-column production. The labile sources of nitrogen available that supports the dry season primary producers ultimately fish will be investigated in chapter 5.

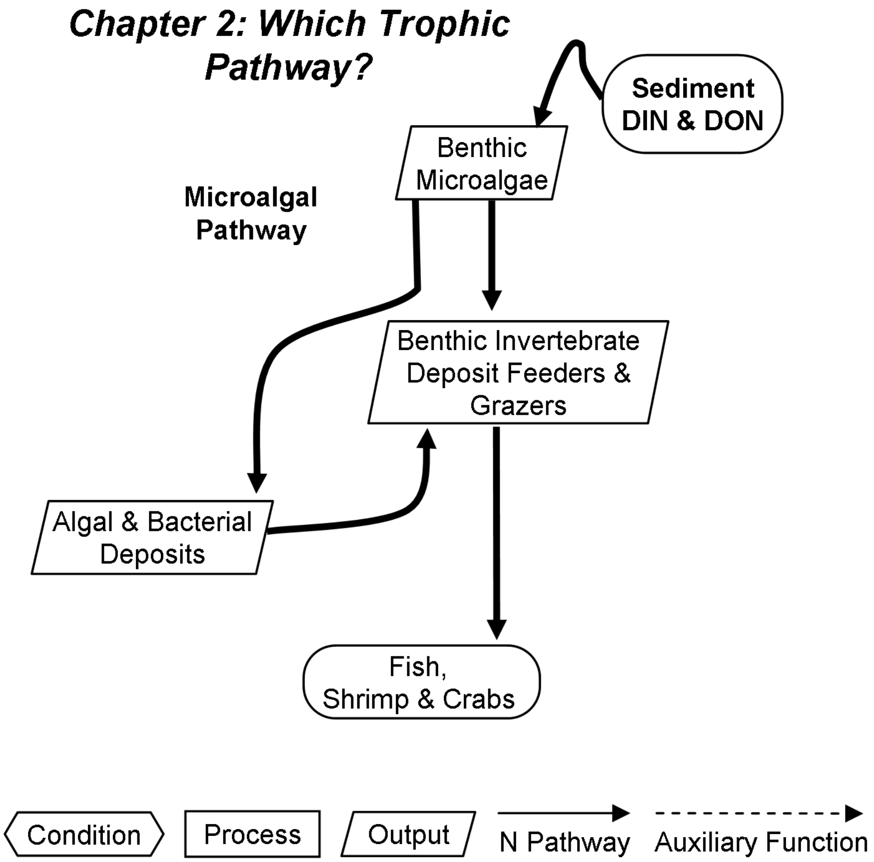

Figure 2.13: Dry Season Microalgae Trophic Pathway. Arrows indicate that benthic microalgae supports a benthic microalgae based community during the dry season.

Implications for Management:

Microalgae as a Food Source

The traditional food-web based on vascular plant detritus was not observed as a dominant pathway in this study. Nitrogen only minimally appeared to enter foodwebs through the breakdown vascular plants and through the assimilation of nitrogen by bacterial colonies.

Managers should be compelled to consider microalgae as a major food source in nursery habitats; in nearly all systems studied here, microalgae ultimately supported fish biomass. Furthermore, managers must have a means to gauge the processes that control the availability of this food source. Specifically managers must measure the processes that control 1) the abundance of microalgae 2) the locations where deposition of water-column microalgae occur, and 3) the locations of high benthic microalgae survivability. Effects of light, depth, and residence time on particle deposition and microalgae survivability are reviewed in great detail in Figures 1.2-1.7. Hydrological modeling and an understanding of stream geomorphology may be adequate to predict locations of microalgae deposition and locations of high benthic microalgae survivability; however, it is unlikely that these models will adequately predict the absolute abundance of these primary producers. In nitrogen limited systems, nitrogen controls the abundance of microalgae and ultimately the abundance of fish. Therefore managers must have a means to identify 1) the potential sources of nitrogen available to these nursery habitats and 2) how nitrogen is delivered from these potential nitrogen sources to nursery habitats.

Nutrients and Fish Production

In the following chapters, nitrogen sources and delivery mechanisms are identified with the assumption that nitrogen has a positive curvilinear relationship with benthic microfauna and fish abundances up to a critical point where the relationship either levels-off or reverses (Caddy 1993). Identifying the relationship between fish abundance and nitrogen loads requires a comprehensive dataset of nitrogen concentrations, stream flow measurements, and nekton abundances. This data is not available for the watersheds studied here, and therefore, this study cannot unambiguously define the potential effects of increasing loads to these habitats. However, in some instances this study will use limited chlorophyll concentration datasets downloaded from the environmental protection agency’s (EPA) *storet* website and nekton abundance datasets available from Greenwood et al. (2009) and Tampa Bay (2008) to approximate relationships between nitrogen loads and fish abundance. These plots are intended to be used as exploratory tools to provide clues about whether adding nitrogen to these systems would deter or support fish production.

Although not observed here, it should be noted that in systems with very low nitrogen inputs and in shaded areas, it is likely that vascular plant detritus indirectly supports fish biomass. This study does not preclude the importance of vascular plants to juvenile fish as structural refuges from predation.

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CHAPTER 3:

THE AGRICULTURAL FOOTPRINT ON SOUTHWEST FLORIDA FISH TISSUE

Introduction

The ability to transform the atmospheric nitrogen pool into food has undoubtedly played an important role in the burgeoning global population; agricultural application of Haber-Bosch industrial fertilizer has been estimated to be responsible for sustaining half of all humanity in 2008 (Erisman et al. 2008). Unfortunately, nitrogen use efficiency in agriculture is far from perfect, and only 17% of applied fertilizer ends up in crop biomass (Braun 2007). The large remainder of this labile nitrogen may be denitrified or exported to a manifold of atmospheric, terrestrial, aquatic, or marine pools (Erisman et al. 2008). Because of this inefficiency, nutrients derived from agricultural application have often been inculpated of evoking natural resource degradation via eutrophication. Copious amounts of nutrients may create algal blooms that decay and cause lethal hypoxic events in many receiving waters.

Nutrients and Eutrophication

The oxymoronic term “nutrient pollution” has been applied to non point-source nutrients derived from anthropogenic land use that results in hypoxia (Smith 1998), seagrass declines (Greening and Janicki 2006), and fish kills (Clarholm et al. 1988). Oftentimes, nutrient pollution is associated with estuarine systems that have been over-utilized and under-protected, with adverse economic consequences including the degradation of habitats essential for fish production (Braun 2007); Chatterjee (2009) has concluded that eutrophication of freshwater regions alone accounts for over a four billion dollar annual loss to the United States.

In consideration of the potentially high economic costs of eutrophication, the United States Environmental Protection Agency (EPA) has implemented strict laws to prevent the adverse effects of eutrophication. Section 303(d) of the Clean Water Act requires states to establish a total maximum daily load (TMDL) of pollutants in water-bodies considered to be impaired by pollution. Consequently, this has put pressure on agricultural communities to implement stronger pollution controls (Bosch et al 2006).

Nutrients Support Food Webs

Although nutrients have been widely perceived by ecosystem managers and ecologists as damaging to ecosystems, they are incontrovertibly a key building block for fish production. Cushing (1975) coined the term “agricultural model” that relates fish biomass to the amount of nutrients supporting the food chain. This theory has been confirmed by positive relationships between primary production and fish landings observed by Nixon (1982). Further evidence of this phenomenon was provided by Josefson and Rasmussen (2000) who found a positive curvilinear relationship between nitrogen load and benthic microfauna up to a critical point at which the relationship either leveled-off or reversed. These results support the models proposed by Caddy (1993) that relate responses of fish yield to nutrient loads in a variety of semi-enclosed seas. Caddy (1993) proposed that shapes of fish catch to biomass curves would be dependent on the benthic or pelagic nature of trophic pathways leading to a particular fish catch. Especially notable were the benthic foodwebs, described as extremely susceptible to hypoxia under high nutrient loads. In a recent study, Oczkowski and Nixon (2008) defined the nutrient enrichment scale associated with the Caddy (1993) models in the tropical lagoons of the River Nile in Egypt and concluded that anthropogenic nitrogen initially increased higher trophic level productivity to a critical point after which an exponential decline in fish landings occurred. Oczkowski et al (2008) also reported that half of hard clam biomass (*Mercenaria mercenaria*) in Narragansett Bay is supported by anthropogenic nutrient sources.

Florida Agriculture and Recreational Fishing Economies

Agriculture is an integral economic sector in Florida, second only to tourism that is driven by the region's billion dollar recreational fishing industry. Sub-estuary receiving waters that drain agricultural watersheds support a variety of economically important juvenile fish and because many of these species utilize these habitats as feeding nurseries (Killam et al. 1992; Peebles et al. 2007), often deriving their biomass via hypoxia-susceptible, benthic trophic pathways (Hollander and Peebles 2004).

Nutrients, a byproduct of Florida's second most important economic sector, may either support or degrade fish nursery habitats that foster Florida's chief economic sector. Therefore, before implementing policy, it is crucial to understand the relationship between anthropogenic nitrogen and fish abundance because excessive nutrient export from agriculture may destroy the economically important benthic-dependent nursery fish habitats, while completely shutting off nutrient support or completely removing water supply would result in no fish biomass. To prevent the collapse of these two economic sectors, appropriate nutrient policy must be established, but first, the pathway that links nutrients derived from specific agricultural land use to fish must be defined.

Water Management and Hydrology

Another key issue affecting both economic sectors, especially in Southwest Florida, is water management, and there is a growing emphasis on using rivers to support agricultural water demands rather than groundwater. Traditionally, agricultural operations withdraw large amounts of groundwater for irrigation, which has recently been shown to cause salt intrusion into drinking water sources. On the other hand, groundwater irrigation processes provide a mechanism of water and nutrient delivery, especially during Southwest Florida's dry season (March through May) where agricultural groundwater withdrawals provide streams with baseline-flow, the flow of water entering a stream. Since rainfall and surface runoff are the major processes that deliver land-use nutrients to fish habitats, and these processes peak from June through September (wet season), it is essential to take into account seasonal hydrological connectivity when defining nitrogen pathways between land use and fish.

Stable Isotopes Identify Nutrient Pathways

Stable nitrogen isotopes have been widely used to define nitrogen pathways in a variety of sub-estuaries. These tracers are appropriate for determining the effects of nutrients on fish biomass because the isotopic composition of the actual organisms and the dissolved species present in aquatic ecosystems inherently provide information on coupling between watershed nutrients and receiving waters. Dissolved inorganic nitrogen (DIN) supports primary production downstream, but also carries with it information on its land-use origin in the form of stable isotopes of nitrogen and oxygen (Kendall 1998). For example, land use types such as agriculture often export nitrate with very heavy $\delta^{15}N$ values due to large amounts of $^{14}NH_3$ and $^{14}N_2$ released during the processes of ammonia volatilization and denitrification, which occur after fertilizer application. DIN derived from treated sewage and septic systems undergo similar biogeochemical processing. DIN sources derived from urbanized soils often have a wide variety of isotopic values because of the various forms of nitrogen processing that occur in urban watersheds. If developed land use does not play a role in delivering nitrogen to ecosystems, then the isotopic composition of nitrate would likely indicate a non-land-use specific source of nitrogen such as the light values of NO_x present in atmospheric deposition or light nitrogen derived from nitrogen fixing bacteria (Kendall 1988). Consequently, many studies utilize the nitrogen isotopes of dissolved nitrate as an instantaneous measure of nitrate's land use origins (Fogg et al. 1988, Rock and Mayer 2006), while other studies utilize the nitrogen isotopic composition of sessile primary consumers such as clams (Oczkowski et al 2008) or mussels (McKinney et al. 2001, 2002) to acquire a more time averaged source signal.

This study uses nitrogen stable isotopes of fish muscle in 14 Southwest Florida watersheds to identify the pathways between different land-use types and the resident fish species that occupy receiving waters. This study aims to 1) define the specific agricultural, urban, and natural land use types that influence the isotopic composition of nitrogen in fish muscle, 2) determine each influential land use type's specific contribution to the isotopic composition of nitrogen in fish muscle, and 3) interpret the values of the isotopic signatures that are associated with these land use types to interpret watershed connectivity pathways. To clarify the effects of hydrology on these linkages, this study was conducted during Southwest Florida's wet and dry seasons.

Methods

Project Scopes and Study Sites

Isotope data reported here originate from three investigations. The first, Hollander and Peebles (2004), identified the primary producers that support upper-trophic-level biomass in four Southwest Florida tidal river sub-estuaries (Figure 3.1, left panel). The second and third investigations, the "tidal streams study" (Tampa Bay 2008) and Greenwood et al. (2009) targeted smaller tidal stream sub-estuaries (Figure 3.1, right panel). Fish isotope data from all three studies are considered here.

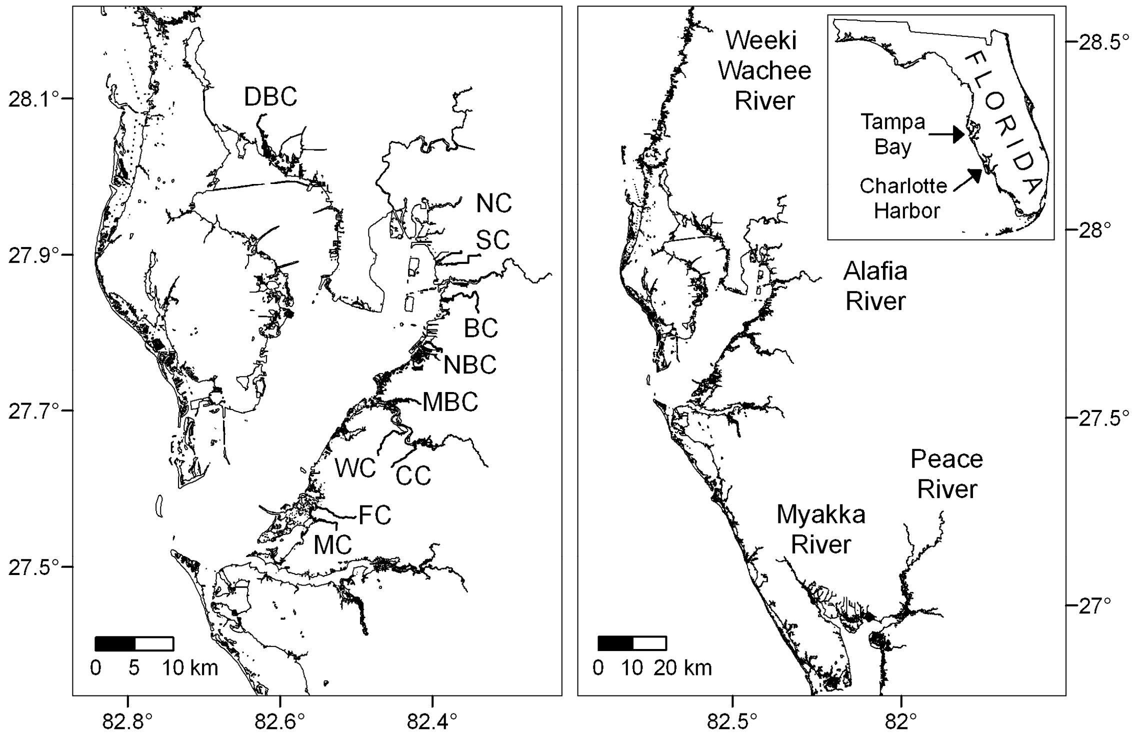

Figure 3.1: Study Sites to Determine Nutrient Sources in Watersheds with Rural Land Use. Watersheds studied include DBC = Double Branch Creek, NC = North Archie Creek, SC = South Archie Creek, BC = Bullfrog Creek, NBC = Newman Branch Creek, MBC = Marsh Branch Creek, WC = Wildcat Creek, CC = Curiosity Creek, FC = Frog Creek, MC = McMullen Creek, WWR = Weeki Wachee River, AR = Alafia River, MY = Myakka river, PR = Peace River.

Sample Collection and Processing

This study aims to define the nitrogen linkages between land use and fish in diverse Southwest Florida tidal fish nursery habitats by relating land use to fish isotopes in 14 different sub-estuaries, instead of focusing within individual systems. Resident fish species including rainwater killifish (*L. parva*) and silversides (*Menidia spp.*) were collected from most sub-estuaries. In cases where these species were unavailable, alternative estuarine species including tidewater mojarra (*E. harengulus*), hogchoker (*T. maculates*), and clown gobies (*M. gulosus*) were collected. These species were also determined to occupy similar trophic positions in all systems (Greenwood et al. 2009) and are supported by benthic trophic pathways (Hollander and Peebles 2004). In this case, sessile resident species are appropriate for defining nutrient sources because they incorporate nitrogen across a wider spatial and temporal scale within each sub-estuary compared to the instantaneous concentration and isotope ratio measurements of dissolved species such as nitrate and ammonium or the short-term averaged isotopic composition of primary producers.

In Hollander and Peebles (2004), fish samples were collected from 2 to 4 different sites along sub-estuary reaches ranging from 5 km to 25 km depending on sub-estuary length. Each sampling site was only visited once for sample collection during each season. Dry season sampling dates ranged from May to June of 2003, while wet season dates ranged from September to October of 2003. Greenwood et al. (2009) collected fish from approximately 1-2 sites along sub-estuary reaches ranging from 1 km to 3 km. Each sampling site was only visited once for sample collection during each season. Dry season sampling occurred in May 2007, while wet season sampling occurred in September 2007. In the tidal streams project, samples were collected at 4-5 sites per sub-estuary with individual sub-estuary reaches ranging approximately from 1 to 4 km. Each sampling site was visited twice for sample collection during both seasons. Fish were collected monthly from March to June for dry season sampling and from August through November for wet season sampling.

Samples of fish tissues were obtained using mid-channel trawls (Hollander and Peebles 2004 study) and shoreline seines (all studies). All samples were limited to tissues (primarily muscle) located posterior to the peritoneal cavity. All solid samples were dried at 50°C, powdered, and frozen until isotopic analysis.

Stable isotope nitrogen values were measured using a Carlo Erba 2500 Series I elemental analyzer equipped with a zero-nitrogen blank auto-analyzer that was coupled to a Finnigan Mat Delta Plus XL stable-isotope mass spectrometer. All samples were run in duplicate using Spinach Leaves (NIST 1570a) as an internal reference material, and N isotope values are reported in conventional per mil notation (‰) relative to the air international standard. Instrumental standard deviation remained under 0.3‰.

Land use geographic information system data and watershed boundary geographic information system data for the entire Southwest Florida region were downloaded from the official SWFWMD website. According to the metadata files, both 2004 and 2006 land use data were categorized according to the Florida Land Use and Cover Classification System (FLUCCS), and the features were photo-interpreted at 1:12,000 using 2004 or 2006 1-meter color infrared digital aerial photographs. The watershed boundary data were interpreted and digitized from the USGS 1:24,000 quadrangles, and the boundaries were categorized according to the USGS Hydrologic Unit Classification coding system. Using these two datasets, percent area coverage for each land use type was calculated using the spatial analyst function in ArcGIS version 9.

Statistical Approach

Because watersheds dominated by agricultural landscapes are rapidly being urbanized, this study utilized a multiple regression bootstrapping approach to asses the contribution of each land use type to fish $\delta^{15}N$. This technique is generally used in situations where data are expensive, information is sparse, assumptions of normality are unclear, or where further data are difficult to acquire (Henderson 2005).

The percent of total watershed area covered for 27 land use classifications were calculated in each watershed. To remove effects of collinearity, correlated land use types and land use types assumed to have similar stable isotopic properties were grouped resulting in 12 uncorrelated, independent land use group variables. For each of the 14 watersheds (n=14), the relative area of 12 land use groups were considered as possible explanatory variables that influence the response variable of each watershed's average fish $\delta^{15}N$ values. All land use and isotopic data are summarized in Table 3.1.

Table 3.1: Land Use and Isotope Data Summary for Rural Watersheds. Relative area of watershed covered for grouped land use types: ROW = row crops, vineyards; TCR = tree crops; FCR = fallow crops; AGO = feeding operations, specialty farms; RHI = high density residential, institutional; RLO = low, medium density residential; GLF = golf courses, other recreational; EXT = commercial, extractive, industrial; TU = transportation, utilities, communication; FOR = upland forested, wetland forested; UNF = upland non-forested; WET = vegetated wetlands. Wet and dry season fish isotope data includes mean, sample size (n) and standard error (STE), and species include: L = *L. parva*, M = *Menidia spp.* E = *E. harengulus*, T = *T. maculates*, G = *M. gulosus.*

System	Size	Land Use Group												Wet δ^{15}N	Dry δ^{15}N
	(km^2)	ROW	TCR	FCR	AGO	RHI	RLO	GLF	EXT	TU	FOR	UNF	WET	n, STE	n, STE
AR	1093	3.1	4.5	14.0	0.4	3.1	16.4	0.7	39.6	1.6	10.3	4.4	1.9	10.69 EM 4, 0.9	12.14 M 9, 0.21
NC	24	0.0	0.0	12.5	0.1	30.3	11.2	1.8	14.3	7.6	5.8	9.7	6.7	9.12 GT 10, 0.44	9.18 LM 6, 0.38
SC	10	0.0	0.0	2.1	0.1	14.7	8.9	0.0	25.7	8.3	11.5	22.3	6.5	8.54 M 2, 1.04	9.10 M 3, 1.0
BC	76	15.2	2.8	17.0	3.2	5.7	13.5	0.4	3.3	2.6	16.1	17.4	2.8	11.43 L 3, 0.18	13.17 E 2, 0.34
CC	57	29.3	11.3	17.3	0.2	0.0	4.7	0.0	0.0	2.3	21.1	8.8	4.9	13.19 L 5, 0.09	15.91 LM 40, 0.11
DBC	70	0.2	0.2	3.6	1.6	19.1	14.3	5.3	3.9	4.6	28.4	12.2	6.6	9.52 LM 6, 0.40	10.16 L 3, 0.39
FC	24	6.4	9.5	12.6	0.3	6.4	12.5	3.5	0.8	11.0	20.6	14.2	2.3	11.18 LM 20, 0.29	12.26 LM 15, 0.29
MBC	17	10.1	4.4	12.4	2.0	6.3	29.4	0.7	6.5	3.6	9.1	15.0	0.4	11.32 TE 6, 0.23	10.46 LM 6, 0.32
MC	8	5.4	0.7	25.1	0.2	19.6	23.5	2.9	3.1	1.9	11.3	4.9	1.5	9.59 L 10, 0.10	9.47 L 5, 0.08
MR	1550	5.7	2.2	19.0	0.2	0.9	11.7	0.5	1.1	0.6	20.2	22.2	15.5	9.26 M 9, 0.22	9.54 GM 5, 0.10
NBC	6	1.6	0.0	43.8	0.3	22.0	4.8	1.4	0.5	4.4	9.1	7.6	4.7	10.22 L 4, 0.12	9.57 LM 5, 0.47
PR	6079	1.8	17.0	27.7	0.3	1.3	7.7	0.7	13.4	0.9	11.5	9.0	8.8	11.25 M 10, 0.27	12.24 M 33, 0.11
WC	9	7.7	34.9	19.3	0.0	1.6	4.8	6.4	0.3	0.4	6.9	13.9	3.8	13.16 LM 35, 0.15	13.05 LM 20, 0.18
WWR	99	0.1	0.0	0.3	0.0	4.9	36.4	3.2	2.5	2.5	32.1	8.2	9.9	9.04 LM 8, 0.34	9.66 LM 25, 0.11

A bootstrap algorithm was then executed to randomize the observations and to conduct a forward stepwise multiple regression to select up to 3 significant land use variables that explain fish δ^{15}N variability. The selected land use groups and permutation based p-values (1000 repetitions) were then automatically recorded in a matrix. This process was repeated 10,000 times to ensure data robustness; no single observation could dominate the selection process. The Use of 10000 bootstrap samples provided reproducible results with smooth distributions. Land use variables were further analyzed if selected in more than 33% of bootstrap regressions and if the 50th percentile of the variable's p-value distribution was less than 0.001.

Defining Specific Land Use Coefficients

The magnitudes of the coefficients assigned to land use variables are likely a function of the isotopic composition associated with land use as well as the amount of nitrogen exported or removed from that particular land use type. Because the ranges of these coefficients may provide important predictive information about nitrogen pathways in ecosystems, the confidence intervals around the coefficients were calculated by bootstrapping the coefficient parameters using Statistics 101 Version 1.4 with a non-parametric bootstrap algorithm modified from Simon (1997), similar to those described by Draper and Smith (1998); 10,000 bootstrap samples were constructed that recorded the distribution of each coefficient's values. Confidence intervals for each of the significant land use types were determined by calculating the 5th and 95th percentile from each of the coefficient's distributions.

Defining Specific Land Use Contributions to Fish Biomass

The contribution of specific land use types to the nitrogen isotopic composition of fish was determined by constructing bootstrapped squared correlation coefficient (R^2) distributions for each significant land use group variable. In every bootstrap sample, predicted fish isotopes were first calculated using linear multiple regression by plugging in a single land use group variable that explained the most fish $\delta^{15}N$ variability. Predicted fish isotopes were then correlated against observed values, and the resulting R^2 parameter was recorded, representing the contribution from the first chosen land use group variable. The contributions from other land use groups were calculated by plugging in the land use variable that explained the remaining variability of the previous model's residuals. Total R^2 parameters were then calculated to determine the variability explained by all the variables already entered into the model, and the variability explained by the most recently added land use variable was calculated by subtracting preceding total R^2 value from the most recent total R^2 value. This process was repeated for each chosen land use variable in every bootstrap sample. R^2 distributions were then created by repeating this process 10,000 times. This methodology provided ranges of the percent variability explained by each land use group, and it also identified situations in which two land use types were competing to explain the majority of fish $\delta^{15}N$ variability. Model outputs containing different land use variables with highly bimodal, overlapping R^2 distributions were considered indicative of the model inconsistently choosing one land use type over another. If these variables also had similar coefficient distributions, variables were combined, and models were rerun to produce new coefficient and R^2 distributions.

The total fish $\delta^{15}N$ variability explained by the complete model (adjusted R^2) was also determined using a bootstrap methodology in which all selected variables and coefficients were used to calculate predicted fish isotope values for each bootstrap sample. These predicted fish isotope values were regressed against observed values, and the resulting adjusted R^2 values were recorded for each bootstrap sample to create a distribution of total explained fish $\delta^{15}N$ variability.

These three steps were repeated for both wet and dry season fish $\delta^{15}N$ data to establish seasonal specific influences of land use on fish tissue.

Results

Land use Types that Influence Fish Stable Nitrogen Isotope Composition

Selected land use groups were not seasonally identical (Figure 3.2). Tree crops, row crops (including vineyards), and vegetated wetlands variables were highly significant

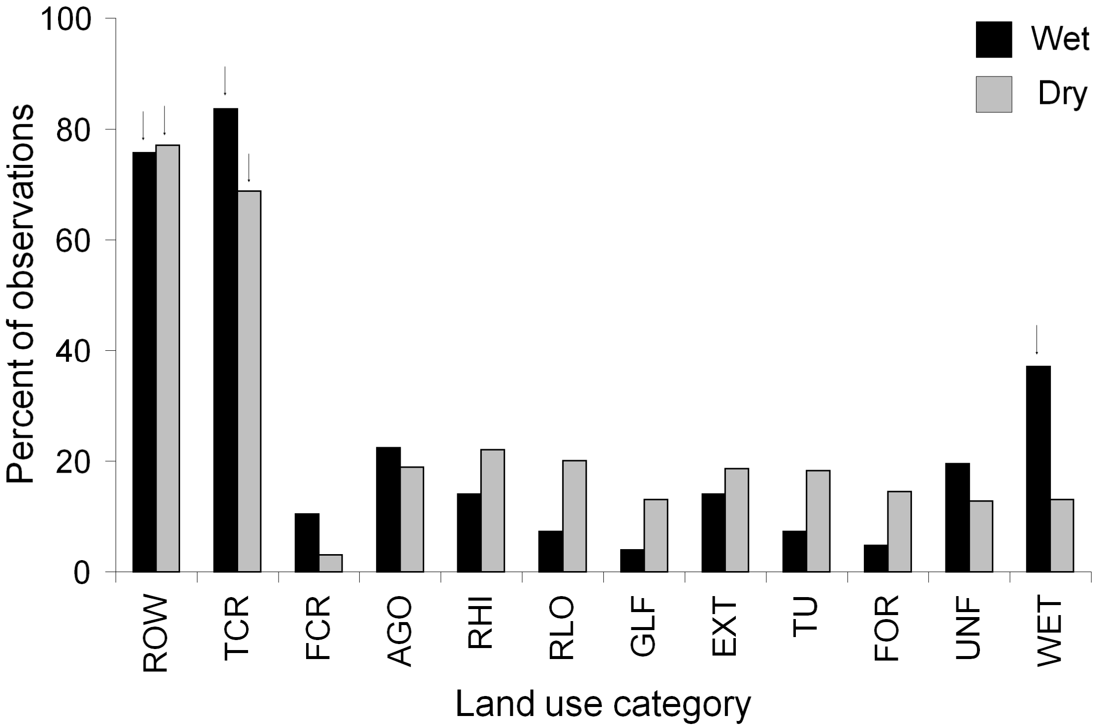

Figure 3.2: Results of Bootstrap Program Used to Select Land Use Types that Influence Fish Stable Nitrogen Isotope Variability. Percent of the 10,000 bootstrap observations (y-axis) in which a specific land use type was selected (x-axis) is depicted for both wet (black) and dry season (grey) data. Arrows indicate whether the selected land use type was significant based on permutation based p values ($p < 0.001$).

during the wet season (median $p < 0.001$). In contrast, row crops were highly significant during the dry season, and tree crops played a comparatively minor role. In the wet season analysis, row crops and tree crops had overlapping bimodal R^2 and coefficient distributions, so these variables were grouped into a single row and tree crop variable. New bootstrap parameters were calculated and are reported here. The original medians of the individual row crop and tree crop R^2 distributions were 0.36 and 0.56, respectively.

Defining Specific Land Use Coefficients

The wet season coefficient distributions for the combined row and tree crops variable and the vegetated wetlands variable are depicted in Figure 3.3. The row and tree crop group had coefficient magnitudes as expected assuming that agriculture areas

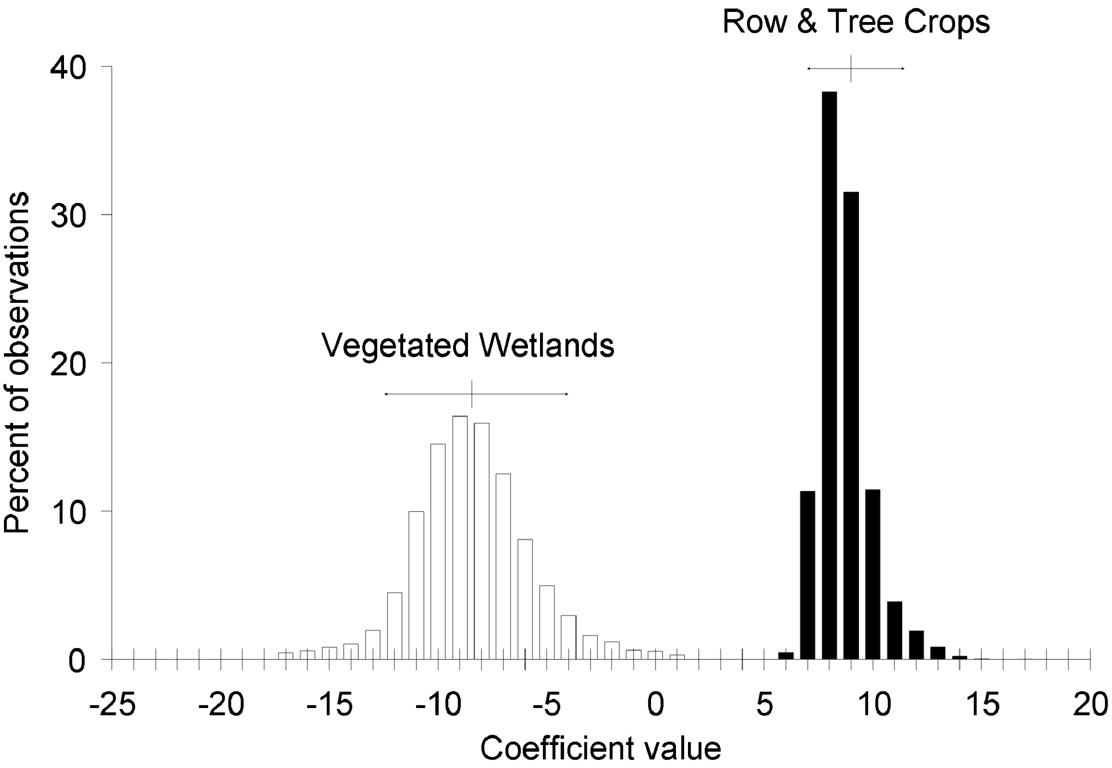

Figure 3.3: Wet Season Coefficient Distribution for Rural Land Use Watersheds. Bootstrap distributions of coefficient values (x-axis) assigned to land use variables that explain wet season fish $\delta^{15}N$ variability. Y-axis: percent of the 10,000 bootstrap observations where a specific coefficient value was selected. Ranges indicate the 5th, 50th, and 95th percentile of each distribution.

export nitrogen with heavy $\delta^{15}N$ values (Kendall 1997). Wetlands on the other hand had a coefficient distribution centered on a negative value, which may imply nitrogen removal from the system, nitrogen transformation, or wetland export of low $\delta^{15}N$ nitrogen.

The dry season coefficient distributions of the row crops variable and the tree crops variable are depicted in Figure 3.4. Both row crops and tree crops had coefficient magnitudes as expected assuming that agriculture areas export nitrogen with heavy $\delta^{15}N$ values. Row crop and tree crop variables were not combined during the dry season because these variables did not have overlapping R^2 or coefficient distributions. The tree crops coefficient distribution was centered on a lower value when used to predict dry season fish $\delta^{15}N$ values compared to wet season fish $\delta^{15}N$ values. The row crops

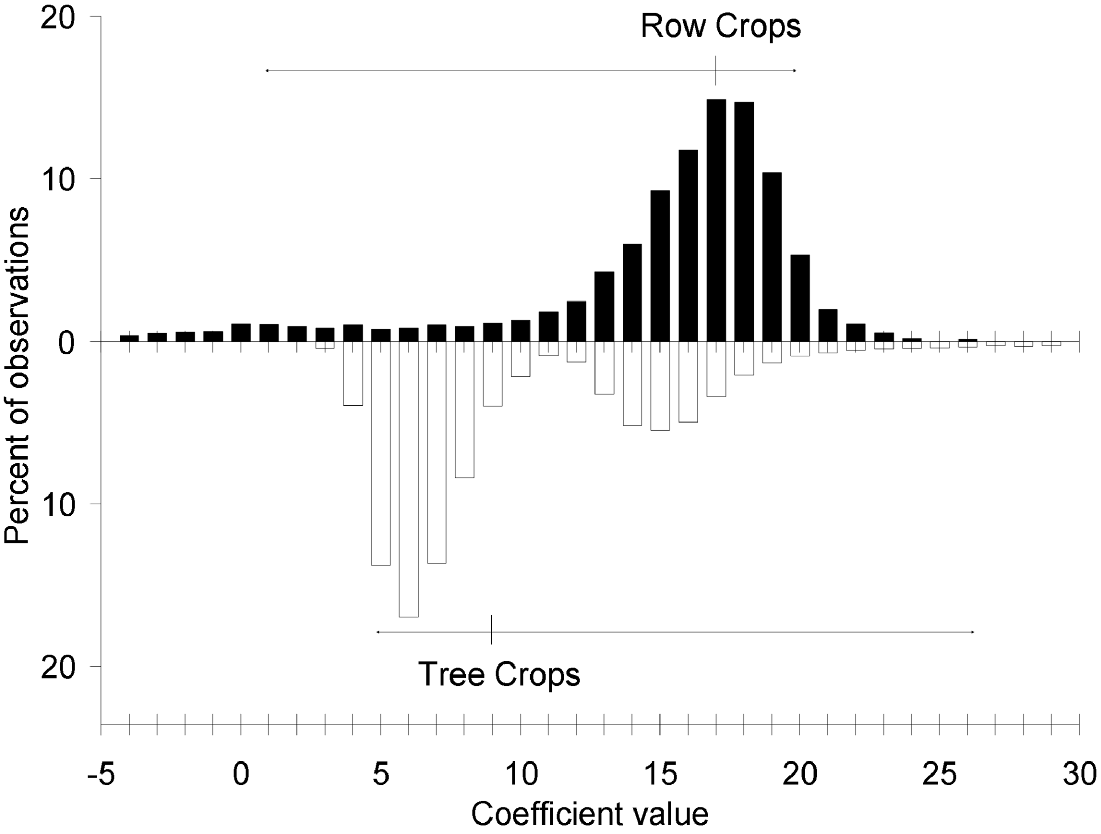

Figure 3.4: Dry Season Coefficient Distribution for Rural Land Use Watersheds. Bootstrap distributions of coefficient values (x-axis) assigned to land use variables that explain dry season fish $\delta^{15}N$ variability. Y-axis: percent of the 10,000 bootstrap observations where a specific coefficient value was selected. Ranges indicate the 5th, 50th, and 95th percentile of each distribution.

coefficient distribution was centered on a higher value when used to predict dry season fish $\delta^{15}N$ values compared to wet season fish $\delta^{15}N$ values.

Defining Specific Land Use Contributions to Fish Biomass

The wet season R^2 distributions for row and tree crops, vegetated wetlands, and overall adjusted R^2 are depicted in Figure 3.5. Row crops and tree crops explained 88% (50th percentile) of fish $\delta^{15}N$ variability, while wetlands explained 6% of the variability. Overall adjusted R^2 values were high; 92% (50th percentile) of fish $\delta^{15}N$ variability was explained using only these two land use groupings. Since none of the distributions were

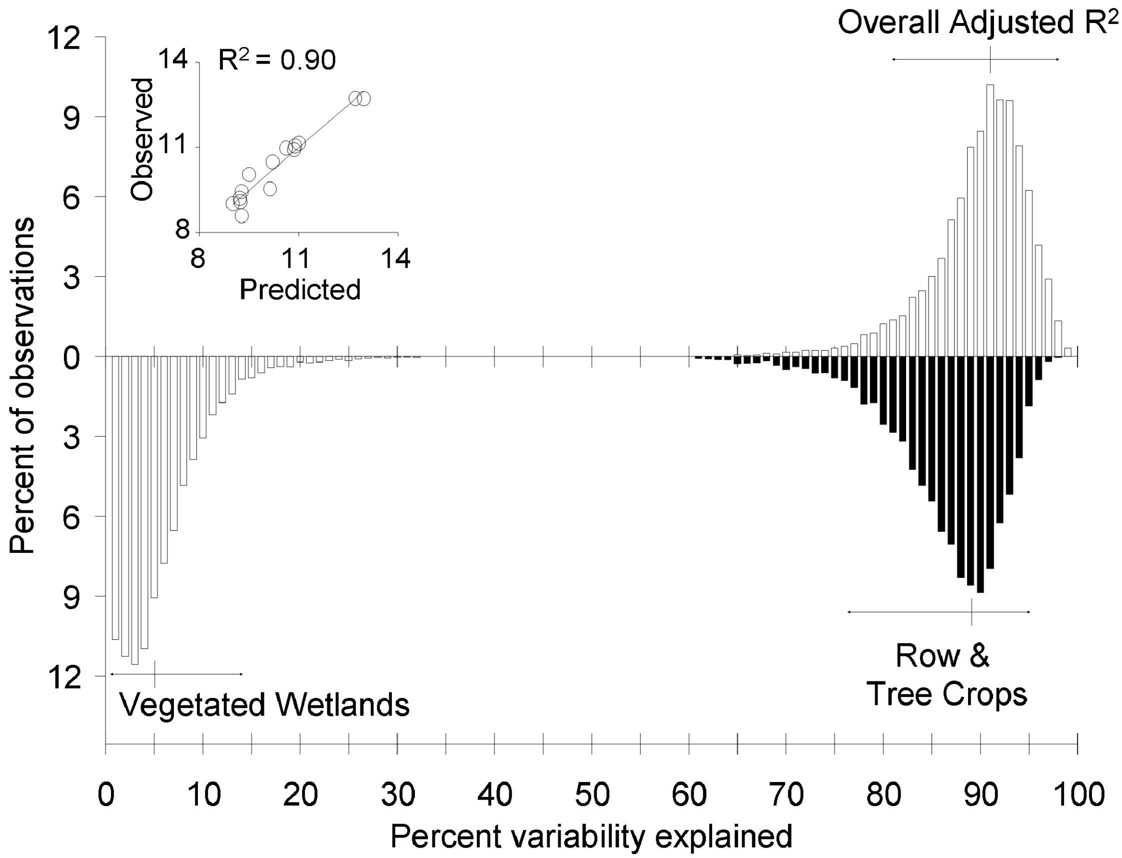

Figure 3.5: R-Squared Distributions of Land Use Variables that Explain Wet Season Fish Stable Nitrogen Isotope Variability. Bootstrap distributions of the R^2 values of individual land use types are presented as well as the total adjusted R^2 describing the amount of explained wet season fish $\delta^{15}N$ variability (x-axis) using ordinary least square multiple regression. Percent of observations on the y-axis is the percent of the 10,000 bootstrap observations (y-axis) in which a specific R^2 value was calculated. Ranges indicate the 5th, 50th, and 95th percentile of each distribution. Also included are the results of the non-bootstrapped OLS multiple regressions with adjusted R^2 value.

bimodal, the regression models did not consistently choose row and tree crops over wetlands or vice versa

Dry season R^2 distributions for the row crops and the tree crops land use groups are depicted in Figure 3.6. Rows Crops explained 67% (50th percentile) of the variability of fish $\delta^{15}N$, while tree crops explained 15% (50th percentile) of the variability of fish $\delta^{15}N$. Adjusted R^2 values were low compared to the wet season; 78% (50th percentile) of fish $\delta^{15}N$ variability was explained using these two land use variables. The distributions were much wider during the dry season compared to the wet season. This poor model

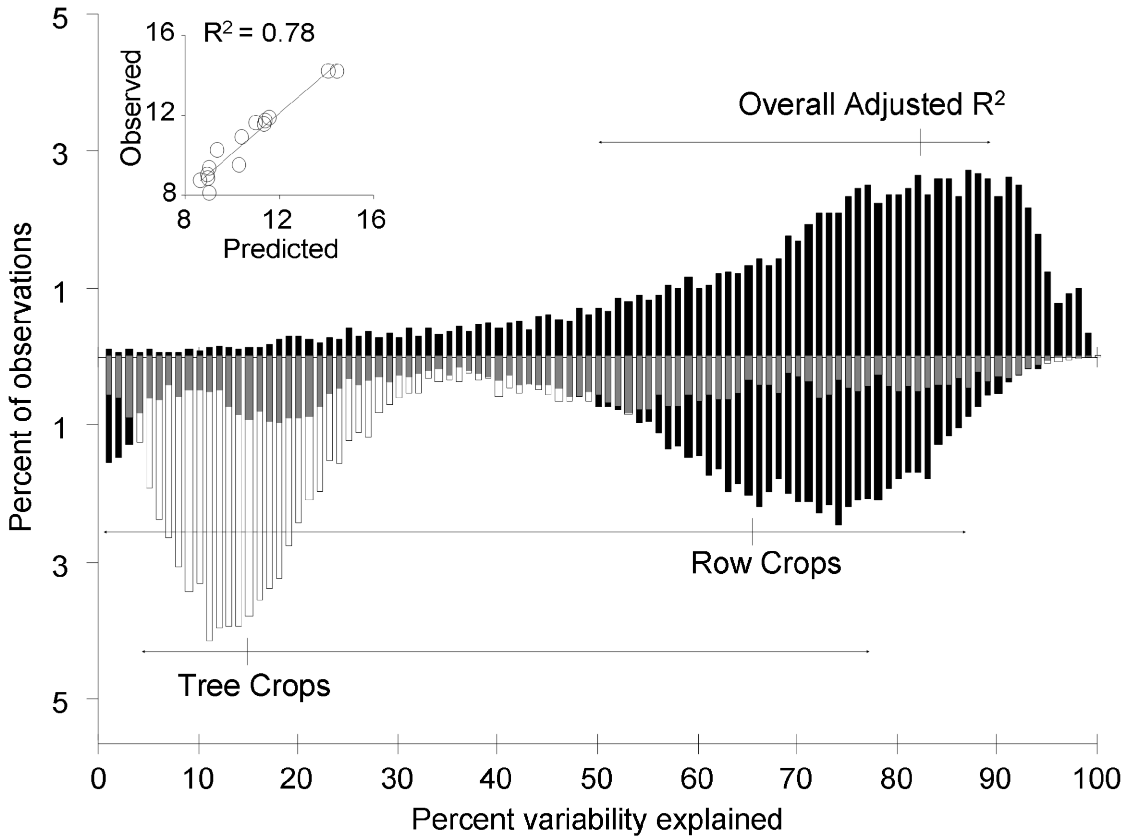

Figure 3.6. R-Squared Distributions of Land Use Variables that Explain Dry Season Fish Stable Nitrogen Isotope Variability. Bootstrap distributions of the R^2 values of individual land use types are presented as well as the total adjusted R^2 describing the amount of explained dry season fish $\delta^{15}N$ variability (x-axis) using ordinary least square multiple regression. Percent of observations on the y-axis is the percent of the 10,000 bootstrap observations (y-axis) in which a specific R^2 value was calculated. Ranges indicate the 5th, 50th, and 95th percentile of each distribution. Gray areas represent overlapping distributions. Also included are the results of the non-bootstrapped OLS multiple regressions with adjusted R^2 value.

performance implies inconsistent relationships between land use and fish during the dry season.

Discussion

The land use types that explained fish $\delta^{15}N$ variability were row crops, tree crops, and vegetated wetlands. Results imply that these specific sections of the watershed export or intercept more nitrogen than their relative areas would suggest. Figure 3.7 compares the mean percent relative area covered to the median percent variability explained according to R^2 distributions (Figure 3.5 and 3.6).

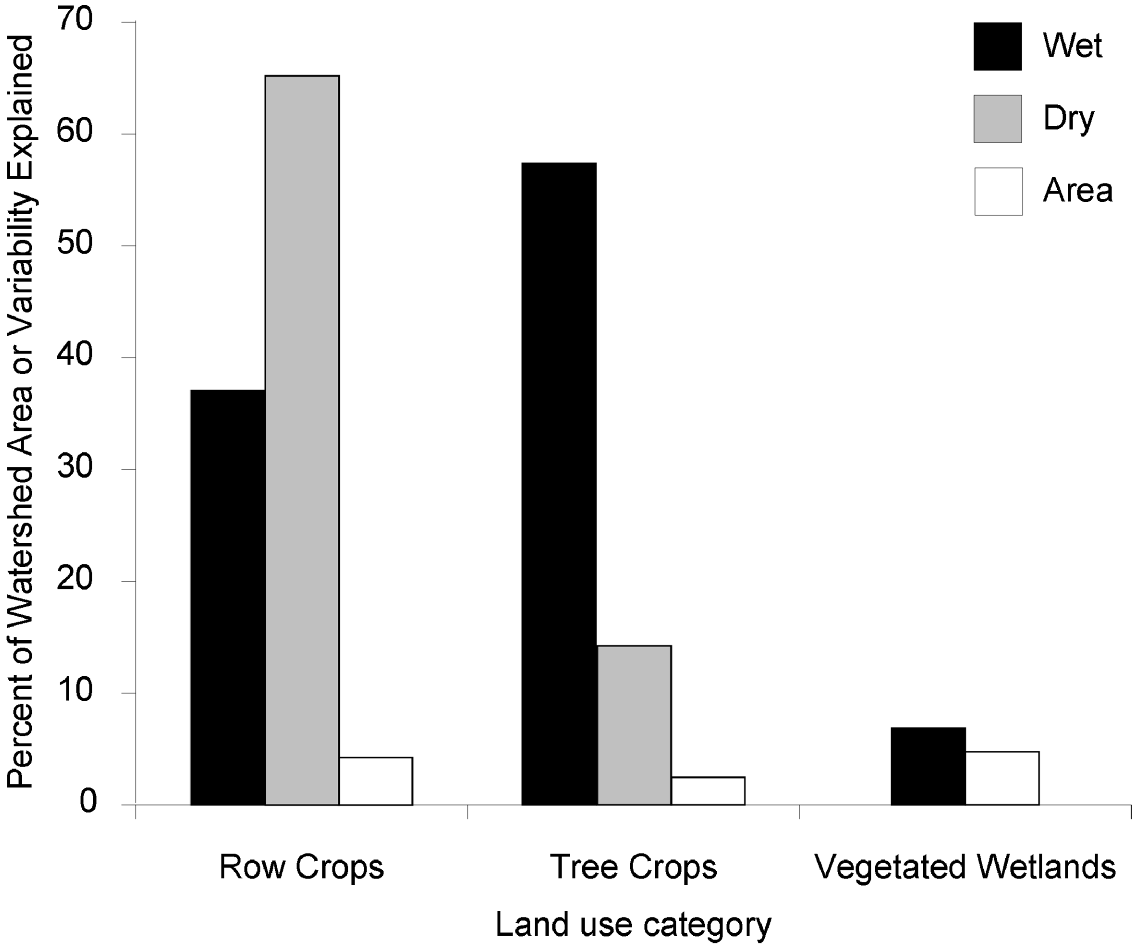

Figure 3.7: Comparison of Land Use Area and the Percent of Fish Stable Nitrogen Isotope Variability Explained. Comparison between each land use variable's mean relative area of watershed covered (white bars) and the percent of fish $\delta^{15}N$ variability explained. Results are depicted for both wet (black) and dry season fish $\delta^{15}N$ data (gray).

These results provide insights about the amount of nitrogen exported by a given area of specific land use. During the wet season, row crops and tree crops export copious amounts of nitrogen per unit area of land covered. Cumulatively, these variables explained 88% of the variability of fish $\delta^{15}N$ during the wet season, despite covering an average of less than 10% of watershed area. Vegetated wetlands on the other hand only transformed or removed an intermediate amount of nitrogen per unit land use area; during the wet season, this variable explained 6% of fish $\delta^{15}N$ variability, covering 5% of watershed area. During the dry season, row crops also likely export a large amount of nitrogen per unit area. Row crops explained 67% of fish $\delta^{15}N$ variability, but tree crops only explained 15% of fish $\delta^{15}N$ variability. This is not surprising, since tree crops are not actively fertilized during the winter months, while row crops are processed year round. Results suggest that agricultural landscapes export a large amount of nitrogen per unit area, while landscapes that remove and transform nitrogen do not operate as efficiently per unit area. Large wetland areas would likely be required to remove nitrogen exported by a relatively small amount of agricultural land-use.

Application to a Nitrogen Isotope Connectivity Model

Section 304(a)(1) of the Clean Water Act requires the development of water-quality criteria that accurately reflect the latest scientific knowledge. Current TMDL calculations may be consistently misrepresented downstream due to intervening processes such as selective burial of different forms of nitrogen in sediments and by various microbial processes that convert and release nitrogen and perhaps most importantly by fundamental differences in the hydrologic connectivity between different types of land cover and downstream waters. This study provides crucial information to help unravel the intricate nitrogen cascades that occur between anthropogenic watershed land use and the organisms that occupy receiving waters.

Mechanistic mass balance isotope equations can complement TMDL methodology by assigning different land use types as nutrient sources or sinks. A differential equation that describes the seasonal time dependence of the ratio of ^{15}N to ^{14}N in fish tissue for a given watershed can be described in terms of a watershed's nitrogen sources and sinks (McCauley 1999) as:

$$\mathbf{N}\frac{\mathbf{DR_{FISH}}}{\mathbf{dt}} = \sum_{\mathbf{SOURCES}} \mathbf{\Phi_{SOURCE}}\left[\mathbf{R_{SOURCE}} - \mathbf{R_{FISH}}\right] - \mathbf{R_{FISH}} \sum_{\mathbf{SINKS}} \mathbf{\Phi_{SINK}}\left[\mathbf{\alpha_{SINK}^{-1}} - \mathbf{1}\right] + \mathbf{k}$$

where R is the ratio of ^{15}N to ^{14}N, N is the total number of moles of nitrogen in a fish, Φ is flux in $(mol)(season)^{-1}$, α is the kinetic isotope fractionation factor associated with bioavailable nitrogen, and k is trophic isotopic enrichment.

Since R can be expressed relative to the air $\delta^{15}N$ international standard as $\delta^{15}N = ((R_{SAMPLE})(R_{STANDARD})^{-1} - 1)(1000)$, at steady state the differential equation can be simplified (McCauley 1999) as:

$$\boldsymbol{\delta^{15}N_{FISH} = \sum(X_{SOURCE})(\delta^{15}N_{SOURCE}) + \sum(X_{SINK})(\varepsilon_{SINK}) + k}$$

where $\boldsymbol{\varepsilon_{SINK}} = 1000(\alpha_{SINK} - 1)$.

The empirical multiple regression equation can be rearranged into the simplified mechanistic steady state equation. The multiple regression equation used in this study is in the form:

$$\boldsymbol{\delta^{15}N_{FISH} = \beta_0 + (\beta_1)(A_1) + (\beta_2)(A_2) \ldots + (\beta_x)(A_x) + e}$$

where A_X are the variables of percent watershed area covered for land use type x, β_x represent the model's fitted coefficients for A_x, and e is the residual error term. The percent of watershed area covered (A_x) is assumed to be related to the fraction of total nitrogen flux (X) if the amount of nitrogen a specific land use type exports or absorbs per unit of relative area is corrected for. This can be achieved by appending a land use specific nitrogen connectivity factor (C_x) to A_x. X in the steady state equation becomes equivalent to the product of C_x and A_x. By rearranging the coefficients and variables of the multiple regression equation into nutrient source and nutrient sink groupings, the mechanistic mass balance equation become apparent as:

$$\boldsymbol{\delta^{15}N_{FISH} = \sum(\delta^{15}N_{SOURCE})(C_{SOURCE})(A_{SOURCE}) + \sum(\varepsilon_{SINK})(C_{SINK})(A_{SINK}) + k}$$

where A_{SOURCE} represents A_x variables that act as nutrient sources, and the product of $(\delta^{15}N_{SOURCE})(C_{SOURCE})$ represent the regression β_x coefficients for the nutrient source A_x variables. Similarly, A_{SINK} represents A_x variables that act as nutrient sinks, and the product of $(\varepsilon_{SINK})(C_{SINK})$ represents regression β_x coefficients for nutrient sink A_x

variables. The constant k represents trophic isotopic enrichment as well as the nutrient sources and sinks not explained by the variables in the model.

Therefore, β_x inherently contains information on both the isotopic composition and fractionation associated with specific nutrient source and nutrient sink land use types as well as the connectivity of specific land use types. C_{SOURCE} corrects for the amount of nitrogen exported per unit area of nutrient source land use type, and C_{SINK} corrects for the amount of nitrogen removed and transformed per unit area of nutrient sink land use type. Therefore, the wet season multiple regression equation:

$$\delta^{15}N_{FISH} = (\beta_{CROPS})(A_{CROPS}) + (\beta_{WETLANDS})(A_{WETLANDS})$$

can be transformed to the mechanistic equation as:

$$\delta^{15}N_{FISH} = 9 + (\delta^{15}N_{CROPS})(C_{CROPS})(A_{CROPS}) + (\varepsilon_{WETLANDS})(C_{WETLANDS})(A_{WETLANDS})$$

where the k is the median constant determined by bootstrapping, and the subscript CROPS refers to the row and tree crop nutrient source parameters, and the subscript WETLANDS refers to wetland nutrient sink parameters. Fish collected in this study are generally considered secondary consumers. An isotopic enrichment of 6‰ is expected assuming a 3‰ increase in $\delta^{15}N$ per trophic level (Peterson and Fry 1987) and a trophic level of 3. The remaining 3‰ is unexplained.

For the parameter products represented in Figure 3.3, $\delta^{15}N_{CROPS}$ can be estimated as 10‰. Crop soils are first fertilized by Haber-Bosch fertilizer with an original $\delta^{15}N$ equal to 0-2‰. The soil processes of ammonia volatilization, nitrification and denitrification result in heavy residual agricultural source nitrates with a mean $\delta^{15}N$ value of approximately 10‰ (Kendall 1998).Since β_{CROPS} is the product of $\delta^{15}N_{CROPS}$ and C_{CROPS}, dividing $\delta^{15}N_{CROPS}$ (10‰) by β_{CROPS} (~10) results in a C_{CROPS} value of 1, which may imply that rows crops and tree crops export a relatively large amount of nitrogen per unit area in agreement with Erisman et al. (2008).

Vegetated wetland soils and plants are commonly recognized as nitrogen sinks (Reddy et al. 1989, Gersberg et al. 1983). Generally, it is assumed that in major denitrification zones, the release of light dinitrogen leaves the residual nitrate pool enriched in ^{15}N (Kendall 1998); fish that occupy watersheds with excessive wetland nitrogen removal should therefore have isotopic compositions positively correlated with wetland area. The negative relationship here implies that wetlands must also be major zones of nitrogen transformation. Reinhardt et al. (2006) reported a similar phenomenon using an isotope mass balance approach in a constructed wetland; incoming nitrates were consistently isotopically heavier compared to exported nitrates, even during periods of high nitrogen removal. This was attributed to the complex nitrogen cascades that occur in wetlands. $\varepsilon_{WETLANDS}$ may account for these dynamic reactions that remove and transform nitrogen in wetland soils. These kinetic and equilibrium fractionation processes include but are not limited to denitrification, nitrification denitrification coupling, anammox, ammonia volatilization and assimilation (Kendal 1998).

If it is assumed that the same amount of nitrogen that is exported per unit relative area of agriculture is either transformed or removed as it is transported to a unit area of vegetated wetland (i.e. $C_{WETLANDS} = C_{CROPS} = 1$), it can be assumed that $\varepsilon_{WETLANDS}$ is equal to $\beta_{WETLANDS}$. Values for $\beta_{WETLANDS}$ can then be compared directly to those of Reinhardt et al. (2006) as an exercise to determine whether transformation or removal processes dominate in these vegetated wetlands.

During periods of copious denitrification, Reinhardt et al. (2006) reported $\varepsilon_{WETLANDS}$ as approximately -3‰, while during periods of high nitrogen transformation and minimal removal, $\varepsilon_{WETLANDS}$ was reported as approximately -15‰. Interestingly, Reinhardt et al. (2006) found that nitrogen removal in the sediments co-occurred with an oxygenated water column, while nitrogen transformation in the sediments co-occurred with a partially anoxic water column. In the current study, the intermediate $\varepsilon_{WETLANDS}$

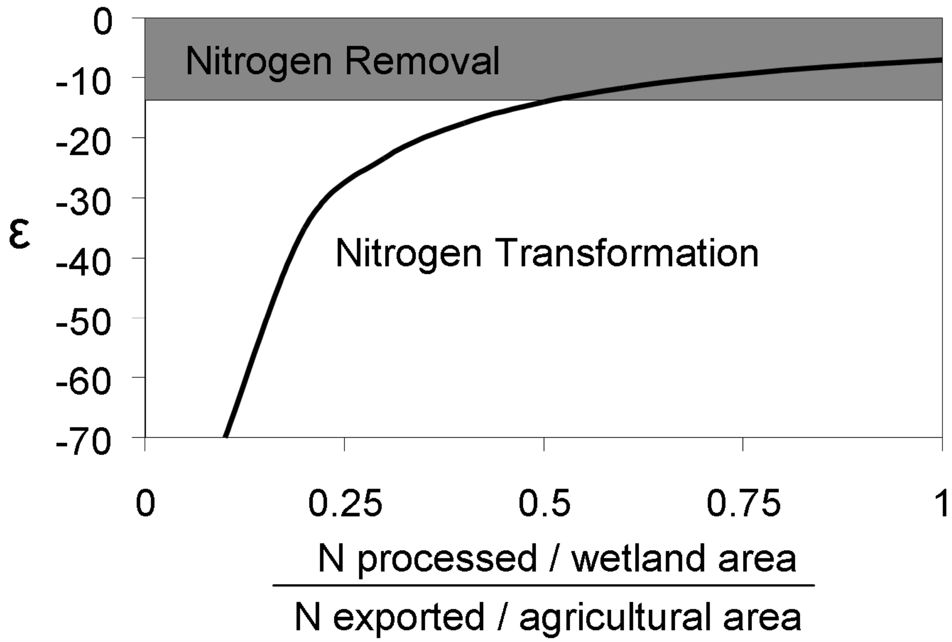

Figure 3.8: Nitrogen Fractionation and Wetland Process. Relationship between the ratio of $C_{WETLANDS}$ to C_{CROPS} (x-axis) and the nitrogen isotopic fractionation that occurs in vegetated wetlands assuming that the $(C_{WETLANDS})(\varepsilon_{WETLANDS}) = -7$ as in Figure 3.3. Area representing nitrogen transformation and nitrogen removal are derived from Reinhardt et al. (2006).

value of -7 (Figure 3.3), may imply that nitrogen removal processes slightly outweigh transformation processes in vegetated wetlands. Figure 3.8 shows possible values of $\varepsilon_{WETLANDS}$ for different values of the ratio of $C_{WETLANDS}$ to C_{CROPS}. Assuming $(C_{WETLANDS})(\varepsilon_{WETLANDS}) = -7$, $C_{CROPS} = C_{WETLANDS}$, and $C_{CROPS} = 1$; as long as a unit of wetland area processes at least 50% of the nitrogen derived from a unit area of agriculture, these areas are expected to be zones of net nitrogen removal.

Since the regression coefficients are equivalent to the product of the connectivity factor and the isotopic composition of a given source, the same wet season equation manipulation can be applied to the dry season data. The simple dry season regression equation:

$$\boldsymbol{\delta^{15}N_{FISH} = (\beta_{ROW})(A_{ROW}) + (\beta_{TCR})(A_{TCR}) + \beta_0}$$

can be reconstructed as:

$$\boldsymbol{\delta^{15}N_{FISH} = (C_{ROW})(\delta^{15}N_{ROW})(A_{ROW}) + (C_{TCR})(\delta^{15}N_{TCR})(A_{TCR}) + 9}$$

where the k is equal 9, and the subscript ROW designates row crops nutrient source parameters, and the subscript TCR designates tree crops nutrient source parameters. Fish collected during this season are also generally considered secondary consumers, and an isotopic enrichment of 6‰ is expected assuming a 3‰ increase in $\delta^{15}N$ per trophic level (Peterson and Fry 1987). The remaining 3‰ is unexplained by the model. Though, the total variability explained during the dry season was relatively low (Figure 3.6), the β_{ROW} values implied row crops are nutrient sources (Figure 3.4).

The values of $(C_{ROW})(\delta^{15}N_{ROW})$ were higher in the dry season compared to the wet season. Because of the reduced sheet flow during this period, it is unlikely that the amount of nitrogen exported to the watershed increased. Instead, the increased product parameter values likely reflect differences in isotopic transformation; reduced mixing of agricultural soils with low $\delta^{15}N$ rainwater nitrate (Kendall 1998) could account for the increased C_{ROW} values. As in the wet season, nitrogen exported from row crops will impact the natural baseline of aquatic ecosystems, but the method of delivery changes. Instead of daily convective thunderstorms, pumped groundwater used for irrigation could provide the water required to transfer nitrogen to adjacent aquatic ecosystems. Groundwater containing DIN with high $\delta^{15}N$ values may also be responsible for the increased values of β_{ROW}. Reduced model performance during the dry season (Figure 3.6) may be indicative of the large amounts of in-situ nutrient recycling that occurs within the sediments during this period. This could alter the isotopic composition of the original land use isotopic signatures delivered during high nitrogen flux periods.

Conclusions

Figure 3.9 incorporates information from the wet-season trophic pathways depicted in Figure 2.9 with major finding found in this chapter. Nutrient transport during Southwest Florida’s wet season is a dynamic process in which row crop and tree crop

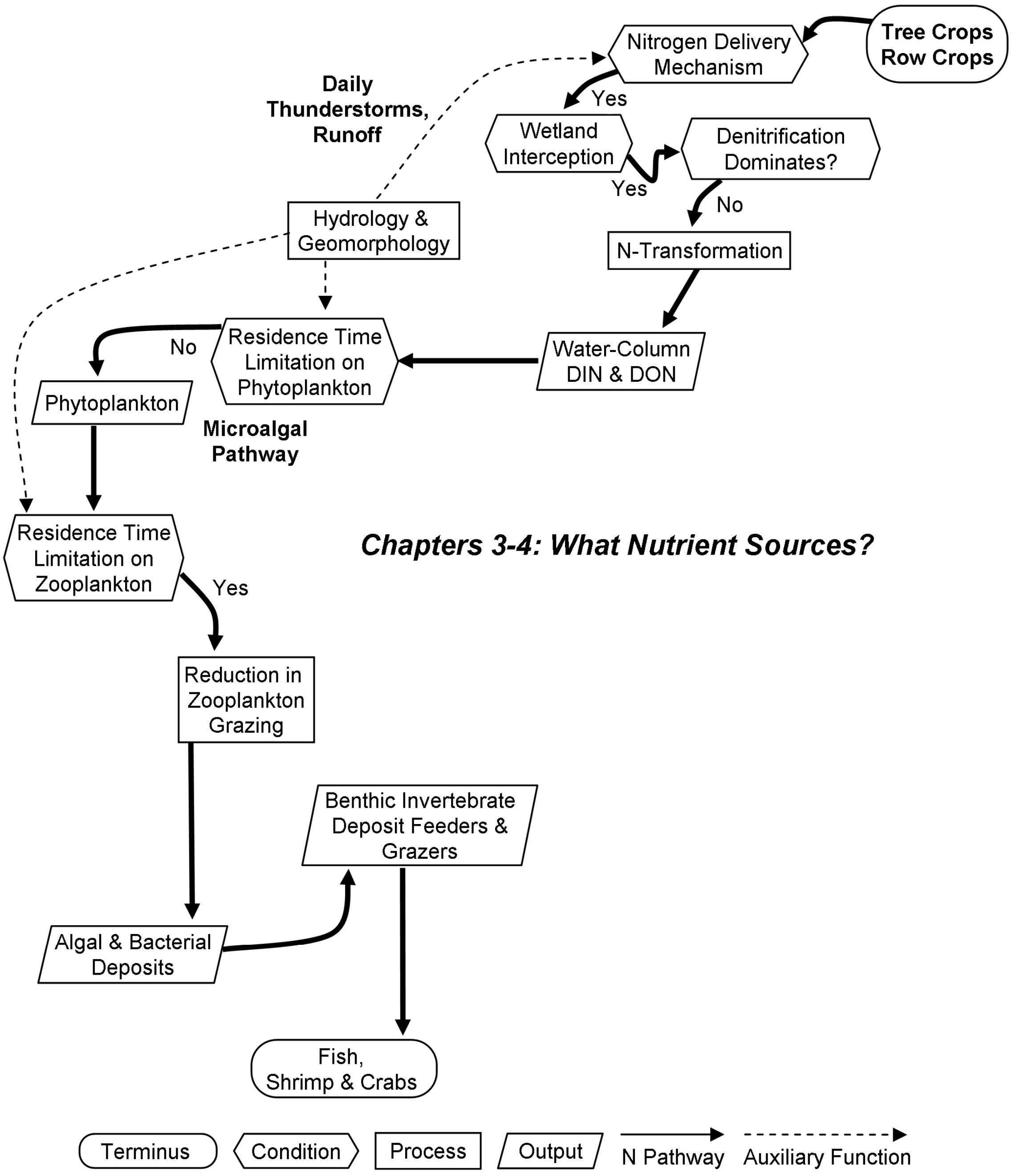

Figure 3.9: Nutrient Sources Supporting Wet Season Pathways in Rural Watersheds. Arrows indicate that row crops and tree crops ultimately support trophic systems during the wet season. Wetlands act as both zones of nitrogen transformation and removal. However, wetland denitrification cannot compensate for the high nutrient loads to these systems during the wet season.

agricultural land use exports heavy nitrogen via runoff. The water required for this transport mechanism is provided by daily thunderstorms and a high water table. As runoff is filtered via wetland soils, some nitrogen is removed from the system on its path to reaching aquatic ecosystems, but there is not nearly enough wetland cover to intercept all incoming nitrogen, and much of the nitrogen intercepted by wetlands is transformed instead of removed. Once the exported nitrogen reaches aquatic ecosystems, it is incorporated into algal biomass. This algal biomass is transferred to resident estuarine fish species via invertebrate trophic intermediates either through a benthic microalgal-based foodweb or through water column phytoplankton that have been deposited to the benthos forming a phytoplankton-deposit based foodweb (based on fish-gut studies from Tampa Bay (2008)). In the watersheds studied here, the ultimate source of nitrogen to these systems is the fertilizer applied to tree crops and row crops during the wet season.

In the dry season row crops are also the major contributors of nitrogen to nearby aquatic ecosystems, but tree crops are much less influential. In this case, pumped agricultural groundwater provides water to transport nutrients to the foodwebs in nearby aquatic ecosystems, but the in-situ nutrient recycling that occurs during the dry season likely confounds the model's ability to predict fish $\delta^{15}N$ based strictly on land use. This processing alters the isotopic composition of the original land use isotopic signatures which were highly predictable during the wet season. Chapter 5 investigates sedimentary nitrogen recycling in depth, where the dry season pathway model depicted in Figure 2.12 will be developed further.

Implications for Management

Nitrogen Loads and Fish Production

To establish effective nitrogen loading polices that ensure maximum fish production, the relationship between nitrogen loads and total fish production must be well-defined. Caddy (1993) described functions that predict fish production from nitrogen

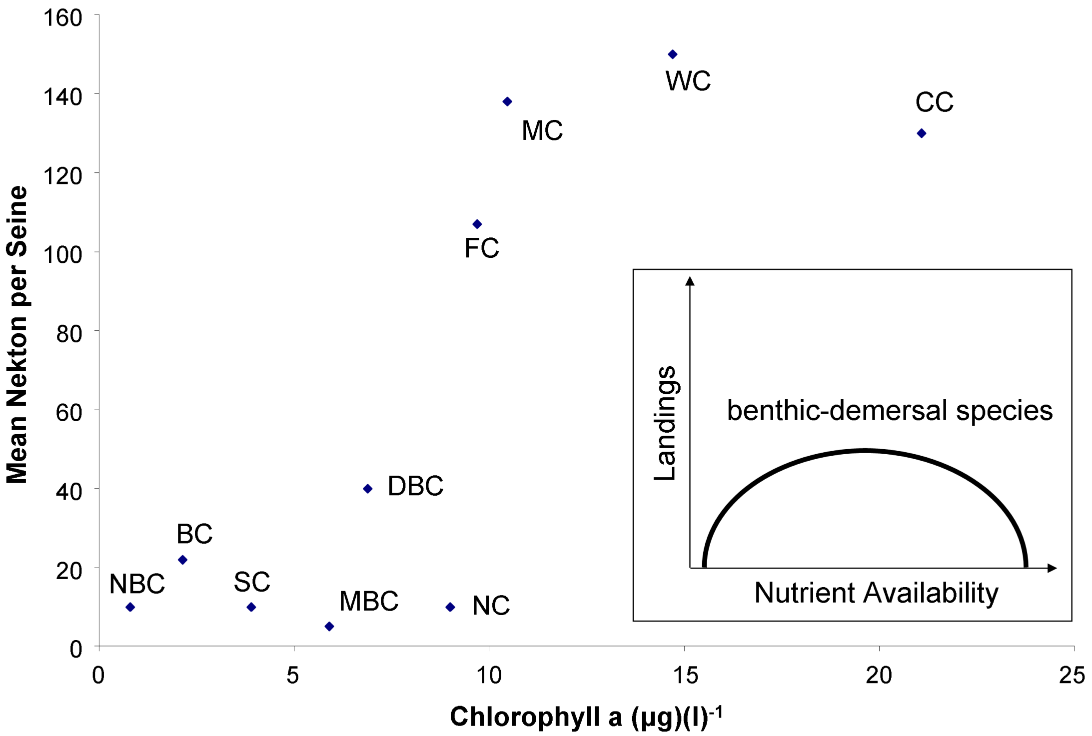

Figure 3.10 Nekton Abundance versus Chlorophyll in Rural Watersheds. Geometric annual mean of nekton per seine plotted against annual mean chlorophyll a concentrations. Data compiled from Greenwood et al. (2009), Tampa Bay (2008), and EPA *Storet* nutrient data. Data was available for DBC = Double Branch Creek, NC = North Archie Creek, SC = South Archie Creek, BC = Bullfrog Creek, NBC = Newman Branch Creek, MBC = Marsh Branch Creek, WC = Wildcat Creek, CC = Curiosity Creek, FC = Frog Creek, and MC = McMullen Creek. Also included is a modification of Caddy's (1993) model that depicts the response of benthic demersal fish landings to nutrient availability.

loads; fish production was positively related to nitrogen loads up to a point where it either leveled-off or significantly dropped due to the adverse effects of nutrient pollution (Figure 3.10). Managers should aim to identify this function in Southwest Florida, so the potential threat of increased nitrogen loads can be accurately assessed in each watershed.

Chlorophyll seems to have a positive relationship with nekton abundance in the systems studied here (Figure 3.10). Assuming chlorophyll concentrations are related to nutrient availability, an increase in nitrogen loads seems unlikely to cause a reduction in fish abundance in the majority of the systems studied. Possible nursery habitats at risk may be those located in Wildcat Creek and Curiosity Creek. Nutrients in these systems originate from the application of fertilizer on row crops and tree crops. Therefore, to reduce the threat to nursery habitats in Curiosity and Wildcat Creek, either the amount of fertilizer applied to these landscapes should be reduced or irrigation methods should be modified to increase the efficiency of fertilizer uptake. The use of drip irrigation instead of groundwater pumping may reduce agricultural water demand and reduce nitrate contamination to nearby waters (Pier and Doerge 1995). It should be noted that in some cases the data points in Figure 3.10 were constructed by averaging three monthly observations of chlorophyll a concentrations, and none of the patterns are statistically significant. This figure should only be considered a clue to the true relationship between nutrient loads and fish abundance.

Connectivity Factors as Measurable Targets

Once the relationship between fish abundance and nitrogen loading is established for this geographic region, the connectivity factors discussed in this chapter may be used as manageable targets. Connectivity factors represent the relative amount of nitrogen exported per unit land use area. Managers can target specific values of connectivity to specific types of land use. For example, in this study row and tree crops were determined to have a connectivity factor of 1. If managers implement a fertilizer reduction policy to these watersheds and want to determine the policy's effectiveness, the study described in this chapter can be repeated and the connectivity factors can be recalculated. If a connectivity factor of less than 1 is calculated for row and tree crops, the policy can be considered a success, since less nitrogen is exported per unit area.

The methodology described in this chapter uses fish from different watersheds to derive connectivity factors. Data collected from single watersheds cannot provide enough information to derive these coefficients. Therefore, to properly use the connectivity factors mentioned here, managers should assign watersheds to groups before calculating nitrogen connectivity for specific land use types. An effective strategy that uses connectivity factors would be to calculate factors for a group of watersheds before the implementation of nutrient policy and compare the pre-implementation values with factors calculated for the same group of watersheds after policy implementation. Managers must attempt to keep watersheds in the same groupings before adopting this approach for long term nitrogen loading management.

Wetlands and Nitrogen Remediation

Wetlands and riparian buffer zones are commonly used in watersheds for nutrient remediation with the goal of avoiding eutrophication in nearby aquatic habitats. The coefficients assigned to wetlands may indicate that riparian buffer zones and wetlands are effective at intercepting and removing nitrogen on its pathways from landscapes to nursery habitats. But, these results are based on $\delta^{15}N$ values calculated by Reinhardt et al. (2006). The fact that the coefficients assigned to wetlands had negative values implies that nitrogen transformation processes must occur as nitrogen flows through wetlands. Excessive denitrification in wetlands would result in the outflow of high $\delta^{15}N$ DIN (Kendall 1998); however, in this case fish that occupied nursery habitats adjacent to landscapes with abundant wetland cover had relatively low $\delta^{15}N$ values. Thus, wetlands processes must produce nitrogen with low $\delta^{15}N$ values. Dissimilatory nitrate reduction to ammonium, nitrification, and remineralization produce low $\delta^{15}N$ nitrogen that could eventually incorporate into fish biomass.

In some instances wetlands may actually act as a source of nitrogen. Fisher and Acreman (2004) reviewed evidence for nitrogen removal in wetlands. In most cases the authors found enough evidence to support the conventional notion that wetlands remove nitrogen, but interestingly, during high flow events the presence of wetlands could actually increase nitrogen loads. This phenomenon could occur during Southwest Florida’s wet season where a high flow of autochthonous and allochthonous particulate nitrogen passes over wetlands. If these particles originate from vascular plants with low $\delta^{15}N$ values, the negative coefficients may actually be associated with a source instead of a sink. DIN remineralized from vascular plants with low $\delta^{15}N$ values could be delivered to nursery habitats and incorporated into fish biomass.

Data presented in this study suggest that there is a need to determine whether wetlands and riparian zones are removing, transforming, or delivering nitrogen. The assumption that wetlands remove nitrogen may not be true in all cases. To accurately define the role of wetlands on nitrogen loads, independent studies should be conducted in the local watersheds studied here. Nitrogen species and isotopic compositions should be measured at the inflow and outflow locations of specific wetlands or riparian zones. The ability of wetlands to remove nitrogen must be established before managers use wetlands as nitrogen remediation tools.

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CHAPTER 4:

URBAN INFLUENCES ON THE ISOTOPIC COMPOSITION OF FISH MUSCLE

Introduction

Global burgeoning coastal population growth and urbanization has led to coastal ecosystem deterioration, prompting policy-makers to set limits on freshwater withdrawals and labile nutrient loads. Tampa Bay's recent dramatic population increase and associated land cover transformation is Florida's paradigm of this phenomenon; the original rural watersheds once dominated by agricultural and natural landscapes are now over 16% urbanized (Greening and Janicki 2008). Juvenile fish nurseries in sub-estuary receiving waters are the potential ecosystems at risk; these habitats receive drainage from Tampa Bay's urbanized watersheds and support a variety of economically important fishes and crustaceans. During their juvenile stages, species such as snook, red drum, striped mullet, tarpon and blue crab populations utilize these habitats as feeding nurseries (Killam et al. 1992; Peebles et al. 2007) often deriving their biomass via hypoxia susceptible benthic trophic pathways (Hollander and Peebles 2004).

There are also economic interests at stake; nutrients, a byproduct of Florida's real estate and urban development industries could interfere with tourism, which is driven by the region's billion-dollar recreational fishing industry. Therefore, before implementing policy in urbanized areas, it is crucial to understand the relationship between anthropogenic nitrogen and fish because excessive nutrient export from urbanized watersheds may destroy the economically important nursery fish habitats as seen in many polluted systems, while completely shutting off nutrients from these areas could result in reduced fish biomass (Caddy 1993). To prevent the collapse of multiple economic sectors, appropriate nutrient policy must be established, but first, the pathway that links nutrients derived from specific urban nutrient sources to fish must be defined.

Water Management in Urban Areas

Another key issue affecting both economic sectors, especially in Southwest Florida, is water management; the major challenge in this region is to balance urban water requirements with aquatic habitat water requirements. The basic management strategy is public water preservation and water recycling; the Southwest Florida Water Management District (SWFWMD) highly limits residential lawn water use during the dry season (March through May), and current policy supports reclaimed water utilization for golf course and public park maintenance. Furthermore, water is not only a fundamental requirement of aquatic ecosystems but is also the means of transporting land-derived nutrients to juvenile fish nursery habitats. Because water management, juvenile habitat quality, and land use are intimately linked, and because this region's climate has well defined wet and dry periods, it is essential to take into account seasonal hydrological connectivity when defining nitrogen pathways between urban land use and fish.

Nitrogen Sources in Urban Areas

The Tampa Bay estuary receives nitrogen from various watershed sources. Pribble et al. (2001) has reported that two-thirds of the nitrogen in Tampa Bay originates from non-point sources, one-fifth originates from atmospheric deposition, and only one-tenth originates from well defined point sources. Potential nitrogen sources in this region may include septic systems, lawn fertilizer use, leaky sewage pipes as well as nitrogen oxides derived from fossil fuels delivered via atmospheric deposition (Valiela et al. 1990, Anisfeld et al. 2007, Gu et al. 2009). Furthermore, local nutrient sinks may exist, which could include retention ponds (Jansson 1994) and removal by intact local sewage systems.

Defining Nutrient Sources with Stable Isotopes

Stable nitrogen isotopes have been widely used to define specific nitrogen sources in a variety of urbanized sub-estuaries. These tracers are appropriate for determining the effects of nutrients on fish biomass because the nitrogen isotopic composition ($\delta^{15}N$) of the actual organisms and the dissolved species present in aquatic ecosystems inherently provide information on coupling between watershed nutrients and receiving waters. Dissolved inorganic nitrogen (DIN) supports primary production downstream but also carries with it information on its land-use origin in the form of stable nitrogen isotopes (Kendall 1998). For example, urbanized areas containing septic systems and treated sewage often export nitrate with very heavy $\delta^{15}N$ values due to large amounts of $^{14}NH_3$ and $^{14}N_2$ released during the microbial processes of ammonia volatilization and denitrification. DIN derived from residential soils often have a wide range of isotopic values. Freshly applied Haber-Bosch fertilizers applied to residential soils have low $\delta^{15}N$ values equal to atmospheric elemental nitrogen. If developed land use does not play a role in delivering nitrogen to ecosystems, then the isotopic composition of nitrate would likely indicate a non-land-use specific source of nitrogen such as the light values of nitrogen oxides (Kendall 1988) present in atmospheric deposition or nitrogen derived from diazotrophic bacteria. Stable isotopes are tools that have the ability to constrain the number of potential nutrient sources in urbanized areas, and therefore many studies utilize the nitrogen isotopes of dissolved nitrates to acquire an instantaneous measure of nitrate’s land use origins (Fogg et al. 1988, Rock and Mayer 2006, Anisfeld et al. 2007). Other studies utilize the nitrogen isotopic composition of sessile primary consumers such as clams (Oczkowski et al. 2008) or mussels (McKinney et al. 2001, 2002) to acquire a more time averaged source signal.

Objectives

This study uses nitrogen stable isotopes of fish muscle in 14 urbanized watersheds in Tampa Bay to identify the nitrogen pathways between urbanized land use and the resident fish species that occupy receiving waters. This study aims to 1) define the point and nonpoint sources that specifically influence the isotopic composition of nitrogen in fish muscle, 2) determine each source's relative contribution to the isotopic composition of nitrogen in fish muscle, and 3) interpret the values of the isotopic signatures that are associated with these nitrogen sources to interpret watershed connectivity pathways. To clarify the effects of climate and hydrology on these linkages, this study will be conducted during Southwest Florida's wet and dry seasons.

Methods

Because manifold nitrogen sources and processes exist in urbanized and residential watersheds, it is difficult to predict the isotopic composition of nitrogen exported from these landscapes to receiving waters. This study applies a three-pronged approach to identify the anthropogenic sources of nitrogen to the juvenile fish nursery habitats present in the receiving waters of urbanized watersheds. Average nitrogen isotopic compositions of fish are empirically related to *nutrient source variables* of 1) specific nutrient point source data, 2) sociological U.S. census data assumed to be indirectly related to specific types of nitrogen processing, and 3) GIS land use data. Since various types of data were used in this study, each dataset was normalized by subtracting the mean and dividing by the standard deviation. This techniques was used to aid comparison between the variables

Study Sites and Data Collection

Fish isotope data reported here originate from Greenwood et al. (2009), which identified the primary producers that support upper-trophic-level biomass in urbanized tidal sub-estuaries (Figure 4.1). Two resident fish species, rainwater killifish (*L. parva*) and silversides (*Menidia spp.*) were collected from most sub-estuaries. In cases where these species were unavailable, alternative estuarine species including tidewater mojarra (*E. harengulus*), mosquitofish (*G. holbrooki*), and gulf killifish (*F. grandis*) were collected. These species were also determined to occupy similar trophic positions in all systems (Greenwood et al. 2009) and are all at least partially supported by benthic trophic pathways (Hollander and Peebles 2004). Sessile resident species were assumed to be appropriate for defining nutrient sources because they incorporate nitrogen across a wider spatial and temporal scale within each sub-estuary compared to the instantaneous concentration and isotope ratio measurements of dissolved species such as nitrate and ammonium. Fish were collected from approximately 1-2 sites along sub-estuary reaches ranging from 1 to 3 km. Samples of fish tissues were obtained using shoreline seines. All samples were limited to tissues (primarily muscle) located posterior to the peritoneal cavity. All solid samples were dried at 50°C, powdered, and frozen until isotopic analysis. Each sampling site was only visited once for sample collection during each season. Dry season sampling occurred in May 2007, while wet season sampling occurred in September 2007.

Stable isotope nitrogen values were measured using a Carlo Erba 2500 Series I elemental analyzer equipped with a zero-nitrogen blank auto-analyzer that was coupled to a Finnigan Mat Delta Plus XL stable-isotope mass spectrometer. All samples were run in duplicate using Spinach Leaves (NIST 1570a) as an internal reference material, and $\delta^{15}N$ values are reported in conventional per mil notation (‰) relative to the air international standard. Instrumental standard deviation remained under 0.3‰.

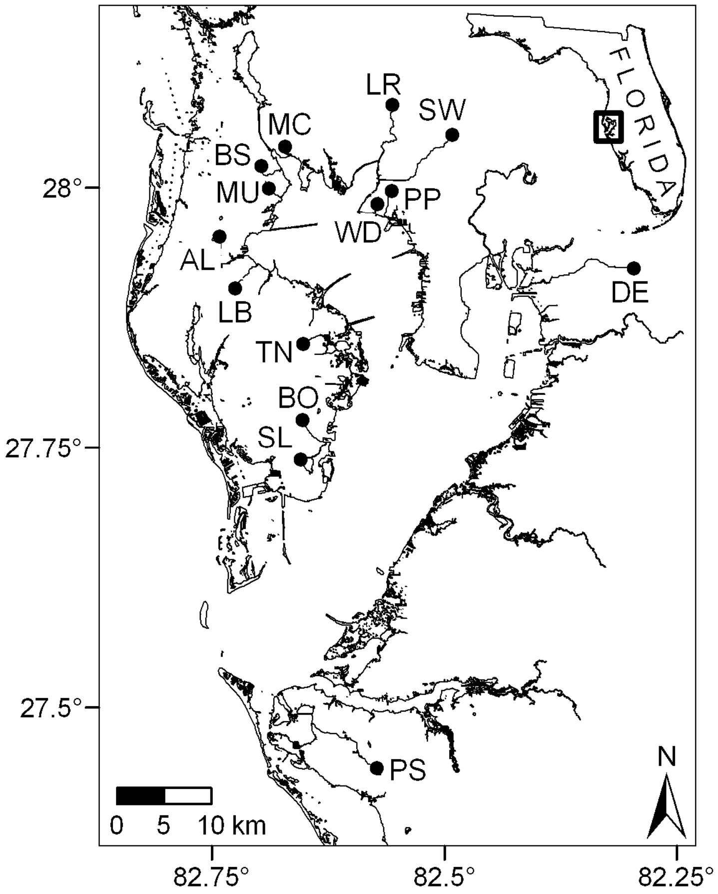

Figure 4.1: Urban Watershed Study Sites. AL = Allen Creek, BS = Bishop Creek, BO = Booker Creek, DE = Delaney Creek, LB = Long Branch Creek, LR = Lower Rocky Creek, MC = Moccasin Creek, MU = Mullet Creek, PS = Wares Creek, PP = Peppermound Creek, SL = Salt Creek, SW = Sweetwater Creek, TN = Tinney Creek, WD = Wood Creek.

Land use geographic information system data and watershed boundary geographic information system data for the Tampa Bay region were downloaded from the official SWFWMD website. According to the metadata files, 2006 land use data were categorized according to the Florida Land Use and Cover Classification System (FLUCCS), and the features were photo-interpreted at 1:12,000 using 2006 1-meter color infrared digital aerial photographs. The watershed boundary data were interpreted and digitized from the USGS 1:24,000 quadrangles, and the boundaries were categorized according to the USGS Hydrologic Unit Classification coding system. Using these two datasets, percent area coverage for each land use type was calculated using the spatial analyst function in ArcGIS version 9.

Point source GIS data included sewage processing plants and septic systems. Sociological U.S. census data included population density and poverty status data. U.S. census data for the year 2000 were downloaded from the SWFWMD website, and 2007 point source data were downloaded from Florida Department of Health website. Specific census data were selected based on their likelihood to be correlated with a type of human behavior influencing nitrogen isotopes and export. These data included population densities and income statistics with the assumption that 1) larger populated areas are likely to export more nitrogen and 2) urban lands with human inhabitants living under different economical conditions will export nitrogen with different isotopic compositions because impoverished humans are unlikely to purchase copious amounts of lawn fertilizer and are more likely to have leaky sewage pipes compared to more affluent residents.

Watershed-specific census, land use, and point source parameters were calculated using the spatial analyst function in ArcGIS version 9. Since each variable was measured in widely different units, all data were standardized prior to empirical modeling by subtracting the mean and dividing by the standard deviation, so regression parameter coefficients could be compared. To explore potential seasonal changes in hydrological connectivity, each of these models was run using wet and dry season fish stable nitrogen isotope data. To calculate each watershed’s poverty status, percent of population impoverished (family of four with an income less than $22,050) was first calculated for each census tract within the watershed. Next, overall watershed poverty status was calculated by averaging tract-specific poverty values weighted by tract areas. Watershed specific population density was calculated by summing the population in all tracts within a watershed and dividing by the total watershed area.

Statistical Approach

Because the potential *nutrient source variables* studied here are not necessarily normally distributed and because data were only available for a relatively low number of watersheds, this study utilized a bootstrapped multiple regression approach to asses the contribution of each nutrient variable to fish $\delta^{15}N$. This technique is generally used in situations where data are expensive, information is sparse, assumptions of normality are unclear, and where further data are difficult to acquire (Henderson 2005).

Each of the following steps was repeated for both wet season and dry season fish $\delta^{15}N$ data to identify the seasonally-specific influences of urbanization on fish tissue.

Data Analysis: Pinpoint the Sources that Influence Fish Stable Nitrogen Isotope Composition

Land use types that were assumed to have a similar isotopic composition were grouped, resulting in 6 land use *nutrient source variables* in addition to the 2 census *nutrient source variables* (population density, percent impoverished) and the 2 point data *nutrient source variables* (septic tanks, sewage processing plants). For each of the 14 watersheds (n=14), the different *nutrient source variables* were considered as possible explanatory variables that explain the response variable of each watershed's average fish $\delta^{15}N$ value. All land use and isotopic data are summarized in Table 4.1.

After all *nutrient source variables* were standardized to facilitate comparison between variables, a bootstrap program using the R programming language was executed to first randomize the observations and then to conduct a forward stepwise multiple regression to select up to 3 *nutrient source variables* that explain fish $\delta^{15}N$ variability. The selected land use groups and permutation based p-values (1000 repetitions) were automatically recorded in a matrix. This process was repeated 10,000 times to ensure data robustness; no single observation could dominate the selection

Table 4.1: Nutrient Source Variables and Isotope Data Summary for Urban Watersheds. Point sources and nonpoint sources include: Sew = number of sewage processing plants; Sep = number of septic tanks. Census data includes: Pop = population density in humans per square km; Pov = percent of inhabitants living below poverty line. Relative area of watershed covered for grouped land use types: AG= agriculture; IND = industrial and transportation; REC = recreational; UP = Upland habitats; URB = urban areas beside recreational; WET = wetlands. Wet and dry season fish isotope data includes mean, sample size (n) and standard error (STE), and species included: L = *L. parva*; M = *Menidia spp.*; E = *E. harengulus*; F = *F. grandis*; G = *M. gulosus*.

Site	Size	Point & Census Data				Percent Watershed Area						Fish Isotope Data	
	km	Sep	Sew	Pop	Pov	AG	IND	REC	UP	URB	WET	Wet $\delta^{15}N$ (n, STE)	Dry $\delta^{15}N$ (n, STE)
AL	21.8	711	0	1444	0.1	3.9	2.2	2.1	0.7	89.3	1.8	10.76 LG (6, 0.29)	10.89 L (4, 0.42)
BS	3.7	7	1	854	0.1	0.1	6.8	1.0	2.0	88.1	2.0	8.91 GLM (9, 0.21)	9.29 LM (6, 0.25)
BO	14.5	5	0	1215	0.4	1.7	15.6	4.8	0.0	77.8	0.1	11.73 M (3, 0.43)	10.85 G (3, 0.20)
DE	47.8	1024	0	457	0.1	13.0	6.3	0.6	7.5	69.2	3.5	11.89 LM (6, 0.10)	12.62 LM (6 0.22)
LB	6.1	9	0	1257	0.2	4.1	7.0	0.2	4.4	83.4	0.9	9.35 LG (5, 0.37)	NA
LR	29.9	314	0	699	0.1	9.3	5.7	2.9	3.7	65.8	12.7	9.96 LM (6, 0.34)	10.67 L (6, 0.22)
MC	11.4	1	0	551	0	5.4	7.7	4.1	8.7	44.5	29.7	9.32 LG (6, 0.25)	9.77 M (2, 0.02)
MU	7.7	30	0	840	0.1	5.4	6.5	1.1	5.6	79.4	2.1	8.6 LG (6, 0.16)	9.35 GL (6, 0.26)
PS	16.2	19	0	1398	0.1	1.6	2.5	7.8	1.5	86.0	0.6	9.42 EM (4, 0.35)	9.24 LM (5, 0.64)
PP	2.9	6	0	1765	0.1	1.5	1.1	0.0	6.0	85.2	6.2	8.37 LG (6, 0.46)	9.59 GL (5, 0.33)
SL	15.0	1	1	1064	0.3	0.4	4.2	9.6	7.1	77.6	1.1	10.38 F (10, 0.16)	NA
SW	24.4	179	0	696	0.2	6.5	27.8	3.4	2.4	56.9	3.0	10.44 LM (5, 0.29)	10.54 L (3, 0.33)
TN	40.0	10	0	481	0.1	2.3	9.3	6.0	3.8	69.5	9.1	10.47 LGM (7, 0.36)	10.62 GM (6, 0.50)
WD	0.9	3	0	709	0.1	0.0	2.6	0.0	3.0	84.3	10.1	9.64 LG (5, 0.13)	10.28 GL (6, 0.38)

process. The Use of 10000 bootstrap samples provided reproducible results with smooth distributions. Individual variables were only further investigated if the 50th percentile of the variable's p-value distribution was less than 0.001, and if the variable was selected in more than 33% of bootstrap regressions for wet season models and more than 50% of the regressions for dry season models (only 12 dry season observations were available) In cases where significant correlation existed among land use, point source, and census variables, the parameters most frequently chosen by the R program were investigated further, and the corresponding correlated variables were discarded.

Data Analysis: Defining Specific Nutrient Source Coefficients

In addition to the predictive value of the coefficients assigned to the *nutrient source variables*, the coefficient signs and magnitudes can provide insight on 1) the types of nitrogen sources that the census variables represent, and 2) the isotopic transformations of nitrogen that occur during transport from source to sink. Therefore, the confidence intervals for coefficient values were calculated by bootstrapping the coefficient parameters using Statistics 101 (ver. 1.4) with a non-parametric bootstrap algorithm modified from Simon (1997), similar to those described by Draper and Smith (1998); 10,000 bootstrap samples were constructed that recorded the distribution of each coefficient's values. Confidence intervals for each of the significant *nutrient source variables* were determined by extracting the 5th and 95th percentile from each of the coefficient's distributions.

Data Analysis: Defining Specific Nutrient Source Contributions to Fish Biomass

The contribution of specific *nutrient source variables* to the nitrogen isotopic composition of fish was determined by constructing bootstrapped squared correlation coefficient (R^2) distributions for each *nutrient source variable* chosen by the R program. In every bootstrap sample, predicted fish isotopes were first calculated using multiple regression by plugging in the single *nutrient source variable* that explained the most fish $\delta^{15}N$ variability. Predicted fish isotopes were then correlated against observed values, and the resulting R^2 parameter was recorded, representing the contribution from the first chosen *nutrient source variable*. The contributions from subsequent *nutrient source variables* were calculated by plugging in the *nutrient source variable* that explained the remaining variability of the previous model's residuals. Total R^2 parameters were then calculated to determine the variability explained by all the variables already entered into the model, and the variability explained by the most recently added *nutrient source variable* was calculated by subtracting preceding total R^2 value from the most recent total R^2 value. This process was repeated for each bootstrap sample. R^2 distributions were then created by repeating this process 10,000 times. This methodology provided ranges of the percent variability explained by each *nutrient source variable*, and it also identified situations in which two *nutrient source variables* were competing to explain the majority of fish $\delta^{15}N$ variability.

Concurrently, the total variability explained by the complete model (adjusted R^2) was determined using a bootstrap methodology in which all selected variables and coefficients were used to calculate predicted fish isotope values for each bootstrap sample. These predicted fish isotope values were regressed against observed values, and the resulting adjusted R^2 values were recorded for each bootstrap sample to create a distribution of total variability explained.

Results

Pinpointing the Sources Influential to Fish $\delta^{15}N$

Selected *nutrient source variables* were not seasonally identical (Figure 4.2).

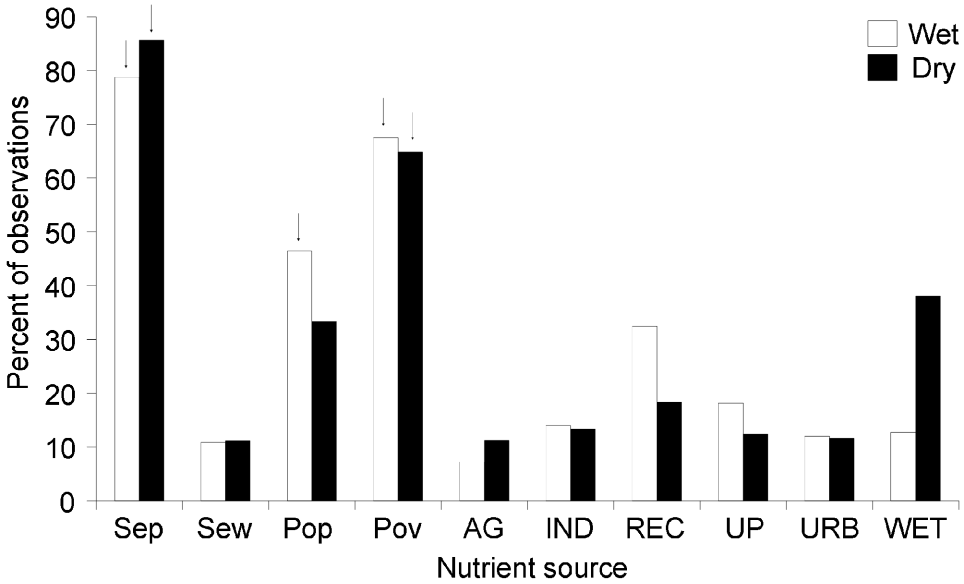

Figure 4.2: Urban Nutrient Source Variables that Explain Fish Stable Nitrogen Isotope Variability. Percent of the 10,000 bootstrap observations (y-axis) in which a *nutrient source variable* was selected (x-axis) is depicted for both wet (white) and dry season (black) data. Arrows indicate whether the selected land use type was significant ($p < 0.001$) using permutation based p-values.

Septic tanks and poverty status explained fish $\delta^{15}N$ variability in both seasons. In contrast, population density only explained fish $\delta^{15}N$ variability during the wet season. It should be noted that wetlands and population density were the third and fourth most-selected variable during the dry season, but neither was selected in more than 50% of the regression models, and, therefore, neither was considered in the further steps of the analysis. Urban land use and population density were correlated as well as septic tanks and agricultural land use, and consequently, the urban land use variable and agricultural land use variable were dropped from the model because septic tanks and population density variables were selected more often in the R program (Figure 4.2).

Defining Specific Nutrient Source Coefficients

The wet season coefficient distributions for the standardized septic tanks, poverty status, and population density *nutrient source variables* are depicted in Figure 4.3.

Poverty status and septic tank variables had similar positive coefficient distributions which may imply associations with water derived from human waste (Kendall 1998). Population density, on the other hand, had a coefficient distribution centered on a negative value which could imply export of low $\delta^{15}N$ nitrogen. Nitrogen oxide fumes derived from automobile fumes would be expected to have a relatively low $\delta^{15}N$ value (Kendall 1998). Daily rainstorms would wash out the nitrogen oxides from the atmosphere onto land and eventually into nursery habitats.

Figure 4.3 also includes the results of a non-bootstrapped OLS multiple regression that used the *nutrient source variables* to explain variability in fish $\delta^{15}N$. The adjusted R^2 value was high 0.85, and highly significant ($p < 0.01$). The model selected septic tanks as the variable explaining slightly more fish $\delta^{15}N$ variability than poverty status. Population density only explained approximately 10% of fish $\delta^{15}N$. The coefficients assigned to all variables were significant ($p < 0.05$), but it should be noted

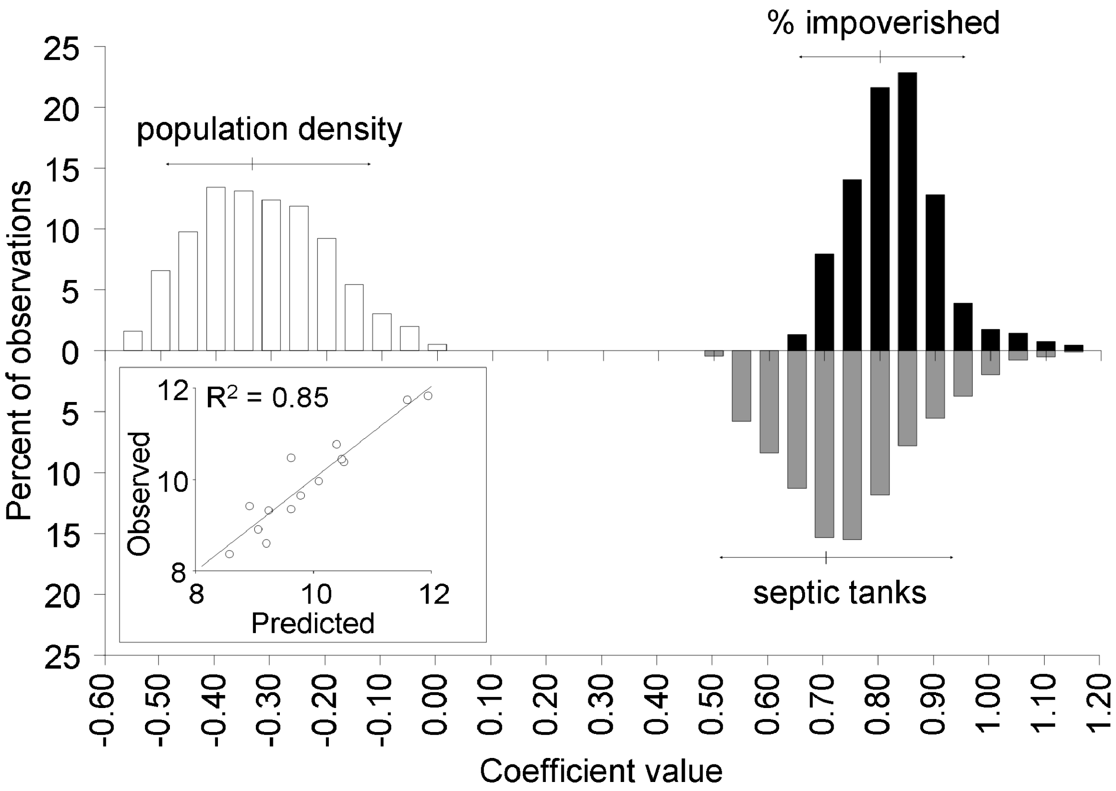

Figure 4.3: Wet Season Coefficient Distribution for Urbanized Watersheds. Bootstrap distributions of coefficient values (x-axis) were assigned to *nutrient source variables* to explain wet season fish δ^{15}N variability. Y-axis: percent of the 10,000 bootstrap observations where a specific coefficient value was selected. Ranges indicate the 5th, 50th, and 95th percentile of each distribution. Also included are the results of the non-bootstrapped OLS multiple regression with adjusted R^2 value.

that these OLS coefficient p values are based on the assumption that the coefficients exhibit parametric distributions. The distributions in Figure 4.3 provide a more appropriate representation of the error associated with the coefficients.

The dry season coefficient distributions for septic tank and poverty status *nutrient source variables* are depicted in Figure 4.4. Both variables had positive coefficient distributions; however, the range of the septic tank distribution was significantly higher in magnitude compared to the poverty status variable, which may imply seasonal changes in the nutrient sources or hydrological connectivity associated with nitrogen related to poverty status. It should be noted that although no dry season data were available for

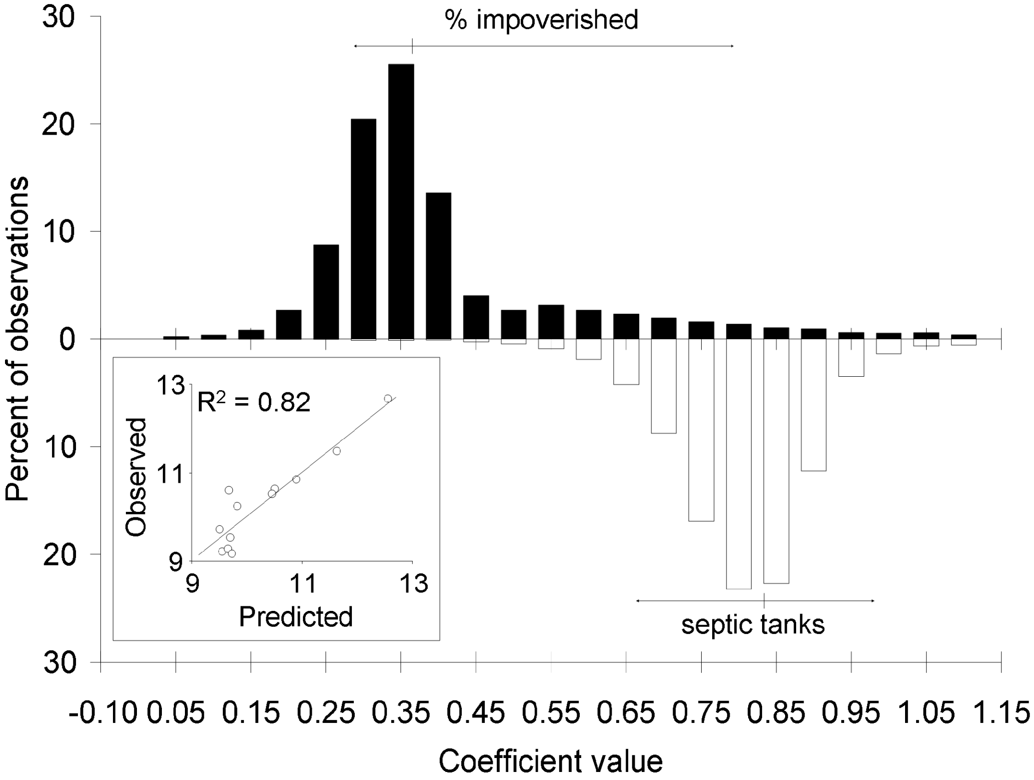

Figure 4.4: Dry Season Coefficient Distribution for Urbanized Watersheds. Bootstrap distributions of coefficient values (x-axis) were assigned to *nutrient source variables* to explain dry season fish $\delta^{15}N$ variability. Y-axis: percent of the 10,000 bootstrap observations where a specific coefficient value was selected. Ranges indicate the 5th, 50th, and 95th percentile of each distribution. Also included are the results of the non-bootstrapped OLS multiple regression with adjusted R^2 value.

Long Branch Creek and Salt Creek, when wet season isotope data were substituted for the missing dry season data, similar results were obtained.

Figure 4.4 also includes the results of a non-bootstrapped OLS multiple regression that used the *nutrient source variables* to explain variability in fish $\delta^{15}N$. The adjusted R^2 value was slightly lower (0.82, $p < 0.01$) compared to results obtained using wet season data. The model selected septic tanks as explaining the majority of fish $\delta^{15}N$ variability. The coefficients assigned to all variables were significant ($p < 0.01$), but it should be noted that these OLS coefficient p values are based on the assumption that the coefficients exhibit parametric distributions. The distributions in Figure 4.4 provide a more appropriate representation of the error associated with the coefficients.

Defining Specific Land Use Contributions to Fish Biomass

The wet season R^2 distributions for poverty status, septic tanks, population density, and overall adjusted R^2 are depicted in Figure 4.5. The septic tank variable explained 40% (50th percentile) of fish $\delta^{15}N$ variability, the poverty status variable explained 37% (50th percentile) of fish $\delta^{15}N$ variability, and population density variable explained 11% of fish $\delta^{15}N$ variability. The bimodal distributions of poverty status and septic tanks imply that these variables were competing to explain the majority of fish $\delta^{15}N$ variability. Overall adjusted R^2 values were high; three variables explained 88% (50th percentile) of fish $\delta^{15}N$ variability.

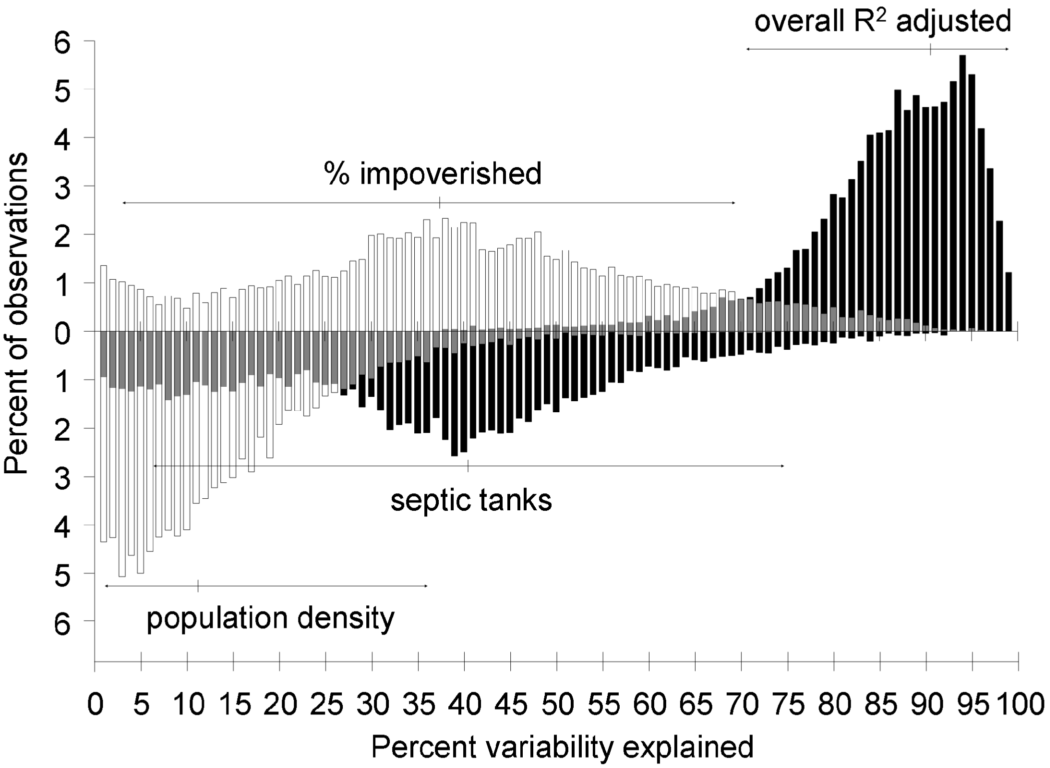

Figure 4.5: R-Squared Distributions of *Nutrient Source Variables* that Explain Wet Season Fish Stable Nitrogen Isotope Variability. Bootstrap distributions of the R^2 values of individual *nutrient source variables* are presented as well as total adjusted R^2 describing the amount of wet season fish $\delta^{15}N$ variability explained (x-axis) using ordinary least square multiple regression. Percent of observations on the y-axis is the percent of the 10,000 bootstrap observations (y-axis) in which a specific R^2 value was calculated. Gray areas represent overlapping distributions. Ranges indicate the 5th, 50th, and 95th percentile of each distribution.

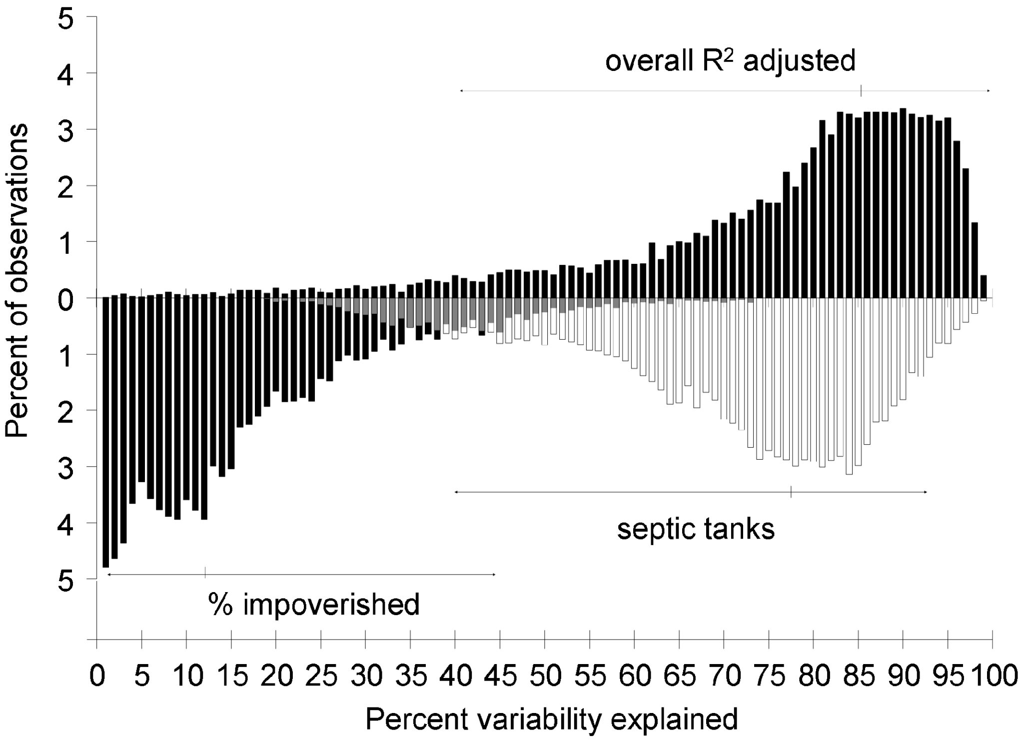

Figure 4.6: R-Squared Distributions of *Nutrient Source Variables* that Explain Dry Season Fish Stable Nitrogen Isotope Variability. Bootstrap distributions of the R^2 values of individual *nutrient source variables* are presented as well as total adjusted R^2 describing the amount of dry season fish $\delta^{15}N$ variability explained (x-axis) using ordinary least square multiple regression. Percent of observations on the y-axis is the percent of the 10,000 bootstrap observations (y-axis) in which a specific R^2 value was calculated. Gray areas represent overlapping distributions. Ranges indicate the 5th, 50th, and 95th percentile of each distribution.

The dry season R^2 distributions for poverty status, septic tanks, and overall adjusted R^2 are depicted in Figure 4.6. The septic tank variable explained 72% (50th percentile) of fish $\delta^{15}N$ variability, while poverty status variable explained 12% (50th percentile) of fish $\delta^{15}N$ variability. Adjusted R^2 values were slightly lower compared to the wet season adjusted R^2 values; 83% (50th percentile) of fish $\delta^{15}N$ variability was explained; however, only two *nutrient source variables* were used in this model. Septic tanks explained the majority of the variability during dry season, whereas during the wet season, septic tanks and poverty status were competing to explain the majority of the variability.

Discussion

Biogeochemistry Represented by Nutrient Source Variables

The major point source, nonpoint source, and sociological impacts that controlled fish tissue nitrogen isotope variability differed seasonally. During the wet season, fish nitrogen isotopes were influenced by the nitrogen derived from septic tanks, nitrogen associated with poverty status, and nitrogen associated with population density. In contrast, during the dry season, the influences on fish nitrogen isotopes were septic tanks and inhabitant poverty level (Figure 4.2). Unlike the influential *nutrient source variables*, the overall adjusted R^2 values were high and similar during both seasons; both R^2 values had magnitudes greater than 0.80 with a difference of less than 0.05, with slightly higher values using wet season data (Figures 4.5 and 4.6).

Nitrogen processes associated with *nutrient source variables* can be hypothesized from the signs and magnitudes associated with the coefficients. The biogeochemistry driving the positive sign and relatively high magnitude of the septic tank coefficient (Figures 4.3 and 4.4) is straightforward. Septic tank nitrogen with high $\delta^{15}N$ values (Kendall 1998) is delivered to nursery habitats by groundwater. The septic tank $\delta^{15}N$ signature propagates through the food web and causes the $\delta^{15}N$ values of fish to increase.

The positive coefficient assigned to the poverty status variable reveals the differences in biogeochemistry that occurs on landscapes occupied by humans with different financial situations (Figures 4.3 and 4.4). Affluent residents can afford to irrigate lawns regularly and apply commercial fertilizer. Commercial fertilizer is derived from atmospheric nitrogen with a $\delta^{15}N$ value of approximately 0‰ (Kendall 1998). Irrigation water delivers this light nitrogen to nearby nursery habitats where it is incorporated into the foodweb. Conversely, the observed high $\delta^{15}N$ nitrogen associated with impoverished areas is likely due to a more passive process in terms of anthropogenic activity; in census tracts with impoverished inhabitants, lawn care is not a priority. Furthermore, in areas of destitution, perfunctory home maintenance could result in leaky sewage pipes. Both of these processes would have a positive relationship with the $\delta^{15}N$ of DIN supplied to food webs. With less fertilizer application, less nitrogen with light isotopic signatures would be exported to nearby nursery habitats. Furthermore, leaky sewage systems would likely export nitrogen with high $\delta^{15}N$ values associated with human waste (Kendall 1998). As a result, landscapes with affluent residents likely export nitrogen with low $\delta^{15}N$ values while landscapes with impoverished residents likely export nitrogen with high $\delta^{15}N$ values. These two end-members cause the positive coefficient associated with poverty status.

The similarity between the septic tank and the poverty status coefficient values during the wet season (Figure 4.3) implies that the nitrogen exported from highly impoverished areas and from septic tanks have similar $\delta^{15}N$ values and similar hydrological connectivity. The $\delta^{15}N$ values of nitrogen in septic tanks and leaky sewage systems should be identical, since both are derived from animal waste (Kendall 1998). Delivery processes during the wet season should also be similar; the increased water table height enhances groundwater's delivery of septic tank water, and consequently, septic tanks and poverty status *nutrient source variables* have virtually identical behaviors during this season.

The significant amount of fish $\delta^{15}N$ variability explained by the septic tank variable in Figures 4.5 and 4.6 indicates that septic tank nitrogen is delivered to nursery habitats during both seasons. During the wet season, the high water table and fast groundwater flow delivers septic tank nitrogen to tidal nursery habitats. During the dry season, the low water table and slow groundwater flows are still adequate to deliver septic tank nitrogen to receiving waters.

The low amount of fish $\delta^{15}N$ variability explained by poverty status in Figure 4.6 indicates that nitrogen derived from leaky sewage systems and lawn fertilizer does not reach tidal nurseries during the dry season. A low water table and less rainfall would abate the delivery processes, which dominated during the wet season. Furthermore, the reduced coefficient values assigned to the poverty status *nutrient source variable* (Figure 4.4) may indicate that a smaller isotopic gradient exists between the nitrogen delivered from affluent and impoverished communities during the dry season. It is possible that the dry season's small poverty status coefficient became more influenced by lawn fertilizer and irrigation compared to leaky sewage pipes. Lawn irrigation would still provide a delivery mechanism for the light nitrogen applied to lawns in affluent neighborhoods, whereas a similar delivery mechanism for the heavy nitrogen associated leaky sewage pipes is absent, and consequently, a moderate isotopic gradient is apparent during the dry season.

The negative coefficients assigned to the population density variable (Figure 4.3) may imply that nitrogen derived from nitrogen oxide fumes is incorporated into fish tissue during the wet season. In highly populated areas, increased traffic creates nitrogen oxide fumes with $\delta^{15}N$ values ranging from -5 to +0‰ (Kendal 1998). Wet season atmospheric deposition delivers low $\delta^{15}N$ nitrogen to tidal nurseries where the low $\delta^{15}N$ values are incorporated into the foodweb. The failure of the model to select the population density variable during the dry season (Figure 4.2) may be indicative of the seasonal differences in atmospheric deposition as a nitrogen source; Poor et al. (2001) reported that atmospheric nitrogen deposition is considerably higher during the wet season compared to the dry season in Tampa Bay. Caution should be used when interpreting these results, since population density was the fourth most influential variable during the dry season according to Figure 4.2.

As human populations grow on land, watersheds export more nitrogen to aquatic habitats. However, the resulting increases in nitrogen loads and concentrations do not necessarily correspond to changes in the isotopic composition of exported nitrogen. A change in the stable isotopic composition of exported nitrogen would only occur if the types of anthropogenic nitrogen processing change as human populations escalate. In this study, the correlation between watershed population and fish stable nitrogen isotopic composition was minimally significant.

The increased magnitudes and ranges of dry season fish $\delta^{15}N$ values compared to wet season fish $\delta^{15}N$ values may be explained by hydrology and seasonal changes in biogeochemistry. Atmospheric nitrogen oxides with low $\delta^{15}N$ values would become a common nitrogen source for all tidal nurseries during the wet season. Daily afternoon thundershowers provide water to deliver this nitrogen to tidal nurseries and reduce the $\delta^{15}N$ values of fish. In contrast, during the dry season, atmospheric deposition is reduced and the water table is depressed; less common low $\delta^{15}N$ sources would create more heterogeneous signals across different watersheds.

The reduced overall adjusted R^2 values in Figure 4.6 indicates that some fish $\delta^{15}N$ variability remains unexplained. This may be due to large amounts of in-situ nutrient recycling within the sediment that occurs during this period, and denitrification could be partially responsible for the increased $\delta^{15}N$ values during this period.

Interestingly, sewage treatment plant point sources were not influential. This is likely due to the fact that wastewater is highly treated and therefore did not influence fish $\delta^{15}N$. During the wet season, nonpoint sources explained 75% of fish $\delta^{15}N$ variability (septic tanks included), while atmospheric deposition (population density) explained 12% of fish $\delta^{15}N$ variability. This agrees fairly well with values calculated by Pribble et al. (2001) who reported 2/3 of nitrogen in Tampa bay was derived from nonpoint sources and 1/5 was derived from atmospheric deposition.

Conclusions

The pathways identified in this chapter are depicted in (Figure 4.7 and 4.8).

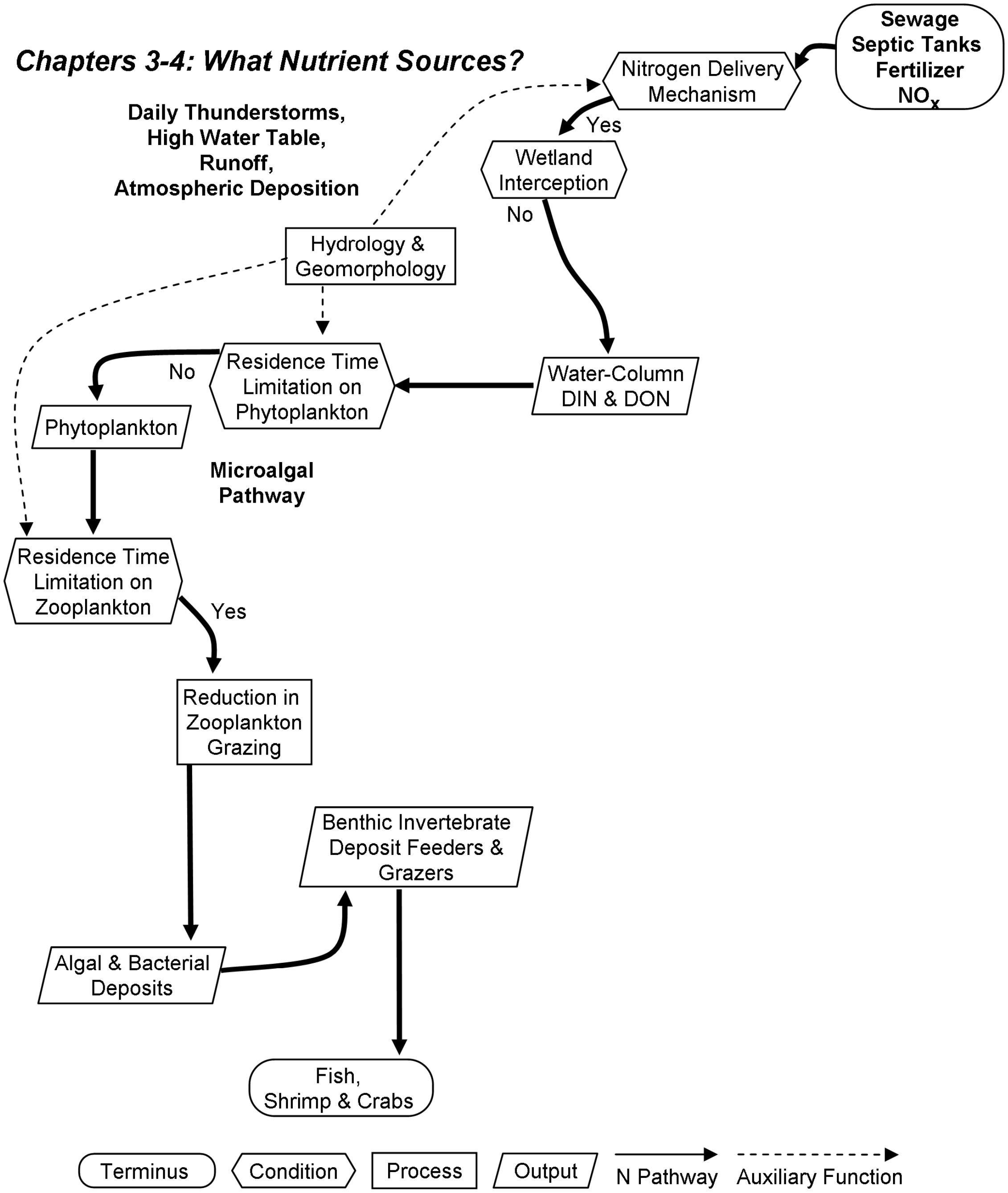

Figure 4.7: Nutrient Sources that Support Wet Season Pathways in Urbanized Watersheds. Arrows indicate that nitrogen derived from leaky sewage pipes, septic tanks, lawn fertilizer, and atmospheric NO$_x$ ultimately support trophic systems during the wet season. Daily thunderstorms, a high water tables are the major nitrogen delivery mechanism in this season.

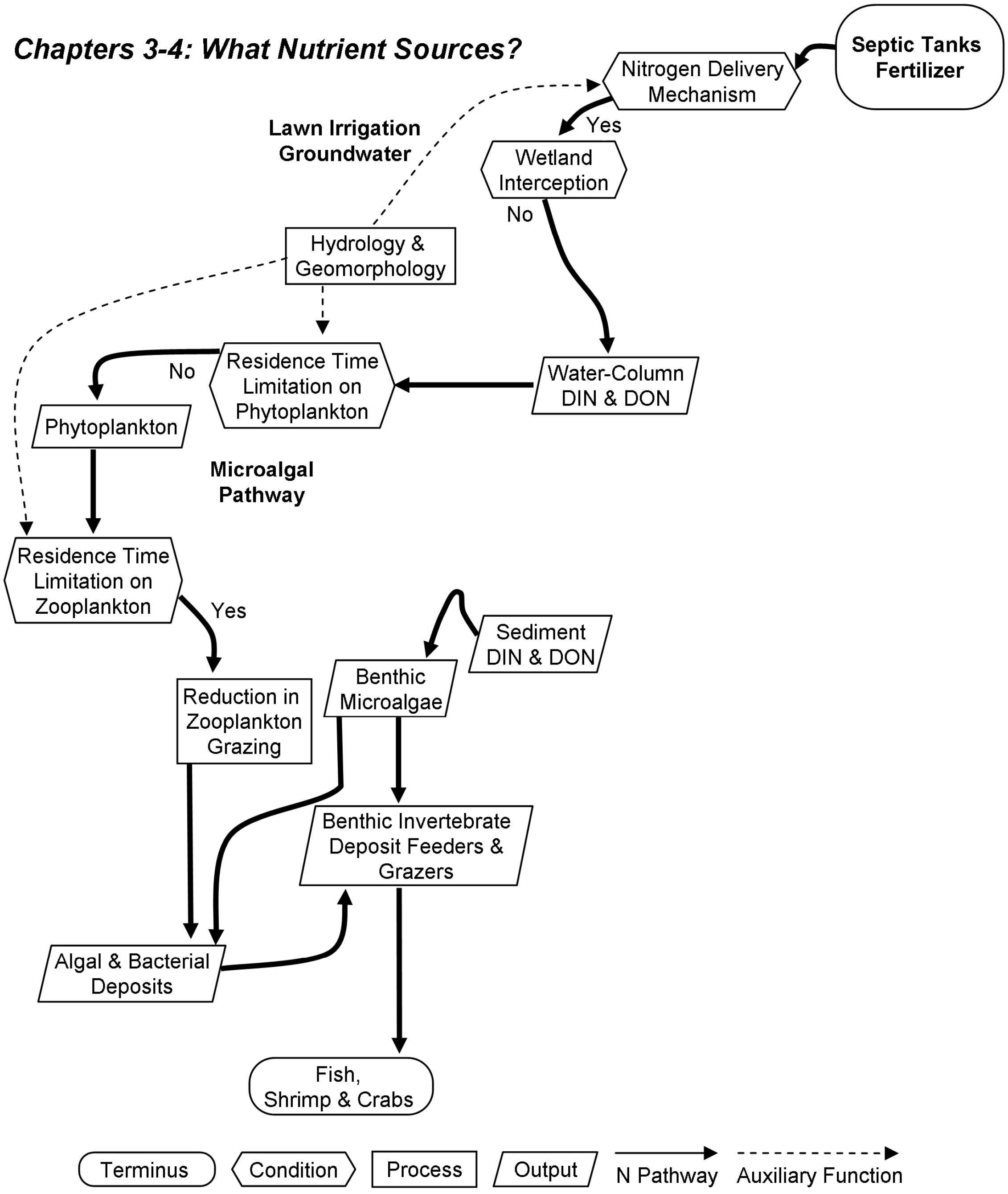

Figure 4.8: Nutrient Sources that Support Dry Season Pathways in Urbanized Watersheds. Thick arrows indicate that nitrogen derived from septic and lawn fertilizer ultimately supports fish because each has a seasonally independent mode of nitrogen delivery, lawn irritation and septic tank water.

The pathways found in this chapter are incorporated into the trophic pathway identified in Chapter 2 using additional information from Tampa Bay (2008).

During the wet season, septic tanks, fertilizer application, leaky sewage pipes, and NO_x in the atmosphere are the major sources of nitrogen to fish nursery habitats. The delivery of nitrogen derived from leaky sewage pipes, septic tanks, and fertilizer is enhanced by a high water table and daily thunderstorms. These thunderstorms also deposit NO_x from the atmosphere to these systems. This nitrogen supports phytoplankton which will deposit under the conditions where water residence time is high and ultimately supports a PBBC during the wet season.

During the dry season, nitrogen sources with rainfall-independent hydrological delivery mechanisms dominate. These include fertilizer delivered by lawn irrigation water and septic tank nitrogen delivered by slow moving groundwater. Poor model performance during the dry season may indicate that sedimentary nitrogen recycling processes play an important role providing nitrogen during the dry season. Data collected by Tampa Bay (2008) indicate that these sedimentary processes would support a BMAC.

Implications for Management

Application to TMDL Policy and the Agricultural Model

The term “nutrient pollution” has been applied to non-point source nutrients derived from anthropogenic land use that result in hypoxia, seagrass declines (Greening and Janicki 2006), and fish kills (Clarholm et al. 1988). Oftentimes, nutrient pollution is associated with estuarine systems that have been over-utilized and under-protected resulting in adverse economic consequences including the degradation of habitats essential for fish production (Braun, 2007); Chatterjee (2009) has concluded that eutrophication of freshwater regions alone accounts for over a 4 billion dollar annual loss to the United States. As a result of the high potential economic costs of eutrophication, the United States Environmental Protection Agency (EPA) has implemented strict laws to prevent the adverse effects of eutrophication by requiring states to establish a total maximum daily load (TMDL) of pollutants in waterbodies considered to be impaired by pollution.

Section 304(a)(1) of the Clean Water Act requires the development of water-quality criteria that accurately reflect the latest scientific knowledge. Though, TMDL calculations may be consistently misrepresented downstream due to intervening processes such as selective burial of different forms of nitrogen in sediments and by various microbial processes that convert and release nitrogen and perhaps most importantly by fundamental differences in the hydrologic connectivity between different types of land cover and downstream waters. The nitrogen cascade is especially complex in urbanized areas with a plethora of potential nutrient sources.

The method used in this study helps to unravel the intricate nitrogen cascades that occur between urban watershed land use and the organisms that occupy receiving waters. Eighty six percent of fish δ^{15}N variability was explained using publicly available GIS point data and U.S. census data. Nitrogen sources and delivery mechanisms correlated with demographic and point source data were considered. This study demonstrated that this already-available data coupled with inexpensive bulk isotopic analyses of fish tissue can be used to form the reasonable conclusion that septic tanks, leaky sewage systems, and atmospheric deposition are the major watershed nitrogen sources to nearby aquatic fish nursery ecosystems. It is highly unlikely that downstream TMDL nutrient measurements could identify these major processes and sources that occur upstream and within watershed.

Nitrogen Loads and Fish Production

This study related the isotopic composition of fish muscle to nitrogen derived from human activity. Although nitrogen derived from urban areas have been widely perceived by ecosystem managers and ecologists as damaging to ecosystems, they are essential for supporting fish production. Cushing (1975) coined the term “agricultural model” that relates fish biomass to the amount of nutrients supporting the food chain. This theory has been supported by positive relationships between primary production and fish landings observed by Nixon (1982). These results are supported by the models proposed by Caddy (1993) which relate responses of fish yield to nutrient loads in a variety of semi-enclosed seas. Caddy (1993) proposed shapes of the fish catch to biomass curves would be dependent on the benthic or pelagic nature of trophic pathways leading to a particular fish catch. Especially notable were the benthic foodwebs, described as extremely susceptible to hypoxia under high nutrient loads. The current study clearly demonstrated nitrogen derived from human activity land use is incorporated into fish tissue.

The clear link between urban land use and Florida’s billion dollar recreational fishing industry beckons the need for an understanding of the relationship between anthropogenic nitrogen and fish biomass in this region because based on Caddy’s (1993) models, excessive nutrient export from urbanized regions may destroy the economically important benthic-dependent nursery fish habitats as seen in many polluted systems, while completely shutting off anthropogenic activity or completely removing water supply could result in no fish biomass. Southwest Florida managers should aim to identify the functions described by Caddy (1993) in the aquatic habitats that receive runoff from urban watersheds. In this way, the potential threat of increased nitrogen loads to nursery habitats can be correctly defined.

Sparse chlorophyll data compiled from the EPA’s *Storet* website and fish abundance data taken from Greenwood et al. (2009) were used to construct a nekton-abundance versus chlorophyll plot (Figure 4.9).

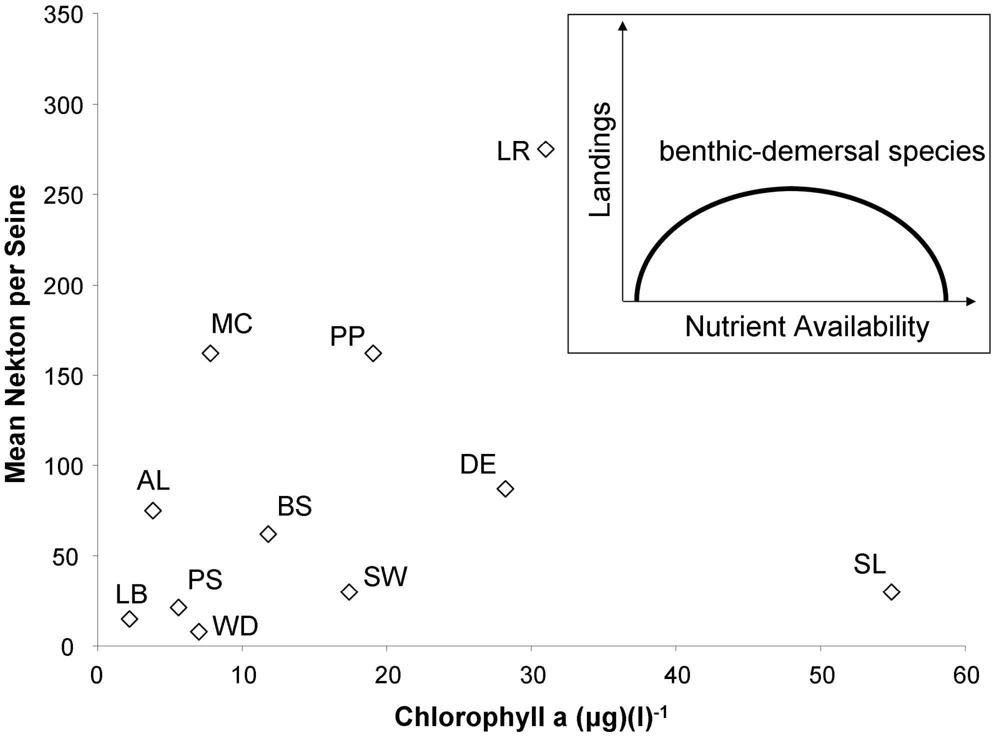

Figure 4.9: Nekton Abundance versus Chlorophyll in Urban Watersheds. Geometric annual mean of nekton per seine plotted against annual mean chlorophyll a concentrations. Data compiled from Greenwood et al. (2009) and EPA *Storet* nutrient data. Data was available for AL = Allen Creek, BS = Bishop Creek, DE = Delaney Creek, LB = Long Branch Creek, LR = Lower Rocky Creek, MC = Moccasin Creek, PS = Wares Creek, PP = Peppermound Creek, SL = Salt Creek, SW = Sweetwater Creek, WD = Wood Creek. Also included is a modification of Caddy's (2000) figure 5 that depicts the response of benthic demersal fish landings to nutrient availability.

In the urban watersheds studied here, Chlorophyll seems to have a positive relationship with nekton abundance up to a concentration of 30 (µg)(l)$^{-1}$. Salt Creek had the highest mean annual chlorophyll concentration and had one of the lowest mean nekton abundance values (Figure 4.9). This creek also happens to receive nitrogen from landscapes occupied by impoverished humans (Table 4.1).

Assuming chlorophyll concentrations are related to nutrient availability, an increase in nitrogen loads seems likely to cause a reduction in fish abundance in many of the systems studied. Possible nursery habitats at risk may be those located in Delaney Creek, Peppermount Creek, and Lower Rocky Creek. Salt Creek already seems to be on the far right side of the Caddy (1993) curve. Ecosystem managers should prioritize reducing nitrogen loads in this system.

Implementing Nitrogen Policy in Urbanized Watersheds

One particularly concerning finding of this study is that impoverished human beings passively export nitrogen to nursery habitats, and these nursery habitats may be in poor condition (Figure 4.9). Fertilizer reduction laws are unlikely to be effective at reducing nitrogen loads in watersheds occupied by impoverished humans. Residents living below the poverty line do not export nitrogen derived from fertilizer, nor do they provide irrigation water to deliver nitrogen to nursery habitats. Instead, nitrogen is passively exported from these landscapes; the flow of groundwater delivers nitrogen derived from broken sewage pipes to nursery habitats. Fertilizer reduction laws in these watersheds would be ineffective. The major challenge to managers is to construct a nutrient policy that is effective in poor neighborhoods where nitrogen sources are passively delivered to nursery habitats. Once policies are established, this study can be repeated with the aim of having less fish $\delta^{15}N$ variability explained by poverty status.

It should be noted that in some cases the data points in Figure 4.9 were constructed by averaging three observations of chlorophyll a concentrations, and none of the patterns are statistically significant. This figure should only be considered a clue to the true relationship between nutrient loads and fish abundance.

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CHAPTER 5:

NITROGEN RECYCLING IN THE SEDIMENTS

Introduction

As juveniles, large proportions of Florida’s snook, red drum, striped mullet, tarpon, yellowfin menhaden, gulf menhaden, bay anchovy and blue crab populations congregate within habitats near the landward estuarine margin (Killam et al. 1992, Peebles et al. 2007). For most of these estuary-dependent species, peak young-of-the-year recruitment occurs during the spring and early summer (Flannery et al. 2002), a time period that includes peninsular Florida’s annual dry season (March to June, Schmidt et al. 2001), or in the case of the two menhaden species, during late winter, which can also be a dry time of year. Because the productivity of these nursery habitats is predominantly nitrogen limited, understanding the sources of bioavailable nitrogen during low-inflow and high-recruitment periods is central to understanding the function of important nursery habitats.

Estuarine nursery habitats are influenced by nitrogen-producing land uses such as urban development, agriculture, and livestock production. However, nitrogen connectivity between the watershed and estuary becomes strongly reduced during dry, high-recruitment periods. In Tampa Bay, water-column chlorophyll levels are typically at their annual minimum during late winter and spring dry periods when water clarity is at its maximum (Hillsborough County Environmental Protection Commission 2001). Once delivered to estuarine nursery habitats, bioavailable nitrogen may ultimately support fish production by assimilation and incorporation into food webs, or it may interfere with fish production by triggering algal blooms and associated hypoxia. Furthermore, different biogeochemical processes may remove or transform different forms of bioavailable nitrogen. Recently, it has been suggested that transformative processes such as dissimilatory nitrate reduction to ammonium (DNRA) have been underestimated, and removal processes such as denitrification have been overestimated (Burgin and Hamilton 2007, Gardner and McCarthy 2009). Whether the ecosystem is sensitive to bioavailable nitrogen as an inhibitor or promoter of fish production, the dominant sources, removal processes, and transformative processes should be identified.

Benthic Microalgae Control of Sedimentary Nitrogen Flux

Benthic microalgae (BMA) often play a major role in trophic dynamics and nitrogen processing in aquatic ecosystems. Hollander and Peebles (2004) found BMA supported the majority of fish biomass in Southwest Florida’s riverine estuaries during the dry season. BMA photosynthesize at the sediment-water interface and use recycled nitrogen produced in the sediments (Kingston 2002). BMA may impact the nitrogen cycle by moderating ammonium flux (Tobias et al. 2003) and by influencing both nitrification (Risgaard-Petersen et al. 2004) and denitrification (Sundback and Miles 2000). The biological and chemical relationships among sediments, BMA, and fish have been researched intensively, whereas the autotrophic sources of nitrogen that undergo biogeochemical processes and food web transfer have received much less attention.

Independence of Carbon and Nitrogen Source in Sedimentary Organic Matter

Most research on sedimentary organic matter (SOM) has focused on carbon. These studies commonly rely on the consistent behaviors of carbon isotopes to distinguish between terrestrial and algal influences on SOM. Distinct oceanic and terrestrial end-members exist, and as a result, these studies (Ogrinc et al. 2005; Hu et al. 2006) often conclude that organic sediments in freshwater regions of estuaries are predominantly derived from terrigenous sources. However, the origins of carbon and nitrogen in SOM may be independent. For example, sediments with upstream plant detritus are often colonized by bacteria that synthesize new complex biomolecules, immobilizing inorganic nitrogen from the surrounding environment in the process. In such cases, the major contribution of organic carbon in the sediments would be upstream plants, whereas the source of organic nitrogen would be the in-situ nitrogen from the surrounding environment. Understanding the source of SOM nitrogen is essential because bulk organic matter can be buried, remineralized, and then either incorporated into microalgal-based foodwebs or utilized by hypoxia-inducing algal blooms.

General Trends in Nitrogen Isotope Fractionation during Organic Decomposition

The complex behavior of bulk stable nitrogen isotopes during remineralization has been studied in a variety of habitats. Investigations of soil systems (Turner et al. 1983) described a discrimination against ^{15}N during decomposition of litter and soil humus. This isotopic enrichment is thought to be the cause of the increased ^{15}N with soil depth. Similarly, in the oceanic water column, the ^{15}N enrichment of particulate matter with depth has also been attributed to isotopic fractionation during remineralization of organic matter (Saino and Hattori 1980; Altabet and McCarthy 1986). Another example was provided by Fogel et al. (1989), who reported that salt marsh grass (*Spartina alterniflora)* became significantly enriched in ^{15}N after just a few months of microbial degradation. Each of these relatively homogenous organic materials became isotopically enriched with time. However, the microbial decomposition or heterogeneous organic matter may exhibit non-uniform fractionation behaviors.

Selective Remineralization of Labile Organic Matter

Although the general trend of microbial enrichment of ^{15}N holds true in many cases, a number of more recent studies have reported more subtle isotopic transformations during remineralization (Lehmann et al. 2002). Bacteria preferentially break down the most labile organic pools first, often with distinct isotopic effects (Altabet 1996). In any organic material, the remineralization rate of autotrophic nitrogenous organic matter is related to the size and lability of its nitrogenous compounds. This is supported by earlier views; Odum et al. (1979) observed that 10-30% of nitrogen in aged upstream tree- and marsh-plant detritus contained refractory non-protein compounds that did not dissolve in either boiling water or strong base. This same study hypothesized that sediments influenced by vascular plants may have the potential to accumulate large amounts of refractory nitrogen. Similarly, Buchsbaum et al. (1991) compared the decay rates of different nitrogenous molecules in detritus derived from either vascular plants or algae. Both types of detritus had a large initial release of labile amino acids, but vascular plant detritus retained 1% of its total amino acids after a two-year period. In contrast, all algal amino acids disappeared in less than two weeks. Moreover, the vascular plant detritus retained up to 30% of its original cell-wall nitrogen after 23 months, whereas algal plant detritus retained less than 14% of this material after 2 months. While examining the remineralization potential of BMA, Tobias et al. (2003) used $^{15}NO_3$ enrichment to conclude BMA provided a low C:N source to sediments that was likely more labile than the bulk SOM that was dominated by high C:N terrestrial or macrophyte-derived sources. Because of this divergent behavior in algal versus vascular-plant remineralization, it is widely believed that vascular detritus does not contribute significant amounts of ammonium to local microalgal production, even in areas where vascular detritus dominates bulk SOM.

Objectives

This study explores the underlying biogeochemistry that supports upper trophic levels in Southwest Florida estuaries during dry periods. Specific objectives were to identify 1) primary producers associated with the labile pools of sedimentary organic matter remineralized in the sediments, 2) primary producers associated with the recalcitrant pools of sedimentary organic matter, and 3) primary producers associated with fish production. This study considers bulk sediment organic matter to be mass-balanced towards— and therefore representative of— the recalcitrant sedimentary organic nitrogen pool, and this study considers sediment porewater ammonium to be representative of the more labile sedimentary organic nitrogen pool. As in previous studies, our approach cautiously uses stable nitrogen isotopes to identify precursor materials with full knowledge that parent materials undergo biogeochemical transformations through time. The directions of most of these transformations are generally predictable (Kendall 1998), and therefore this study formulates conclusions on both the nitrogen sources of labile and recalcitrant nitrogen pools, and then uses these to suggest which types of removal and retention processes dominate in the sediments.

Methods

Study Sites

Data reported here originate from two investigations. The first, Hollander and Peebles (2004), identified the primary producers that support secondary producer biomass (fishes and crustaceans) in four Southwest Florida subestuaries (Figure 5.1, left panel). Hollander and Peebles (2004) focused exclusively on living tissues and therefore no sediment ammonium data were available from their study. The second investigation, the Tidal Streams study (Tampa Bay 2008), had a broader biogeochemical scope and targeted smaller subestuaries (Figure 5.1, right panel), but also emphasized

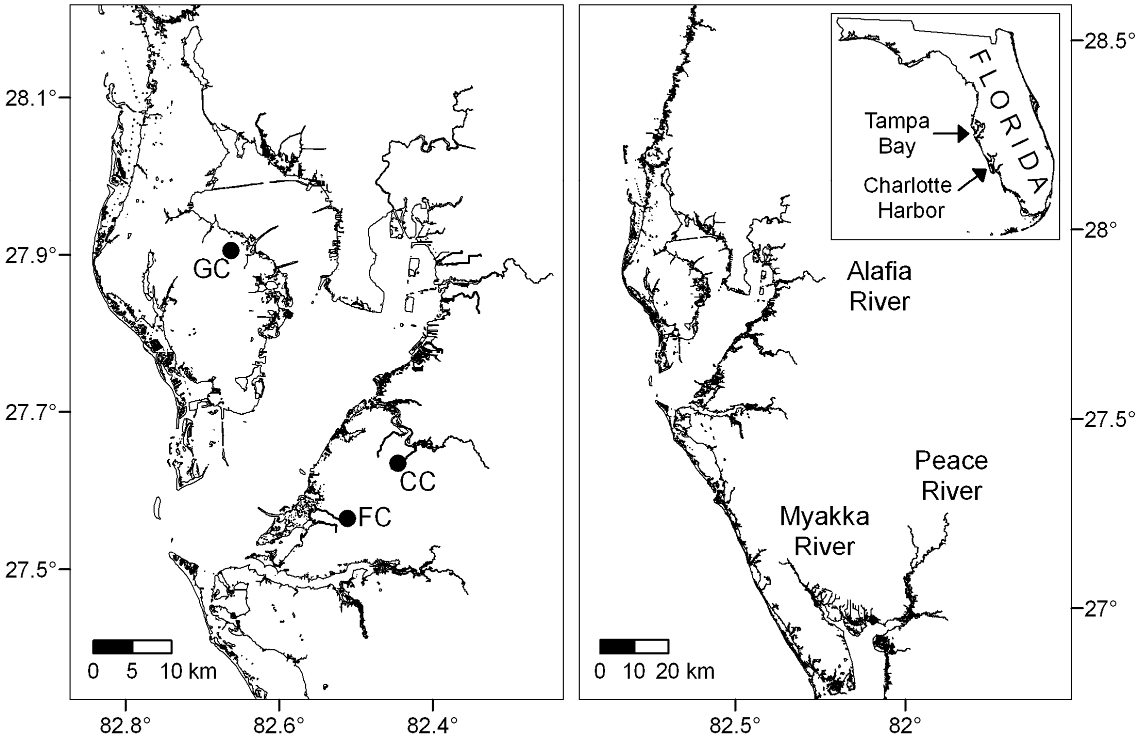

Figure 5.1: Location of six estuarine study sites ("subestuaries"). Left panel identifies three subestuaries sampled by Hollander and Peebles (2004). Right panel identifies three smaller subestuaries sampled during the Tidal Streams study 2008). Curiosity Creek is a tributary of the Little Manatee River.

identification of primary producers that support fish biomass. Data from both studies are considered together, and the effort was to identify sources and processes that were common to all studied locations rather than focusing on individual subestuaries or microhabitats within subestuaries.

Sample Collection and Processing

Samples of upstream trees and shrubs, benthic microalgae, particulate organic matter, sediment organic matter, and fish were collected in triplicate when possible from each subestuary's sampling sites. In Hollander and Peebles (2004), dry-season (May–June 2003) samples were collected at 3 sites per subestuary within the main channel of tidal rivers, ranging from 10 km to 25 km upstream of the tidal-river mouth. In the Tidal

Streams project, samples were collected at 2-3 sites per subestuary that ranged approximately 1 to 4 km upstream of the confluence with a larger channel or open bay waters. For primary producer collection, each sampling site was visited twice during the dry season (March 2006 and again in June 2006). Fishes were collected monthly from March to June, whereas pore water sediment samples for sedimentary ammonium isotopic analyses were only collected during the June sampling period.

Leaves of dominant upstream trees and shrubs (*Taxodium distichum*, *Salix caroliniana*, *Ulmus americana*, *Baccharis halimifolia*, *Quercus laurifolia*, *Carya glabra*, *Myrica cerifera*, *Acer rubrum*, *Quercus virginiana*, *Sabal palmetto*, *Schinus terebinthifolius*, *Juniperus virginiana*, *Serenoa repens*), emergent plants (*Cladium jamaicense*, *Typha spp.*, *Acrostichum danaeifolium*, *Eichhornia crassipes*, *Scirpus spp.*, *Juncus spp.*), and mangroves (*Rhizophora mangle*, *Avicennia germinans*) were collected from the banks of subestuary reaches.

Two methods were used to collect BMA. Hollander and Peebles (2004) grew BMA in-situ on glass plates. The plates were washed, analyzed microscopically, and scraped using a glass microscope slide. These samples were dominated by benthic diatoms, but also included filamentous algae and occasionally small amounts of nonfilamentous green algae and cyanobacteria. In the Tidal Streams study, benthic filamentous algae samples were collected from visible mats growing on the sediment surface.

In both studies, particulate organic matter (POM) samples in the 2.7-30 μm range were obtained via sieving and pressure filtration through pre-combusted glass fiber filters. Microscopic analysis indicated the POM samples from the Hollander and Peebles (2004) study were dominated by diatoms, whereas the samples from the Tidal Streams study were dominated by both diatoms and amorphous detritus. In both studies, bulk sediments were collected as shallow cores (4 cm). These samples were acidified in 0.1 mol L^{-1} hydrochloric acid to remove carbonate prior to isotopic analysis.

All solid samples were dried at 50°C, powdered, and frozen until isotopic analysis. Pore water for sedimentary ammonium ($NH_4^+{}_{SED}$) analysis was extracted by wrapping the bulk sediment samples within fine mesh and pressurizing the wrapped sample with a stainless steel press. The extracted products were then pressure-filtered through a 0.7 µm glass fiber filter, and the ammonium in the filtrate was isolated using the ammonia diffusion method (Holmes et al. 1998). The entire porewater extraction process was either carried out on ice or in glove bags to minimize microbial activity.

Samples of fish tissues were obtained using mid-channel trawls (Hollander and Peebles 2004 study) and shoreline seines (both studies). All samples were limited to tissues (primarily muscle) located posterior to the peritoneal cavity.

C:N and $\delta^{15}N$ values were measured using a Carlo Erba 2500 Series I elemental analyzer equipped with a zero-nitrogen blank auto-analyzer that was coupled to a Finnigan Mat Delta Plus XL stable-isotope mass spectrometer. All samples were run in duplicate using spinach leaves (NIST 1570a) as an internal reference material, and N isotope values are reported in conventional per mil notation (‰) relative to the air international standard. Instrumental standard deviation remained under 0.3‰.

Data Analysis

Primary producer linkages to bulk SOM were evaluated by first subestuary-averaging each primary producer category and SOM $\delta^{15}N$ values. Subestuary-averaging refers to averaging $\delta^{15}N$ values of a specific sample type (i.e. POM, BMA, SOM) collected from all sampling sites within each subestuary to determine that sample type's average value within each subestuary. Linkages between different sample types were then assessed using ordinary least-squares regression of each subestuary-averaged

$\delta^{15}N$ values for different primary producers (vascular plants, POM, BMA) on subestuary-averaged $\delta^{15}N$ values for SOM. Subestuary-averaging was used to account for advection of vascular plant detritus and phytoplankton within each subestuary.

Since $NH_4^+{}_{SED}$ values were not available in the Hollander and Peebles (2004) study, $\delta^{15}N$ data were not subestuary-averaged for determining primary producer linkages to $NH_4^+{}_{SED}$. Instead, sampling-site-specific $\delta^{15}N$ values of $NH_4^+{}_{SED}$ collected from individual sampling sites within each subestuary were compared to sampling-site-specific $\delta^{15}N$ values of primary producers. Specifically, primary producer linkages to $NH_4^+{}_{SED}$ were evaluated using regression of sampling-site-specific $\delta^{15}N$ values from different organic materials (vascular plants, POM, BMA, SOM) on sampling-site-specific $\delta^{15}N$ values for $NH_4^+{}_{SED}$.

Fish linkages to different primary producers were also evaluated using regression. Subestuary-averaged $\delta^{15}N$ values from the biomass-dominant fishes (secondary consumers) bay anchovy (*Anchoa mitchilli*), silversides (*Menidia* spp.), and rainwater killifish (*Lucania. parva*) were plotted against subestuary-averaged primary producer $\delta^{15}N$ values (POM, BMA, upstream plants, emergent vegetation, SOM). In this case, subestuary-averaging was used to offset deposition variability and account for small-scale fish migrations.

Results

Isotope summary statistics are presented in Table 5.1. Reported in Table 5.1 are the mean and standard error of the stable nitrogen isotope compositions of different primary producers, sediments, specific fish species, and sediment porewater ammonium. Mean and standard errors are reported for each sample type in each system. Sediment porewater ammonium was only available for three of the systems studied.

Table 5.1: Isotope Summary Statistics for Sediment Study. Summary statistics for mean and standard error (SE) of $\delta^{15}N$ by subestuary, where SE includes variation among sites within subestuaries as well as instrument standard error. Sample types are benthic microalgae and filamentous algae (BMA), particulate organic matter (POM), bulk sediment organic matter (SOM), upstream plants (UP), sedimentary ammonium ($NH_4^+{}_{SED}$) and fish (scientific names). Sedimentary ammonium was not available from all systems.

Subestuary	Type	mean	SE	*n*	Subestuary	Type	mean	SE	*n*
Alafia	BMA	8.63	1.02	5	Grassy	SOM	2.82	0.22	2
Myakka	BMA	5.41	0.54	6	Frog	SOM	3.46	0.6	3
Peace	BMA	9.07	0.38	13	Alafia	UP	0.59	0.68	19
Curiosity	BMA	11.82	1.8	4	Myakka	UP	-0.27	0.61	15
Grassy	BMA	6.2	0.66	7	Peace	UP	3.05	0.55	33
Frog	BMA	8.95	0.39	4	Curiosity	UP	1.96	1.01	15
Alafia	EM	5.83	0.37	14	Frog	UP	2.6	0.8	10
Myakka	EM	4.33	0.42	28	Alafia	*A. mitchilli*	12.15	0.4	22
Peace	EM	7.57	0.37	46	Alafia	*Menidia spp.*	12.14	0.31	9
Curiosity	EM	5.75	0.48	13	Curiosity	*Menidia spp.*	16.49	0.29	5
Frog	EM	5.43	0.89	5	Frog	*A. mitchilli*	10.59	0.39	5
Alafia	POM	6.52	0.77	3	Frog	*L. parva*	11.67	0.27	5
Myakka	POM	3.32	0.8	3	Frog	*Menidia spp.*	11.62	0.7	5
Peace	POM	6.17	0.62	3	Grassy	*L. parva*	9.72	0.28	10
Curiosity	POM	9.39	0.41	5	Myakka	*A. mitchilli*	10.16	0.26	17
Grassy	POM	4.66	0.72	4	Peace	*L. parva*	12.15	0.32	5
Frog	POM	6.34	0.85	6	Peace	*Menidia spp.*	12.13	0.23	17
Alafia	SOM	2.96	0.69	4	Curiosity	$NH_{4\,SED}^{+}$	6.89	1.94	3
Myakka	SOM	2.08	0.4	3	Frog	$NH_{4\,SED}^{+}$	3.9	2	3
Peace	SOM	4.56	0.71	4	Grassy	$NH_{4\,SED}^{+}$	2.07	0.6	2
Curiosity	SOM	3.84	0.52	3					

Sources of Bulk Sedimentary Organic Material

Regressions of subestuary-averaged $\delta^{15}N$ values for different primary producers against subestuary-averaged $\delta^{15}N$ values for SOM are shown in Figure 5.2. The $\delta^{15}N$ range of subestuary-averaged values for SOM, upstream plants, and emergent vegetation were all quite narrow (2‰ to 4.5‰, 0‰ to 2‰, 4‰ to 6‰, respectively), while the ranges of POM and BMA were much more substantial (3‰ to 9‰ and 6‰ to 12‰). Significant relationships (regression slope $p < 0.05$) were found for upstream plants versus SOM and emergent plants versus SOM, but no significant relationships were found for POM versus SOM or BMA versus SOM.

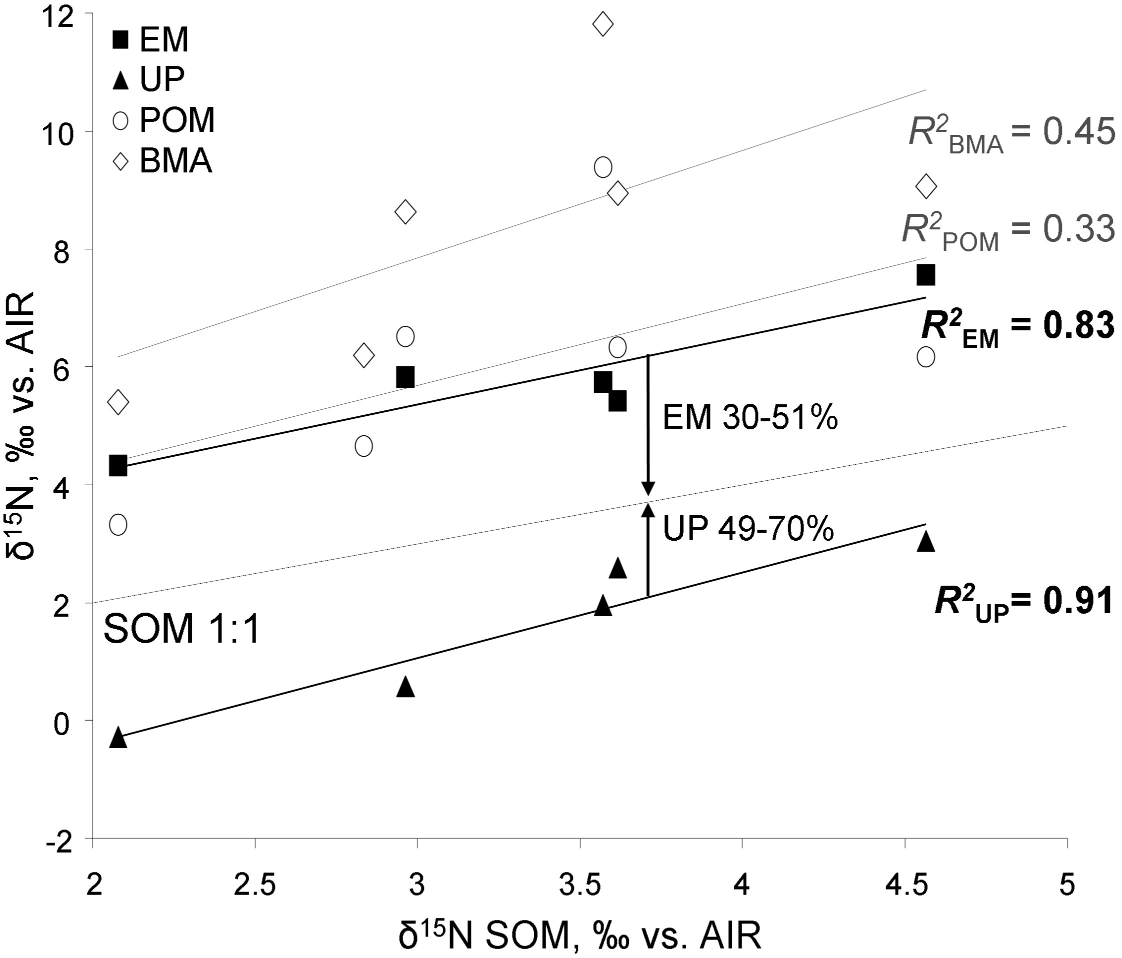

Figure 5.2: Sources of Recalcitrant Organic Matter. Regressions include subestuary-averaged $\delta^{15}N$ values for emergent plants (EM), upstream plants (UP), benthic microalgae and benthic filamentous microalgae (BMA), and particulate organic matter (POM) versus subestuary-averaged $\delta^{15}N$ values of bulk sedimentary organic matter (SOM), with coefficients of determination (R^2). Each point represents each subestuary's average $\delta^{15}N$ value for a specific primary producer versus average SOM $\delta^{15}N$. Solid lines represent the fitted regression for each primary producer. Dark lines represent statistically significant regressions ($p < 0.05$). The broken line identifies a 1:1 relationship (the SOM sink line). Arrows represent the contribution of each potential source to the SOM sink line with ranges of percent contributions calculated from a 2 end member mixing model ($\delta^{15}N_{SOM} = (X)\,(\delta^{15}N_{UP}) + (100-X)\,(\delta^{15}N_{EM})$), where $\delta^{15}N_{SOM} = \delta^{15}N$ of SOM, $\delta^{15}N_{UP} = \delta^{15}N$ of upstream plants, $\delta^{15}N_{EM} = \delta^{15}N$ of emergent plants, and X = % contribution).

Sources of Labile Organic Sedimentary Nitrogen

Regressions of sampling-site-specific $\delta^{15}N$ values for different primary producers and SOM against sampling-site-specific values for $NH_4^+{}_{SED}$ are shown in Figure 5.3. POM, BMA, and $NH_4^+{}_{SED}$ all had wide ranges (4‰ to10‰, 5‰ to 15‰, 0‰ to 11‰), while the range of SOM $\delta^{15}N$ values was extremely narrow (2‰ to 4‰). Significant

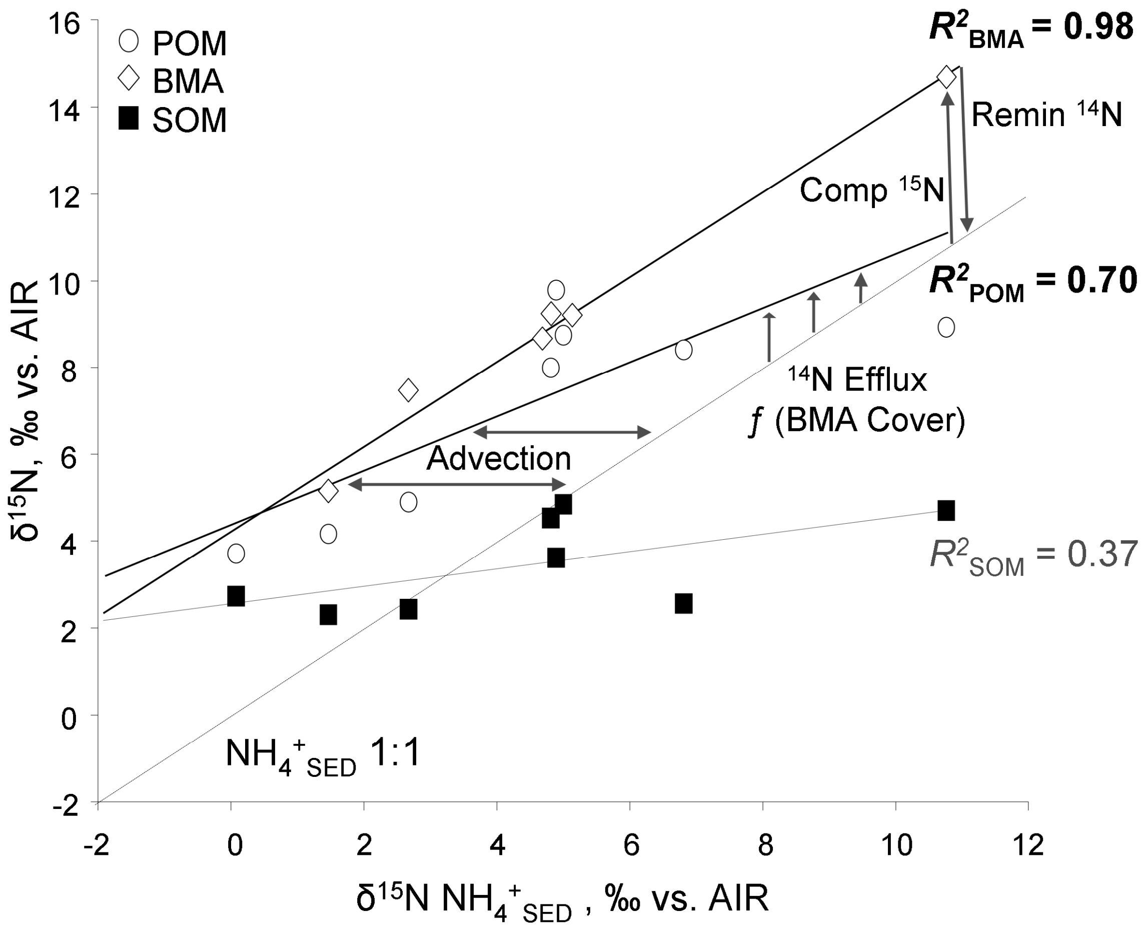

Figure 5.3: Sources of Labile Organic Matter. Regressions of sampling site-specific δ^{15}N values for benthic filamentous microalgae (BMA), particulate organic matter (POM) and surficial bulk sedimentary organic matter (SOM) versus δ^{15}N values for sedimentary ammonium ($NH_4^{+}{}_{SED}$) with coefficients of determination (R^2). Each point represents sampling-site-specific δ^{15}N values for BMA, POM, or SOM versus that site's δ^{15}N value for $NH_4^{+}{}_{SED}$. Dark lines represent statistically significant regressions ($p < 0.05$). The broken line identifies a 1:1 relationship (the $NH_4^{+}{}_{SED}$ line). Arrows represent the isotopic transformations between each primary producer and $NH_4^{+}{}_{SED}$. Vertical arrows between BMA and $NH_4^{+}{}_{SED}$ represent bacterial remineralization of ^{14}N of BMA material (Remin) and forced BMA uptake of ^{15}N due competition with nitrification competition (Comp). Vertical arrows between POM and $NH_4^{+}{}_{SED}$ represent relative amounts of ^{14}N efflux from sediments that is hypothesized to be related to BMA cover (*f*(BMA Cover)). Horizontal arrows represent hypothesized offsets due to advection.

($p < 0.05$) relationships were found for BMA versus $NH_4^{+}{}_{SED}$ and for POM versus $NH_4^{+}{}_{SED}$, but not for SOM versus $NH_4^{+}{}_{SED}$ (Figure 5.3). SOM was positively correlated with vascular plants in Figure 5.2, and site-specific values of vascular plants were not correlated with $NH_4^{+}{}_{SED}$ (not shown in Figure 5.3)

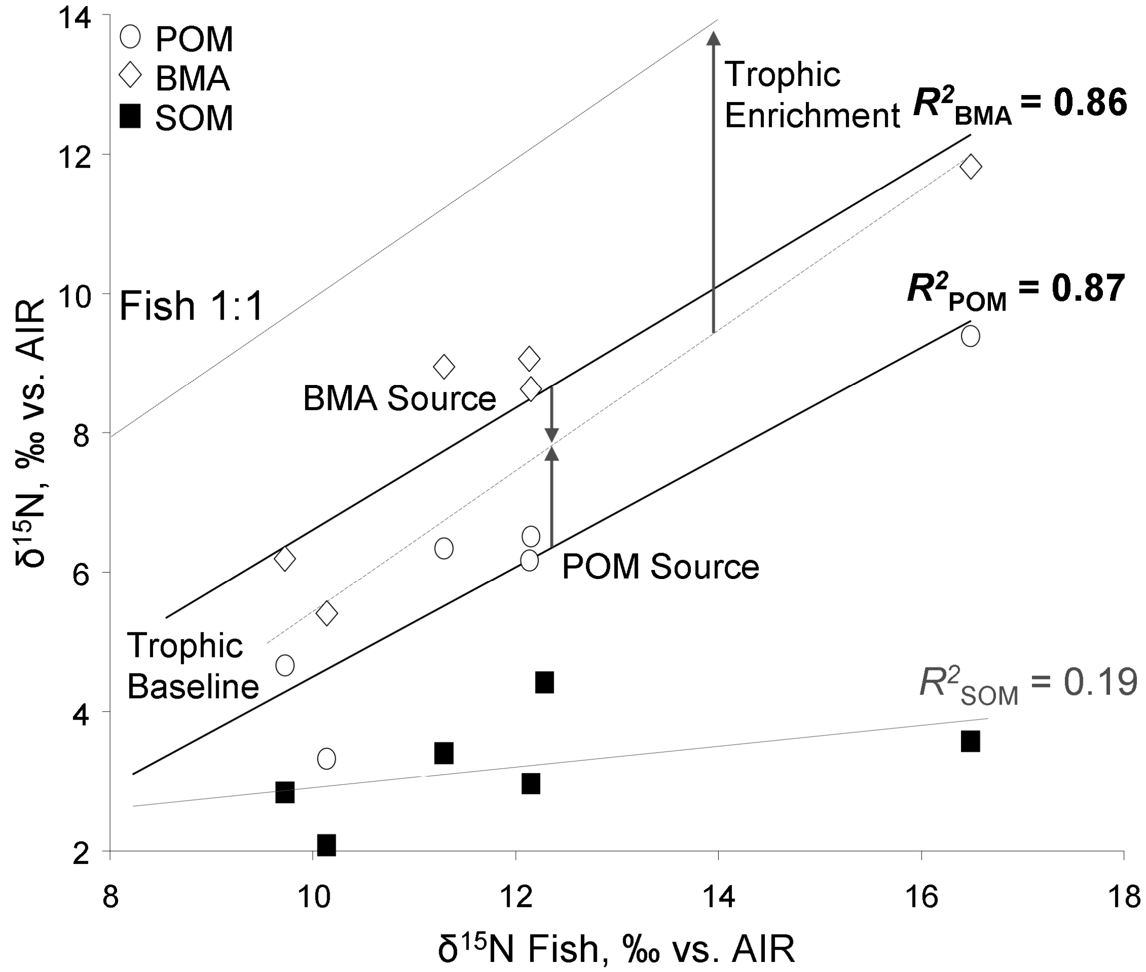

Figure 5.4: Sources of Fish Production. Plot of subestuary-averaged δ^{15}N values for particulate organic matter (POM), benthic microalgae (BMA), and sedimentary organic matter (SOM) versus subestuary-averaged δ^{15}N values for grouped fish taxa that include *A. mitchilli, Menidia spp., and L. Parva* with coefficients of determination (R^2). Each point represents each subestuary's average δ^{15}N value for a specific primary producer or SOM compared to its average δ^{15}N value for the fish taxa. Dark lines represent statistically significant regressions ($p < 0.05$). The broken line represents the trophic baseline calculated from the fish 1:1 line, assuming a trophic position of 2.5 for the grouped fish species with an enrichment of 3‰ per trophic level. Arrows represent the contribution of each primary producer to the trophic baseline and trophic enrichment.

Sources of Fish Production

The linear regression of subestuary-averaged δ^{15}N values for representative fish taxa against subestuary-averaged δ^{15}N values for different organic materials is shown in Figure 5.4. Because of the strong relationship ($p < 0.05$) between SOM and vascular plants (Figure 5.2), SOM is considered representative of upstream plants and emergent plants, as in Figure 5.4. The ranges of subestuary-averaged values for SOM, upstream plants, and emergent vegetation were all narrow (2‰ to 4.5‰, 0‰ to 2‰, 4‰ to 6‰, respectively), whereas the ranges of POM, BMA, and the grouped fish species were wider (3‰ to 9‰, 6‰ to 12‰, 9‰ to 17‰, respectively). Significant relationships were found for fish versus BMA and fish versus POM, but not for fish versus vascular plants or for fish versus SOM.

Discussion

Sources of Bulk Sedimentary Organic Matter

As evident in earlier studies (Ogrinc et al. 2005; Hu et al. 2006), terrestrial materials clearly influenced SOM. Upstream plant detritus and local emergent plants appeared to have strong influences on bulk SOM. Each had good fit with SOM $\delta^{15}N$, and each had slopes near 1:1. If both are entered into 2-end-member isotopic mixing model (i.e., $\delta^{15}N_{SOM}$= (X) ($\delta^{15}N_{UP}$) + (100-X) ($\delta^{15}N_{EM}$), where $\delta^{15}N_{SOM}$ = $\delta^{15}N$ of SOM, $\delta^{15}N_{UP}$ = $\delta^{15}N$ of upstream plants, $\delta^{15}N_{EM}$ = $\delta^{15}N$ of emergent plants, and X = % contribution), then most (49-70%) of the bulk SOM would be attributed to upstream plant detritus. Upstream plant detritus has been observed to be present as bedload in downstream channels, where it appears to remain as a byproduct of past freshwater flood events.

The above scenario takes into consideration the fact that bacteria may preferentially remove labile nitrogen pools from the SOM. For a single type of biochemical polymer, Macko and Fogel (1987) found individual amino acids ranging from 12‰ to 26‰ ^{15}N. Amino-acid nitrogen is likely isotopically distinct from non-protein nitrogen such as the nitrogen molecules associated with cell walls. Assuming that these labile nitrogen pools are primary-producer-specific, regression analysis should still be a valid tool for determining which organic pools are related to SOM. The consistent loss of isotopically distinct material would create primary-producer-specific offsets between parent material (source) and SOM (sink), which should be apparent in the regression results. Primary producers that are associated with SOM should have slopes similar to the 1:1 relationship in Figure 5.2, and emergent and upland plants were the only primary producers that met this criterion.

In the case of fresh, high C:N vascular plant detritus, the slope-matching approach would yield faulty conclusions because bacteria may add isotopically distinct foreign nitrogen to bacteria-detritus complexes (Odum et al. 1979), imparting their own specific isotopic fractionation factors (Hoch et al. 1992). In cases where such processes are dominant, SOM would probably mimic POM, since phytoplankton would obtain nutrients with a similar isotopic composition from the same ambient environment. The results in Figure 5.2 suggest this is probably not the case here because of the different trends of POM and SOM.

Sources of Labile Sedimentary Organic Nitrogen

The strong correlations between microalgae (BMA and POM) and $NH_4^+{}_{SED}$ suggest these materials are closely associated with the labile portions of sedimentary nitrogen. BMA appear to have a particularly close relationship with $NH_4^+{}_{SED}$; the slope of this regression is very close to 1, but is offset from the 1:1 line. This offset does not imply an anomalous fractionation on microalgal assimilation because the ammonium sampled was not BMA's nitrogen source. Our study was designed to identify the source of the bulk labile sedimentary ammonium and not specific nutrient sources for BMA.

It is unlikely that emergent and upland plant detritus contribute significantly to the $NH_4^+{}_{SED}$ pools. Considering vascular-plants were correlated with SOM (Figure 5.2), if the ammonium released from vascular plants contributed significantly to the $NH_4^+{}_{SED}$ pool, then the isotopic composition of SOM would have had the same slope, but with a consistent offset from the 1:1 line in Figure 5.3. This was not the case.

Microalgae and Sedimentary Ammonium Isotopic Divergence

In Figure 5.3, the divergence of these materials away from $NH_4^+{}_{SED}$ is particularly striking and implicates the dominant biogeochemical processes operating in these sediments. The light $NH_4^+{}_{SED}$ values that create the offset is not due to laboratory artifacts because 1) ammonium concentrations were undetectable in the ammonia-diffusion bottles after completing incubations, 2) laboratory standard deviations for incubation duplicates were minimal (<0.5 ‰), and 3) nitrogen was undetectable in the deionized water used to rinse the filtration mesh after porewater extraction.

Ruling out laboratory artifacts, this study proposes various mechanisms behind the BMA and POM offset from $NH_4^+{}_{SED}$. Bacteria selectively remineralizing the light isotopes of microalgal sediments may simply explain the enrichment of POM and BMA compared to $NH_4^+{}_{SED}$; however, a remineralization fractionation of 4‰ (see vertical arrow in Figure 5.3) is considered high (Kendall 1998). Therefore, an additional explanation for the BMA-$NH_4^+{}_{SED}$ offset based on depth-specific sedimentary biogeochemical processes and microalgal-bacterial competition for ammonium follows.

This study bases hypothesis on four assumptions: 1) BMA is in competition with nitrifying bacteria in the top layers of sediment, 2) heavy NH_4^+ is present in the upper layers of sediments due to nitrification processes and competition for light NH_4^+ between bacteria and BMA, whereas light NH_4^+ is produced in the bottom layers by DNRA, 3) concentrations of NH_4^+ are highest in the lower layers of sediments, and 4) this study sampled bulk $NH_4^+{}_{SED}$ which integrates both layers and therefore our $NH_4^+{}_{SED}$ values are mass-balanced towards the $\delta^{15}N$ of the NH_4^+-rich sediments in the deeper layers.

Competition for Ammonium at the Surface

The competition for ammonium between BMA and nitrifying bacteria (Risgaard-Petersen et al. 2004) will likely produce an ammonium-deficient surface microlayer enriched in ^{15}N (vertical arrow in Figure 5.3). Nitrifiers have the first opportunity to execute their extreme fractionation on ammonium (Kendall 1998) with a preferential utilization of $^{14}NH_4^+$, while BMA would incorporate any remaining ^{15}N-enriched reactants. If ammonium becomes unavailable, BMA may consume less-reduced forms of nitrogen such as nitrate undergoing denitrification, a process that greatly concentrates ^{14}N in N_2 and leaves the remaining NO_3^- enriched in ^{15}N (Kendall 1998).

Importance of DNRA in Deeper Sediments

The concentration of ^{14}N in $NH_4^+{}_{SED}$ and the chemical properties of the sediments suggest that the nitrogen transformation processes of DNRA may dominate in these ecosystems. Low nitrate and high organic content, which are characteristics of these sediments, have been shown to be indicative of fermentative DNRA (Nijburg et al. 1997). Furthermore, the study sites were all tidal and therefore have a source of sulfate that will reduce to H_2S via dissimilatory sulfate reduction in the sediment, which has been shown to induce chemoautotrophic DNRA (Brunet and Garcia-Gil 1996). Both fermentative and chemoautotrophic DNRA would likely produce ^{14}N-enriched ammonium. Although the magnitudes of kinetic fractionation for these processes have not yet been defined, it can be assumed that the residual nitrate is heavy compared to the light ammonium produced. If extensive DNRA does occur, then the $\delta^{15}N$ value of the deeper, anoxic sediments would be less than the $\delta^{15}N$ value of the oxygenated sediments occupied by BMA. The $NH_4^+{}_{SED}$ samples collected in this study are likely indicative of DNRA because they appear to be mass-balanced toward the light DNRA products, while the BMA values recorded here are comparatively heavy due to surficial nitrification processes. Sediment porewater nitrate likely originates from the products of nitrification. Denitrification coupled to nitrification would likely leave behind a signature of

^{15}N enriched ammonium. This was not observed in the depth-integrated $NH_4^+{}_{SED}$ $\delta^{15}N$ values, but was evident in the surficial BMA $\delta^{15}N$ values. Our results indicate that transformative processes have greater isotopic impacts than the removal processes in the top 4 cm of sediments. At this depth, ammonium has the potential to be transferred into the overlying water column due to sediment suspension events or bioturbation.

Sedimentary Efflux and POM

Compared to BMA, POM had both a lower R^2 value and a larger offset from the 1:1 line. This is probably because POM is highly susceptible to advection (see horizontal arrows in Figure 5.3). POM collected from a given site does not necessarily deposit at that site or obtain nutrients from that site.

Despite having a lower R^2 value compared to BMA, the slope for POM does suggest a relationship with the $NH_4^+{}_{SED}$. Because low nutrient concentrations are a characteristic of these estuarine areas during the dry season (Hillsborough County Environmental Protection Commission 2001), the POM investigated during the dry season is probably not the original nitrogen source of sedimentary ammonium. Instead, POM is probably a sink. POM was consistently ^{15}N-depleted relative to BMA and had $\delta^{15}N$ values closer to $NH_4^+{}_{SED}$ (Figure 5.3). This may indicate that dry-season POM obtains nutrients from ammonium fluxing from sediments that do not have substantial BMA cover such as at greater hypoxic water depths where BMA would be light limited and nitrifying bacteria would be oxygen limited. In these areas with less BMA and nitrifying bacteria to intercept the ammonium, substantial nutrient flux would be expected. It follows that without substantial competition for ammonium in the sediments, or nitrification to spark nitrification-denitrification coupling in the upper sediments, there would be fewer mechanisms to concentrate ammonium ^{15}N. Consequently, the ammonium fluxing out of the sediments would have a lower $\delta^{15}N$ value (vertical arrows in Figure 5.3), as would any water-column primary producers that strongly relied on this nitrogen source.

Proposed Nitrogen Cycling Pathways

Interpretation of the results depicted in Figures 5.2-5.4 suggests the two dominant nitrogen-cycling pathways described in Figure 5.5. The first is the recalcitrant of Figure 5.5. The recalcitrant cycle involves the accumulation of upstream plant and emergent vascular plant detritus into the sediment where it dominates the bulk isotopic signal but does not significantly influence the sedimentary ammonium signal. These low-nutrient materials are transformed into ammonium very slowly and therefore accumulate and dominate the collective SOM pool.

The second cycle is the labile cycle, depicted on the left side of Figure 5.5 and derived from results presented in Figures 5.3 and 5.4. The labile cycle includes relatively rapid aerobic remineralization of algal detritus, coupled with slower anaerobic remineralization via DNRA, collectively resulting in domination of the sedimentary ammonium pool by labile (microalgal) sources. This labile pool is likely the major supporter of DNRA activity occurring in the deeper sediments and also the ultimate nitrogen source of dry season fish production (Figure 5.4).

According to the model, the large amounts of labile phytoplankton biomass delivered to sediments during the wet season form the original nutrient source of most $NH_4^+{}_{SED}$. After settling, these labile materials pass thorough diagenetic sequences that are controlled by the distribution of sedimentary oxygen. Wet-season POM that settles in aerobic sediments breaks down quickly until oxygen is exhausted, whereas POM buried in anaerobic sediments is likely preserved by slow remineralization rates and may contribute to sediment-water flux during the dry season, where the remineralized nitrogen may or may not be intercepted by competing BMA and bacteria near the

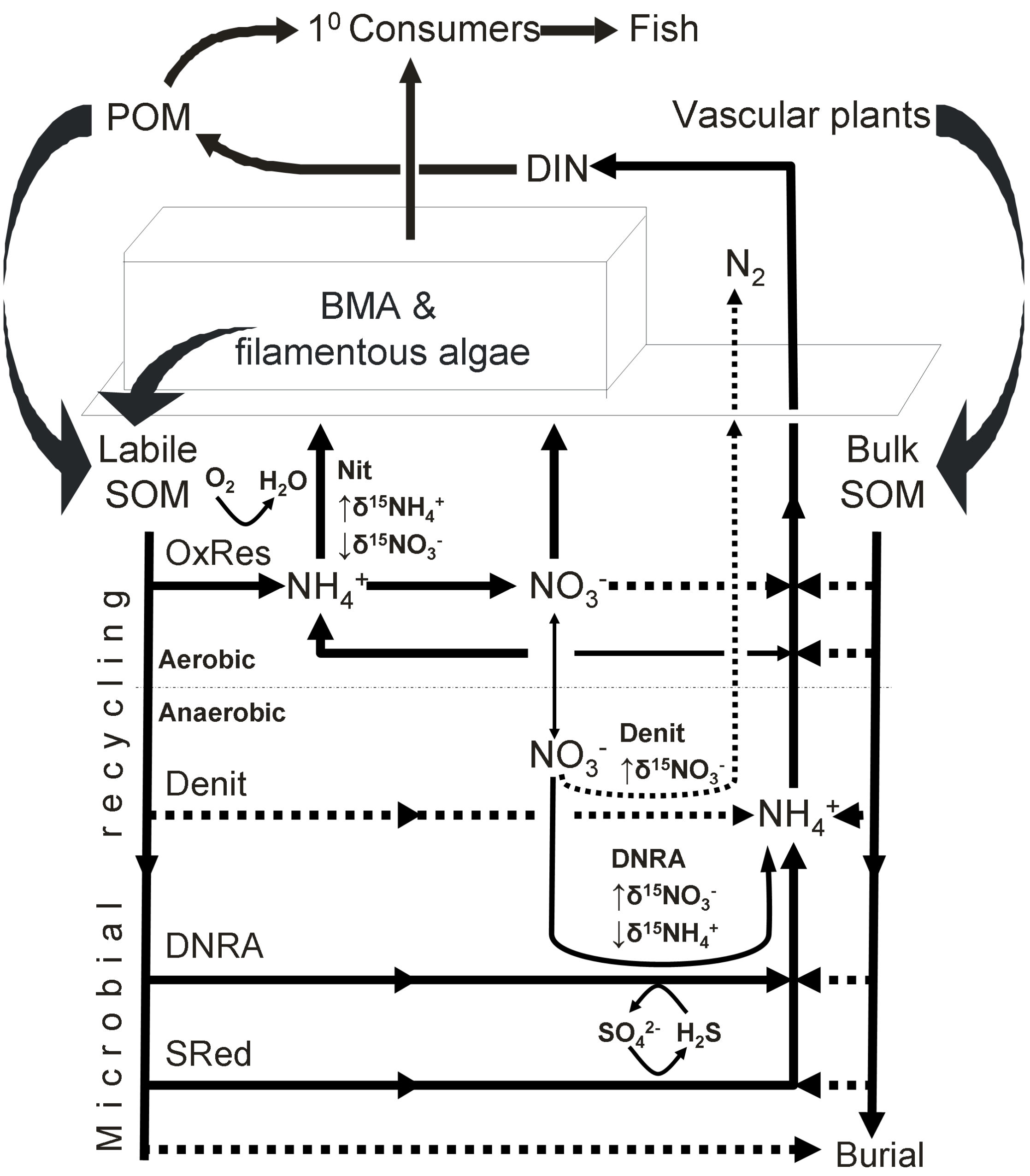

Figure 5.5: Conceptual Model Describing the Two Parallel Nitrogen Cycles. Large, solid arrows represent major nitrogen pathways, while large, broken arrows represent minor nitrogen pathways. Small, solid curved arrows represent major electron acceptors used to break down organic matter as well as sulfur oxidation coupled to dissimilatory nitrate reduction, while small, broken, curved arrows represent minor electron acceptors. Major biogeochemical processes in the model include bacterial oxic respiration (OxRes), nitrification (Nit), dissimilatory nitrate reduction to ammonia (DNRA), and dissimilatory sulfur reduction (SRed), while denitrification (Denit) is considered minor. Transformations of $\delta^{15}N$ are indicated adjacent to respective diagenetic processes. The right side of the Figure depicts the accumulation of recalcitrant upstream plant and emergent plant detritus into sediments. The left side describes the biogeochemical processes and upper trophic level incorporation of labile algal material. It is hypothesized that transformative biogeochemical processes in the deeper sediments enrich $^{15}NH_4^+$ in the oxic layers of sediments and enrich $^{14}NH_4^+$ in the anoxic layers.

cycle that was interpreted from the results in Figure 5.2 and is depicted on the right side sediment-water interface. This competition concentrates ^{15}N in the surficial sediment ammonium. Outward sedimentary ammonium flux is only expected from sediments with little intercepting BMA cover. In this case, ammonium in the surficial sediment does not concentrate ^{15}N and remains isotopically light, allowing the flux of light nitrogen to dry-season POM.

Incorporation into Higher Trophic Levels

The indirect importance of POM and BMA to the biomass to estuarine fishes is evident in Chapter 2. Fishes in these habitats appear to be dependent on microalgal materials as opposed to terrestrial materials. These findings are similar to those by Hollander and Peebles (2004). These consumers, along with macrobenthic organisms (including infaunal metazoans), represent a sink for the selectively recycled (labile) organic nitrogen produced as phytoplankton biomass during the wet season and, to a lesser extent, as benthic-diatom biomass during the dry season. The results in this chapter can be incorporated into the dry season pathway models developed in the previous chapters. Figure 5.6 incorporates the trophic pathway identified in Chapter 2 into the labile benthic recycling processes discussed in this chapter. Ammonium released from the sediments is assumed to be the ultimate source of nitrogen to the trophic pathways during this season, since nitrogen loading is at a minimum during this period with the exception being nitrogen sources with rainfall independent mechanisms of delivery as discussed in Chapters 3-4. Nitrogen delivered by runoff during the wet season is likely the ultimate source of this sedimentary nitrogen. The slow hypoxic breakdown of this material supplies nitrogen during the dry season. The recalcitrant pathway leading from vascular plant to buried nitrogen is independent of the recycling pathway leading to fish production.

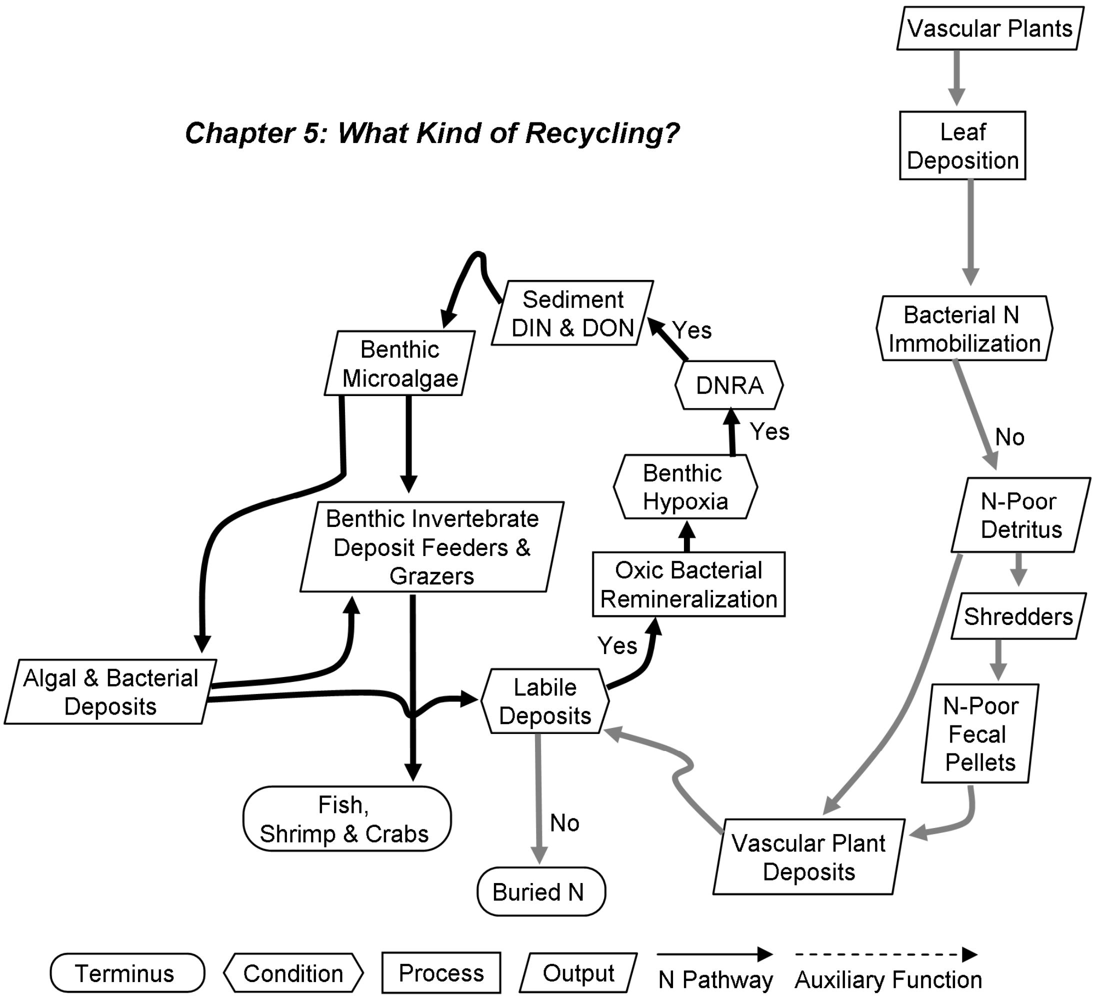

Figure 5.6: Dry Season Microalgae Trophic Pathway and Benthic Recycling. Thick black arrows indicate that recycling of labile algal sediment components support a benthic microalgae based community during the dry season. Thick grey arrows indicate the burial of recalcitrant nitrogen derived from vascular plants. The original source of the nitrogen to the system is likely the high nutrient loading and runoff that occurs during the wet season where phytoplankton deposition peaks. The slow hypoxic breakdown of this material supplies nitrogen during the dry season.

Conclusions

Three general conclusions can be made concerning nitrogen cycling in the study area. First, nitrogen derived from vascular plants such as emergent plants and upstream trees has a close relationship to bulk sediment organic nitrogen. Second, at each site within each subestuary, microalgae were closely connected to sedimentary porewater ammonium but not to the sediment's bulk organic matter, indicating that microalgae support fish biomass. Third, the isotopic depletion of nitrogen in deeper sediments suggests DNRA likely overwhelms denitrification (cf. Gardner and McCarthy 2009), while the isotopic values of BMA are likely associated with nitrification occurring in the oxygenated layers of the sediments. Collectively, these results indicate the bioavailable nitrogen sources are dependent on two nitrogen cycles: 1) algal-derived labile organic matter is recycled to ammonium in the sediments and supports fish populations via BMA and POM, wherein DNRA dominated recycling of buried, wet-season phytoplankton can support dry-season BMA and POM (and fishes), and 2) vascular-plant-derived recalcitrant material accumulates in the sediments and contributes to the bulk organic matter $\delta^{15}N$ signal.

Implications for Ecosystem Management

DNRA Overwhelms Denitrification

This study suggests that DNRA, a major nitrogen retention process, overwhelms denitrification, a major nitrogen removal process. DNRA and sedimentary remineralization have significant implications for managing nitrogen as a total maximum daily loads (TMDLs). Unlike denitrification that removes bioavailable nitrogen, DNRA transforms nitrogen from one bioavailable form to another. This process has been shown to dominate in sediments with high organic content and low nitrate concentrations (Nijburg et al. 1997). Managers should realize that during the dry season, nitrogen in the sediments is transformed rather than removed. The sediments examined in this study should not be considered zones of nitrogen remediation.

DNRA and Total Maximum Daily Loads

If ecosystem managers intend to improve habitat quality by reducing TMDLs, nitrogen concentrations may instead increase as the result of two processes. First, under low nitrate concentrations, DNRA is preferred over denitrification (Nijburg et al. 1997); nitrogen is merely transformed from one labile state to another instead of escaping to the atmosphere as volatile N_2 gas. Second, in estuaries with large amounts of microalgal organic matter stored within anaerobic sediments, the slow burn of the DNRA pathway will delay the recovery of hypereutrophic ecosystems longer than if remineralization were dominated by the denitrification nitrogen removal process. If successful TMDL policy leads to increased dissolved oxygen levels in sediments, then increased bioturbation (increased macrofauinal biomass in newly normoxic sediments) may trigger dominance by the more efficient electron transport system (remineralization through aerobic respiration) for consuming stored labile organic matter, thereby creating negative short-term feedback but ultimately resulting in improved ecosystem condition. Similarly, as infaunal communities recover, increased sediment aeration brought about by increased bioturbation may have an analogous, confounding effect. This oxygenation-bioturbation feedback mechanism will also take place during winter, as cooler temperatures increase dissolved oxygen solubility.

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CHAPTER 6:

THE ROLE OF POLLUTED JUVENILE FISH NURSERY HABITATS

Introduction

Coastal zone habitats provide manifold benefits to society, despite covering only 7% of the ocean's surface. In a coastal temperate setting, over forty types of ecosystem resources were identified and classified as providing provisional, regulatory, and cultural services (Ronnback et al. 2007). Coastal resources are abundant in Florida, the state with the longest coastline in the United States. Encompassing ecological, economical, and cultural dimensions, the most valuable service provided by the coasts of Southwest Florida is the juvenile fish nursery. This critical habitat supports the region's billion dollar recreational and commercial fishing industries, tourism, as well as a fishing culture, cherished by both the local population and vacationers alike.

Role of Tidal Fish Nursery Habitats

Although scientists and ecosystem managers unanimously acknowledge the importance of these habitats, the specific function of juvenile fish nurseries is rarely explicitly defined. The widely accepted principle is that near shore juvenile habitats disproportionally contribute to the production of individuals that recruit to the adult population (Beck et al. 2001). In more technical terms, nursery habitats provide resources that allow fish to have higher fitness values compared to fish in surrounding habitats; in these nurseries, the ratio of mortality to growth (μ/g) is minimized according to the *growth-mortality hypothesis* (Werner and Gilliam 1984).

The Growth Mortality Hypothesis

Several mechanisms have been described that more accurately define the *growth-mortality hypothesis*. In the *bigger is better hypothesis*, mortality is inversely related to size. Juveniles with high growth rates will survive more often than those with low growth rates (Miller et al. 1998). As a fish grows, it becomes a prey item for fewer fish because the smaller the predator to prey size ratio, the smaller the predator’s capture success. As a result, larger individuals of a given age will have a lower probability of mortality compared to smaller individuals of the same age (Leggett and DeBlois 1994). According to the *stage-duration* hypothesis (Chambers and Leggett 1987), if an early ontogenetic stage has a higher mortality rate compared to a later stage, the individuals that change to the lower mortality rate stage at an earlier age will have lower mortality compared to individuals of the same age at the higher mortality-prone stage (Chambers and Leggett 1987). Since mortality decreases as a function of size, the *growth-rate hypothesis* predicts that faster growing individuals will minimize their time existing at small sizes and thus minimize their time being subject to high mortality rates. Faster growing individuals would therefore have a lower probability of mortality compared to slower growing individuals (Shepherd and Cushing 1980). The relatively new *growth-selective predation hypothesis* predicts that slow growing juveniles are more vulnerable to predation compared to their faster growing cohorts, and growth rates directly affect predation mortality (Takasuka et al. 2003). Takasuka et al. (2003) point to potential differences in anti-predator behaviors between slow growers and fast growers; behaviors depicted by fast growers such as better escape activity and faster responsiveness during predator encounters are probable processes behind this hypothesis. Many other studies provide evidence to the *growth-mortality hypothesis*. In-vitro studies have shown that larger larvae obtain food and avoid predators better than smaller larvae (Bailey and Houde 1989), while in-situ studies have shown that survivors of a population are larger at younger ages (Meekan and Fortier 1996), and faster growing cohorts exhibit high rates of survival (Castro and Cowen 1991). Using otolith microstructure analysis, Takasuka et al. (2004) provide evidence for the *growth-selective predation hypothesis*; fish obtained from the guts of predators had slower growth rates compared to their surviving cohorts.

Two characteristics of coastal nurseries, namely structural complexity and high food densities allow fish to grow quickly and hide from predators; these fish will have higher fitness then they would if they occupied surrounding habitats. The relative importance of these characteristics has been debated in the scientific community. High mangrove and marsh grass densities (Reis and Dean 1981), abundant structural complexity (Weinstein and Brooks 1983), and shallow waters (Reis and Dean 1981) have been shown to reduce predation. In contrast, Carr (1994) claims these habitats provide copious nutrition which causes increases in fish growth rates and therefore higher fish fitness. Specific to Southwest Florida's nursery habitats, Hollander and Peebles (2004) identified the primary producers responsible for increased growth rates of juvenile fish; vascular plants in these environments did not support the majority of juvenile fish, rather benthic microalgae provided a crucial role in supporting the food web during dry periods, while deposited phytoplankton supported food webs during wet periods. This suggests that higher water residence time and increased nutrients that support the bases of these food-webs may be the direct cause of these nurseries' high fitness-related habitat values.

Objectives

This study compared the nutritional roles of two impacted coastal nursery ecosystems in the Tampa Bay area and hypothesizes on the mechanisms responsible for the high fitness-related habitat values that are unique to these systems. Because coastal ecosystems are under constant threat of coastal development, this study will also measure the stable isotopes of dissolved inorganic nitrogen (DIN) to determine if upstream anthropogenic nitrogen influences the nutritional and structural resources that support fish.

Methods

Study Sites

This study directly compares a tidal creek (Figure 6.1, bottom) that drains a watershed dominated by urban land use to a highly altered agricultural tidal tributary that drains a watershed dominated by agricultural land use (Figure 6.1, top).

Grassy Creek (Figure 6.1, bottom) is a polluted tidal creek that drains a residential neighborhood and a golf course in developed Tampa Bay. At its upper reaches, Grassy Creek does not rise above sea level and therefore is considered a true tidal creek in this study. This shallow, canopied creek is considered altered because it contains an extensive network of mosquito ditches and because of it artificially receives golf course runoff through a pipe. Nearly the entire sampling area is tidally-influenced. In contrast, Frog Creek (Figure 6.1, top) drains rural south-eastern Tampa Bay. This study actually considers this “creek” a tributary, since its upper reaches are located above sea level. This system has a much deeper channel compared to Grassy Creek and does not have as much mangrove cover. Frog Creek is considered altered because its natural channel has been supplemented by a network of agricultural drainage ditches. Approximately 3 km of the area sampled in Frog Creek was tidally-influenced.

Sample Collection and Processing

Samples of mangroves, benthic microalgae, particulate organic matter, and fish were collected in triplicate when possible from each system’s sampling sites. Samples

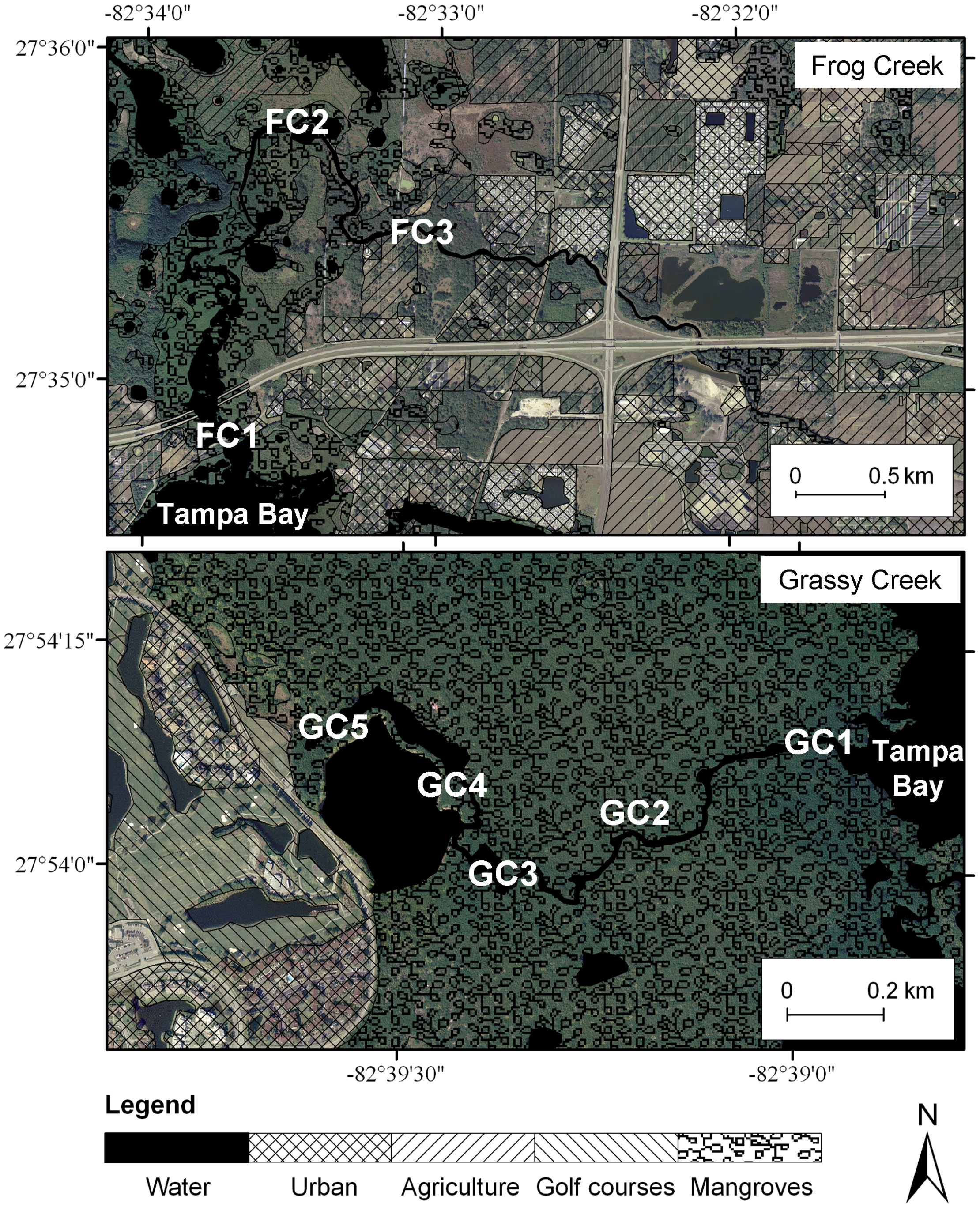

Figure 6.1: Map of Frog Creek and Grassy Creek Study Sites. Top panel identifies study sites of Frog Creek. Bottom panel identifies study sites of Grassy Creek. Land use adjacent to study sites is also displayed. Geographic data were downloaded from SWFWMD website.

were collected at 3-5 sites per system that ranged approximately 1 to 4 km upstream of the confluence with a larger channel or open bay waters. For primary producer and DIN collection, each sampling site was visited twice during the wet season (September and again in December 2006) with the exception being Grassy Creek where DIN samples were only collected in September. Fishes were collected monthly throughout the sampling period.

Leaves of mangroves (*Rhizophora mangle*) were collected from the banks of tributary and creek reaches. Two methods were used to collect benthic microalgae (BMA). Using an in-situ method, BMA were grown on glass plates in the field. The plates were washed, analyzed microscopically, and scraped using a glass microscope slide. These samples were dominated by benthic diatoms, but also included filamentous algae and occasionally small amounts of nonfilamentous green algae and cyanobacteria. In cases where dense microalgal mats were visible, BMA samples were collected by gently removing filamentous algae from the sediment surface.

Particulate organic matter (POM) samples in the 0.2-2.7 μm, 2.7-30 μm, and 30-60 μm range were obtained via sieving and pressure filtration through pre-combusted glass fiber filters. Microscopic analysis indicated the POM samples were dominated by both diatoms and amorphous detritus. Ammonium in the POM filtrate was isolated using the ammonia diffusion method (Holmes et al. 1998). All solid samples were dried at 50^{o}C, powdered, and frozen until isotopic analysis. DIN samples were collected along a salinity gradient to identify upstream and downstream end-members and to identify potential relationships with the primary producers that support fish production.

Samples of fish tissues were obtained using mid-channel trawls and shoreline seines. All samples were limited to tissues (primarily muscle) located posterior to the peritoneal cavity. The δ^{13}C and δ^{15}N values were measured using a Carlo Erba 2500 Series I elemental analyzer equipped with a zero-nitrogen blank auto-analyzer that was coupled to a Finnigan Mat Delta Plus XL stable-isotope mass spectrometer. All samples were run in duplicate using spinach leaves (NIST 1570a) as an internal reference material, and both C and N isotope values are reported in conventional per mil notation

(‰) relative to their respective international standards, Pee Dee Belemnite and Air. Instrumental standard deviation remained under 0.3‰ for both C and N.

Determining the Nutritional Role of Nursery Habitats and Impacts of Nearby Land Use

Juvenile fish nursery habitats were considered to provide a nutritional role if any of the primary producers in the nursery were shown to support the basis of food web that sustains fish production. Bulk carbon and nitrogen stable isotopes of fish, algae, and plant material were used to identify the presence of this nutritional role and to identify which specific primary producers were primarily responsible. Conventional $\delta^{15}N$ versus $\delta^{13}C$ trophic plots that compare the ranges of various primary producers to fish species were used to determine each system's potential nutritional role.

The principle behind $\delta^{15}N$ versus $\delta^{13}C$ trophic plots lies in the two biochemical phenomena that regulate the flow and transfer of isotopic chemical species through biological systems (Peterson and Fry 1987). First, the carbon and nitrogen isotopic composition of primary producers reflect both assimilated nutrient sources and the carbon metabolic pathway used. Second, these tracers can identify the primary producers that support biomass at higher trophic levels because stable isotopes predictably change during biomass transfer from key primary producers upward through the food-chain to fish (Peterson and Fry 1987). This systematic increase per trophic level is 3 to 4 times greater in nitrogen than carbon, and therefore nitrogen isotopic compositions provide a more sensitive indicator of trophic position.

DIN $\delta^{15}N$ analysis can distinguish between food webs supported by anthropogenic nitrogen versus food webs supported by nitrogen originating from Tampa Bay and delivered by the incoming tide. By comparing each system's DIN isotopic gradient with the bulk organic carbon versus nitrogen isotopes plots, the source of nitrogen entering these food webs can be identified.

The principle behind this methodology lies in the phenomenon that the dissolved species present in aquatic ecosystems inherently provide information on coupling between watershed nutrients and receiving waters. DIN supports primary production downstream, but also carries with it information on its land-use origin in the form of stable isotopes of nitrogen (Kendall 1998). For example, land use types such as agriculture often exports nitrate with very heavy $\delta^{15}N$ values due to large amounts of $^{14}NH_3$ and $^{14}N_2$ released during ammonia volatilization and denitrification that occurs after fertilizer application. DIN derived from treated sewage and septic systems undergo similar biogeochemical processing. DIN derived from urbanized soils often has a wide variety of isotopic values because of the various forms of nitrogen processing that occurs in urban watersheds. If developed land use does not play a role in delivering nitrogen to ecosystems, then the isotopic composition of nitrate would likely indicate a non-land-use specific source of nitrogen such as the light values of nitrogen oxides present in atmospheric deposition, the light nitrogen derived from nitrogen fixing bacteria (Kendall 1988), or the influence of seawater nitrates (Peterson and Fry 1987). Consequently, many studies utilize the nitrogen isotopes of dissolved nitrate to determine an instantaneous measure of nitrogen’s land use origins (Rock and Mayer 2006), while other studies utilize the nitrogen isotopic composition of sessile primary consumers such as clams (Oczkowski et al. 2008) or mussels (McKinney et al. 2001) to acquire a more time averaged source signal.

In this case, DIN is used to determine the source of nitrogen in these creeks and to confirm whether this signal is transmitted to fish that occupy each system’s juvenile nursery habitats. This will clarify whether fish obtain nitrogen associated with their nutrition from inside these habitats as opposed to whether fish are simply utilizing these habitats for other survival purposes.

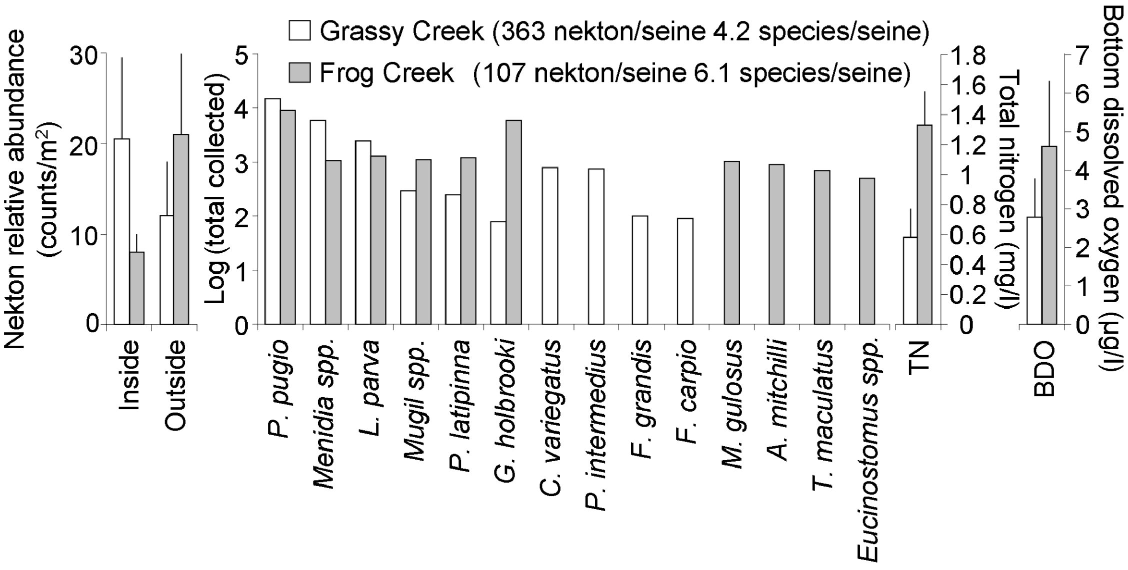

Figure 6.2: Community and Physical-Chemical Parameter Data for Frog Creek and Grassy Creek (adapted from Tampa Bay 2008). Physical-chemical parameters include bottom dissolved oxygen (BDO) and total nitrogen (TN). Community data includes the ten most abundant species in each system. The number of nekton collected outside and inside each system is also reported.

Results

Nekton Abundance and Community Structure Summary

A summary from Tampa Bay (2008) of species abundance, species densities, bottom dissolved oxygen, and total nitrogen for Frog Creek and Grassy Creek is shown in Figure 6.2. More nekton was collected in Grassy Creek compared to Frog Creek though more species were collected per seine in Frog Creek. *P. pugio*, *Menidia spp.*, and *L. parva* were the three most common species caught in Grassy Creek, whereas *P. pugio*, *G. holbrooki*, and *L. parva* were the three most common species in Frog Creek. Differences in community structure between the two systems were not reported as significant (Tampa Bay 2008) according to an analysis of similarities (ANOSIM, R=0.154, p > 0.05). Overall more nekton was collected inside Grassy Creek compared to inside Frog Creek. However, in Frog Creek, most nekton was collected outside the tributary, while in Grassy Creek most nekton was collected inside the creek. These results should be interpreted with caution, since nekton can move freely within a system. Total nitrogen concentration was higher in Frog Creek. Dissolved oxygen was lower in Grassy Creek.

Stable Isotope Analysis: Nutritional Sources and Anthropogenic Impacts

Isotope summary statistics are presented in Table 6.1.

Table 6.1: Summary Statistics for Frog Creek and Grassy Creek. Included are mean and standard error (SE) of δ^{15}N and δ^{13}C for different materials. SE includes variation among sampling events within individual sites and instrument standard error. Sample types are benthic microalgae and filamentous algae (BMA), particulate organic matter (POM), dissolved ammonium (DIN), mangrove (Man), and fish (scientific names). Since fish migrate between sites, statistics for fish are reported on a system wide basis. SE for fish includes variation among sampling events, variation among sites, and instrumental standard error.

Grassy Creek

Type	Site	δ^{15}N	SE	δ^{13}C	SE	n
DIN	1	5.36	NA	NA	NA	1
DIN	2	7.87	NA	NA	NA	1
DIN	3	7.31	NA	NA	NA	1
DIN	4	10.92	NA	NA	NA	1
DIN	5	16.47	NA	NA	NA	1
G. holbrooki	All	8.57	0.22	-25.92	1.45	2
L. parva	All	9.20	0.10	-21.35	0.36	35
Menidia spp.	All	9.77	0.11	-19.78	0.33	20
POM	1	4.26	0.35	-25.35	0.78	3
POM	2	3.46	0.33	-27.23	0.50	2
POM	3	4.03	0.26	-28.37	0.93	3
POM	4	2.89	1.30	-30.85	2.12	3
POM	5	14.90	0.93	-29.40	1.79	4
BMA	1	5.38	0.44	-28.02	0.85	7
BMA	2	4.89	0.67	-28.91	0.89	3
BMA	3	5.48	0.30	-28.32	1.43	3
Man	1	3.38	0.19	-27.25	0.78	4
Man	2	3.35	0.22	-27.86	0.69	3
Man	3	1.81	0.22	-27.27	0.35	3
Man	4	3.02	NA	NA	NA	1

Frog Creek

Type	Site	δ^{15}N	SE	δ^{13}C	SE	n
DIN	1	7.62	NA	NA	NA	1
DIN	2	12.16	0.78	NA	NA	2
DIN	3	11.51	0.17	NA	NA	2
C. undecimalis	All	11.58	0.48	-24.61	0.38	9
G. holbrooki	All	11.13	0.26	-25.99	0.88	5
L. parva	All	10.04	0.28	-20.58	0.40	5
Menidia spp.	All	11.25	0.34	-23.66	0.52	36
Man	1	3.16	0.95	-28.18	0.34	3
Man	2	5.83	0.07	-29.17	0.40	4
POM	1	3.90	0.71	-24.80	0.58	4
POM	2	5.88	1.05	-26.26	0.79	5
POM	3	6.50	1.04	-24.03	0.38	4
BMA	2	6.86	1.15	-26.02	0.90	3
BMA	3	9.49	0.62	-24.89	1.23	5

The δ^{15}N versus δ^{13}C trophic plot for Frog Creek is shown in Figure 6.3. Fish were corrected for δ^{15}N trophic enrichment by subtracting 4.5 from measured δ^{15}N values and 1.5 from measured δ^{13}C values with the assumption that these resident and

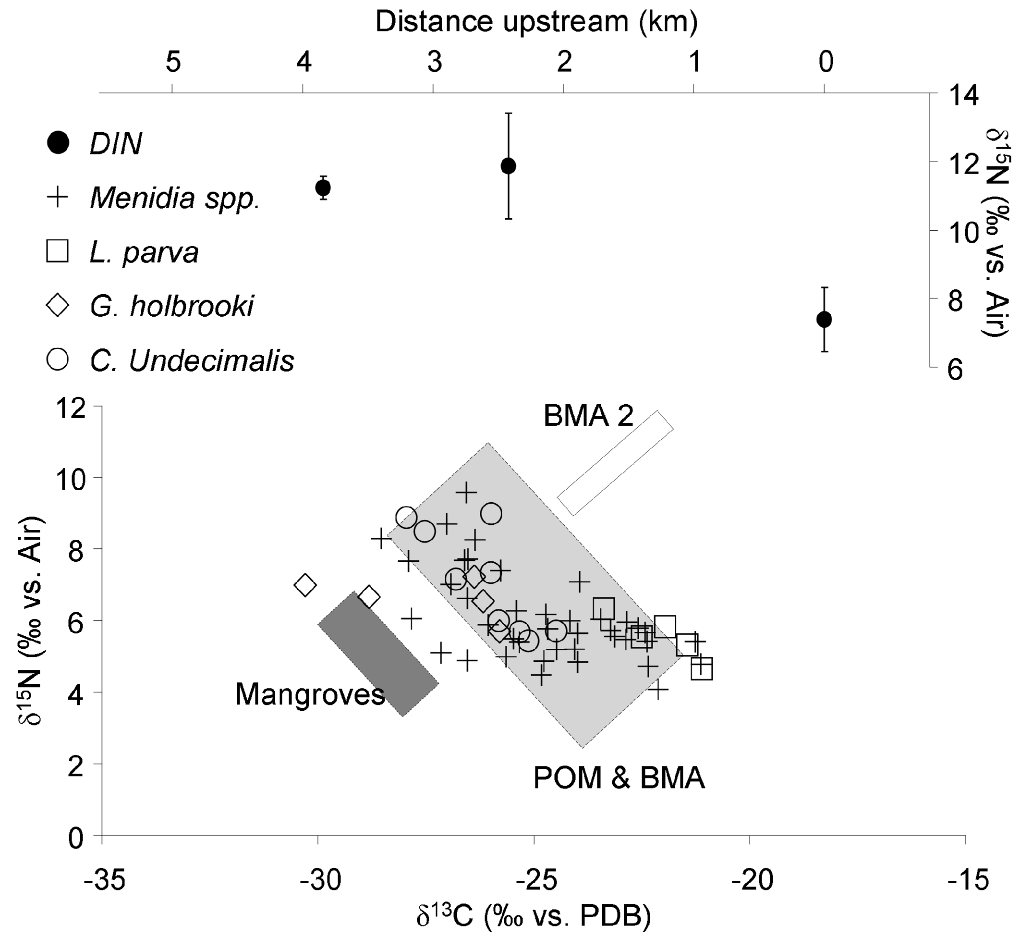

Figure 6.3: Trophic Plot and DIN Stable Isotope Gradient for Frog Creek. Top: DIN δ^{15}N gradient reported against distance from the tributary's mouth. Bottom: δ^{15}N versus δ^{13}C trophic plot. Boxes depict isotopic ranges of each primary producer, and individual fish species observations are depicted as points. Fish species were corrected for trophic level as described in the text. Dotted arrows indicate the amount of anthropogenic nitrogen (N_A) Tampa Bay nitrogen (N_{TB}) that contribute to the primary producers that support Frog Creek's foodweb.

transient fish occupy a trophic level of 2.5 with a trophic enrichment factor of 3 and 1 for nitrogen and carbon respectively. These corrections were based on previous work (Greenwood et al. 2009; Tampa Bay 2008; Hollander and Peebles 2004). In Figure 6.3, rectangles represent the ranges of different primary producers, while each individual fish observation is plotted. If a fish observation is situated within a specific primary producer's rectangle, it is assumed that the primary producer supports the foodweb associated with that fish. Most BMA observations were within the ranges of POM with the exception of BMA collected from the upstream site which is labeled as BMA 2.

A plot of ammonium $\delta^{15}N$ versus distance upstream is recorded above the $\delta^{15}N$ versus $\delta^{13}C$ plot. The upstream, anthropogenic nitrogen end-member is represented as N_A, while nitrogen originating from Tampa Bay is considered the “natural” end-member, represented as N_{TB}. Figure 6.3 clearly shows that the nursery habitats in Frog Creek provide nutrition for its juvenile nursery inhabitants. The nitrogen gradient that decreases from N_A to N_{TB} is also clearly apparent in the fish, and nitrogen derived from agricultural sources seems to be directly incorporated into the food web.

The $\delta^{15}N$ versus $\delta^{13}C$ trophic plot for Grassy Creek is shown in Figure 6.4. Fish were corrected for trophic level as described above. Rectangles represent the ranges of different primary producers, while individual fish observations are plotted. Most BMA observations were within the ranges of POM with the exception of some BMA that were enriched in $\delta^{15}N$, which are labeled as BMA 2. Figure 6.4 fails to show that the nursery habitats in Grassy Creek provide nutrition for its inhabitants. Apparently, the nitrogen and carbon that ultimately supports these fish originate downstream in Tampa Bay. A plot of ammonium $\delta^{15}N$ versus distance upstream provides additional evidence. As in Figure 6.2, N_A represents upstream nitrogen sources, while N_{TB} represents the downstream end-member. Although a strong nitrogen gradient that decreases downstream is clearly apparent as was in Frog Creek, very few fish exhibit an upstream $\delta^{15}N$ signal, unlike the clearly apparent pattern present in Frog Creek. The downstream nutrient source signal that originates in Tampa Bay is incorporated into the fish that occupy this juvenile nursery habitat, rather than the nitrogen signal derived from upstream anthropogenic nutrient sources. Figures 6.3 and 6.4 suggest that the nursery habitats in Grassy Creek and Frog Creek provide two different roles for juvenile fish.

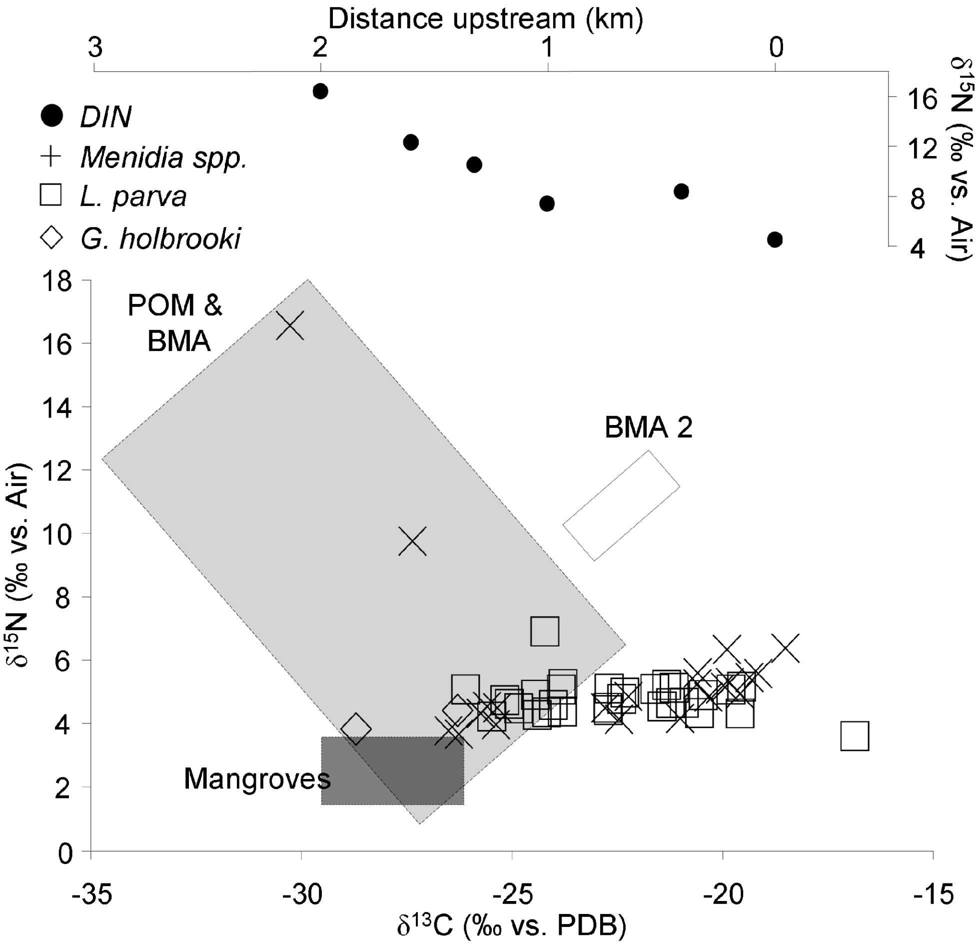

Figure 6.4: Trophic Plot and DIN Stable Isotope Gradient for Grassy Creek. Top: DIN δ^{15}N gradient reported against distance from the creek's mouth. Bottom: δ^{15}N versus δ^{13}C trophic plot. Boxes depict isotopic ranges of each primary producer, and individual fish species observations are depicted as points. Fish species were corrected for trophic level as described in the text. Dotted arrows indicate the amount of anthropogenic nitrogen (N_A) or Tampa Bay nitrogen (N_{TB}) that contribute to the primary producers that support Grassy Creek's foodweb.

Discussion

Specific Nursery Habitat Functions that Increase the Fitness of Juvenile Fish

The different nutritional roles of these two juvenile fish nursery habitats imply that each of these systems has a distinct mechanism that increases fish fitness. In Grassy Creek, the function of the nursery habitat is to shelter fish from predators. Fish fitness is increased because the shallow waters coupled with the complex mangrove root structure decrease predator encounters. In contrast, nursery habitats in Frog Creek undoubtedly provide nutrition for fish. This increases growth rate and possibly synergistically decreases predation mortality, which consequently increases fitness.

System-specific physical−chemical parameters such as oxygen availability may be the cause of the dissimilar functions depicted by these two nurseries. Grassy Creek, a much shallower and more canopied system is characterized by lower oxygen levels compared to Frog creek. These low oxygen levels are likely due to low light levels which reduce photosynthesis.

Nitrogen loads may also be responsible for a high oxygen demand. Nitrogen aids heterotrophic bacteria to breakdown the copious amounts of carbon rich mangrove detritus via aerobic decomposition, an oxygen consuming process. Furthermore, nitrogen may spark primary production in the upstream sunlit regions of Grassy Creek, which may eventually become aerobically remineralized by bacteria as they are transported to the downstream canopied areas of the creek, and a classic hypoxia-causing process would be induced.

Considering the dangerously low oxygen levels, shallow depths, and the presence of dense vegetation, fish likely enter Grassy Creek during high tide. The influx of seawater provides oxygenation and water volume, which increases the number of hiding places available for small fish, while the creek remains shallow enough to prevent entry by larger predators thereby reducing the effectiveness of predators to find their prey. Thus, in the case of Grassy Creek, high fitness-related habitat values are likely due to the fact that these nurseries reduce fish mortality by providing habitats with high structural complexity compared to adjacent Tampa Bay. The trophic plot (Figure 6.4) further supports this notion; fish collected in Grassy Creek obtain nutrition from outside the creek in Tampa Bay. These fish do not incorporate a significant amount of anthropogenic nitrogen delivered from the upstream golf course (Figure 6.4). It is likely that these fish feed on benthic microalgae present in the shallow areas of Tampa Bay during low tide but retreat to Grassy Creek during high tide when seawater influx creates a physiochemical haven for smaller fish wishing to avoid large predators in the flooded shallow habitats of Tampa Bay.

In contrast, the deeper waters of Frog Creek receive adequate sunlight due to low canopy cover. This sparks photosynthesis and sustainable oxygen levels. Consequently, fish do not seem to migrate outside the tributary as was the case in Grassy Creek; Frog Creek's DIN $\delta^{15}N$ gradient is directly incorporated into fish via foodwebs supported by microalgal deposits (Figure 6.3). Upstream nutrients with agricultural $\delta^{15}N$ signatures assimilate into microalgae which deposit and support fish in nearby areas via benthic foodwebs. If these fish were undergoing large migrations within the tributary as was the case in Grassy Creek, no $\delta^{15}N$ gradient would have been apparent. This was not the case. The high fitness-related habitat value of Frog Creek is therefore attributed to the nutrition provided by the nursery habitats within.

Application to Encounter Rate Theory

Encounter rate theory can clarify the contrasting roles of nursery habitats exhibited in this study. Encounter rate theory predicts how predators encounter and consume specific prey items based on reactive distance, the maximum distance at which a predator recognizes a particular type of prey (Eggers 1982). Reactive distance generally increases as prey size increases because a predator will energetically benefit from capturing larger prey as long as handling time does not become an issue. The overall search field of a predator includes a number of prey specific reactive fields with the larger field associated with larger predators and vice versa (Wright and O'Brian 1984).

Encounter rate theory (Wright and O'Brian 1984) predicts that the proportion F_i of prey type i in a given predator's diet will be

$$F_i = \frac{(L_i)^{\alpha}(N_i)}{\sum (L_i)^{\alpha}(N_i)}$$

where L_i is the size length of prey group i, N_i is the relative density of prey group i in the environment, and α is a term that describes the predators reactive search field, which is commonly considered spherical (α=2) for a stationary fish or cylindrical for a swimming fish (α=3).

The geomorphology of Grassy Creek would cause a greater modification of the search fields compared to Frog Creek. The shallow, structurally complex habitats of Grassy Creek would truncate the cylindrical or spherical shapes with two parallel planes, representing the air-water interface and the sediment water interface (Wetterer and Bishop 1985). Since prey reactive fields are size specific, this truncation does not have an identical effect on all sizes of prey and therefore predators are more likely to consume smaller prey items in shallow creeks, since the smaller reactive fields for smaller prey items are less likely to be truncated compared to the larger reactive fields for larger prey (Werner and Hall 1974). The shallow habitats of Grassy Creek would be a better haven for medium sized and larger juvenile fish compared to the deeper habitats of Frog Creek.

The mangrove roots in these habitats and turbid waters of Grassy Creek would further interfere with predator feeding. Manatunge et al. (2000) reported that under laboratory conditions the foraging efficiency of *Pseudorasbora parva* decreased significantly with increasing habitats complexity due to impeding swimming behavior and sight obstruction during foraging which would further limit search fields. In another in-vitro experiment, Barret et al. (1992) found that turbidity did not significantly affect pursuit speed, but had a consistent negative effect on reactive distance.

Role of Mangroves

Since Grassy Creek and Frog Creek both have extensive mangrove forests, and since these systems each have seemingly different functional roles for fish, this study may shed light on the roles mangroves play for fish. Three hypotheses have already been presented (Laegdsgaard and Johnson 2001). In the structural complexity hypothesis, the structural complexity of mangroves provides a zone of lower predation risk compared to surrounding habitats. The structural heterogeneity hypothesis predicts that juvenile fish are attracted to a mangrove habitat's structural heterogeneity. According to the food availability hypothesis, mangrove forests provide a nutritional function for fish; availability of food in mangrove habitats is greater compared to surrounding habitats.

Although this study cannot distinguish between the first two theories, it does present evidence that mangroves may provide multiple functions for fish and that these functions are based on system-specific physical-chemical parameters. In Grassy Creek, the shallow waters and low oxygen levels limit the roles of mangroves to providing protection from predators at high tide. In Frog Creek, the mangrove habitats definitely played a nutritional role but not directly; microalgae within these habitats supported the basis of the foodweb.

Observations presented here contrast with previous studies that claim the functional roles of mangroves for fish are species-specific rather than system-specific. Cocheret de la Morinie et al. (2004) found that the role of mangrove structural complexity on reef fish density depended on whether the species was characterized as nocturnal. Similarly, Laegdsgaard and Johnson (2001) compared fish communities among areas with artificial mangroves with structure, areas without structure, and areas with artificial structure and accumulated fouling algae. Structures with algae attracted the most fish, since algae provided a nutrition source, while areas without structure attracted the fewest number of fish. Species-specific differences were found between fish attracted to the different experimental setups. Laegdsgaard and Johnson (2001) also found that juvenile fish sought structure with the presence of predators but moved to open areas when predators were absent.

Community structure was not significantly different between Frog Creek and Grassy Creek, yet Grassy Creek and Frog Creek are assumed to have high fitness-related habitat values because of different habitat functions identified in this study, i.e., Grassy Creek's structural complexity versus Frog Creek's nutritional role. This indicates that the role of mangroves may depend on system-specific chemical and physical factors and not the types of species that occupy the habitat. Movement of juvenile fish based on the presence or absence of predators as reported by Laegdsgaard and Johnson (2001) may also explain some patterns seen in Grassy Creek. Fish likely exit Grassy creek during low tide to feed when predators are most likely absent and seek shelter in the creek during incoming tide when there is enough volume for predators to occupy the shallow regions of Tampa Bay.

Potential Role of Nutrients

Although both systems had anthropogenic nitrogen inputs as evident by the similar isotopic DIN gradients displayed in Figures 6.3 and 6.4, these upstream nitrogen loads had dissimilar roles in supporting foodwebs; these results are a direct paradigm of nitrogen's ostensible contradictory nature in aquatic ecosystems. Nutrients derived from anthropogenic land use have been shown to result in hypoxia, seagrass declines (Greening and Janicki 2006), and even fish kills (Clarholm et al. 1988). Oftentimes, nutrient pollution is associated with estuarine systems that have been over-utilized or polluted, resulting in adverse economic consequences including the degradation of habitats essential for fish production (Braun, 2007). Grassy Creek, a polluted tidal tributary may be a nursery habitat at risk. The isotopic composition of DIN indicates that the golf course adjacent to Grassy Creek is irrigated by reclaimed water, which has an isotopic composition similar to animal waste (Kendall 1998). Nitrogen exported by the golf course was not incorporated in foodwebs, rather it may be at least partially responsible for the low oxygen levels present in Grassy Creek. The nitrogen would undoubtedly support primary production in the more open regions of Grassy Creek in the upper portions (see Figure 6.1 bottom). Although initially this primary production would produce oxygen, as phytoplankton are transported downstream into the canopied regions, these molecules break down and contribute to hypoxia. Although oxygen levels in Grassy Creek may be naturally low due to low water levels and high temperatures, oxygen levels are further agitated by excessive nutrient inputs from the golf course.

In contrast to Grassy Creek, Frog Creek's juvenile nursery inhabitants incorporate anthropogenic nitrogen. Frog Creek's trophic structure is a clear example of how nitrogen must ultimately support foodwebs in nitrogen limited systems such as the creeks and tributaries of Tampa Bay. Cushing (1975) coined the term "agricultural model" that relates fish biomass to the amount of nutrients supporting the food chain. This theory has been supported by positive relationships between primary production and fish landings observed by Nixon (1982). These results are supported by the models proposed by Caddy (1993) which relate responses of fish yield to nutrient loads in a variety of semi-enclosed seas. Caddy (1993) proposed shapes of the fish catch to biomass curves would be dependent on the benthic or pelagic nature of trophic pathways leading to a particular fish catch. Especially notable were the benthic foodwebs, described as extremely susceptible to hypoxia under high nutrient loads. Furthermore, work by Oczkowski and Nixon (2008) defined the nutrient enrichment scale associated with the Caddy (1993) models in the tropical lagoons of the Nile in Egypt and concluded that anthropogenic nitrogen initially increased higher trophic level productivity to a critical point after which an exponential decline in fish landings occurred. This study has clearly demonstrated that nitrogen derived from agriculture in Frog Creek ends up in fish tissue.

In terms of appropriate ecosystem management, it is critical to determine if nitrogen supports or threatens economically important foodwebs. Chatterjee (2009) has concluded that eutrophication of freshwater regions alone accounts for over a 4 billion dollar annual loss to the United States. Florida is no exception to this potential economic loss; although nitrogen undoubtedly plays a critical role in supporting the foodwebs that support this region's billion dollar recreational fishing industry, it also has the potential to cause ecosystem collapse, a major threat to the services these habitats provide. Based on Caddy's (1993) models, the benthic foodwebs present in these habitats are especially susceptible. Excessive nutrient export from urbanized regions such as the golf course in Grassy Creek may destroy the economically important benthic-dependent nursery fish habitats as seen in many polluted systems, while completely shutting off anthropogenic activity or completely removing water supply in Frog Creek could result in no fish biomass. To prevent the collapse of these economically important habitats, appropriate nutrient policy must be established; the pathways identified here clearly show the potential role that nutrients play on the fish that depend on juvenile nursery habitats.

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CHAPTER 7:

SYNTHESIS

Conclusions

The numerous pathways identified in the previous chapter lead to following conclusions:

1. Benthic microalgae and phytoplankton deposits support the basis of the foodweb.
2. During the wet season in agricultural watersheds, nitrogen derived from row crops and tree crops support fish production with the nitrogen delivery mechanism being daily convective afternoon thunderstorms coupled with runoff.
3. During the dry season in agricultural watersheds, nitrogen derived from row crops and nitrogen transformation in the sediments support fish production. Water from irrigation delivers nitrogen to nursery habitats, and significant nitrogen also originates from sediment recycling.
4. In small urban watersheds, nitrogen derived from septic tanks, lawn irrigation, leaky sewage pipes, and atmospheric deposition support fish production, and unlike the agricultural watersheds these sources (with the exception of atmospheric deposition) are more seasonally consistent because sufficient groundwater flow is available during both seasons to deliver septic tank nitrogen.
5. The specific mechanism that allows nursery habitats to decrease the ratio of mortality over growth rates of juvenile fish is not consistent among systems. These mechanisms are likely dependent on physical-chemical parameters and stream geomorphology.

Generalized Nitrogen Pathway in Tidal Fish Nursery Habitats

Based on the investigations above, in both urbanized and agricultural watersheds, the isotopic composition of fish reveals that anthropogenic nitrogen use and processing influence foodwebs in juvenile fish nurseries. These nutrients may either be derived from agriculture or manifold urban nitrogen sources. Nitrogen is easily transported during the wet season because a high water table and consistent rainfall compliment any human water delivery mechanism at work (i.e. irritation).

These nutrients make their way into phytoplankton which deposit to the sediments in zones of high water residence time. Once deposited, two crucial ecological functions occur. First, benthic deposit feeder consume these deposits which in turn support juvenile fish nursery foodwebs through a phytoplankton based benthic community (PBBC). Second, if the consumption of these deposits is less than the deposition rate, copious amounts of labile material are buried, which in turn may support foodwebs during the dry season when nitrogen loading is low.

During the dry season, deposited phytoplankton deposits are recycled through the slow burning DNRA processes, which provide nitrogen for benthic microalgae. These organisms in turn support benthic communities which ultimately support dry season fish production. A summary of these pathways is shown in Figure 7.1.

If the geomorphology or physiochemical characteristics of these systems is not adequate to set up this efficient nitrogen transfer process to fish as seen in Grassy Creek, the function of these habitats for juvenile fish becomes more of a haven from predators rather than a source of food. Ecosystem managers should adequately define the function of these systems before making any policies regarding nitrogen.

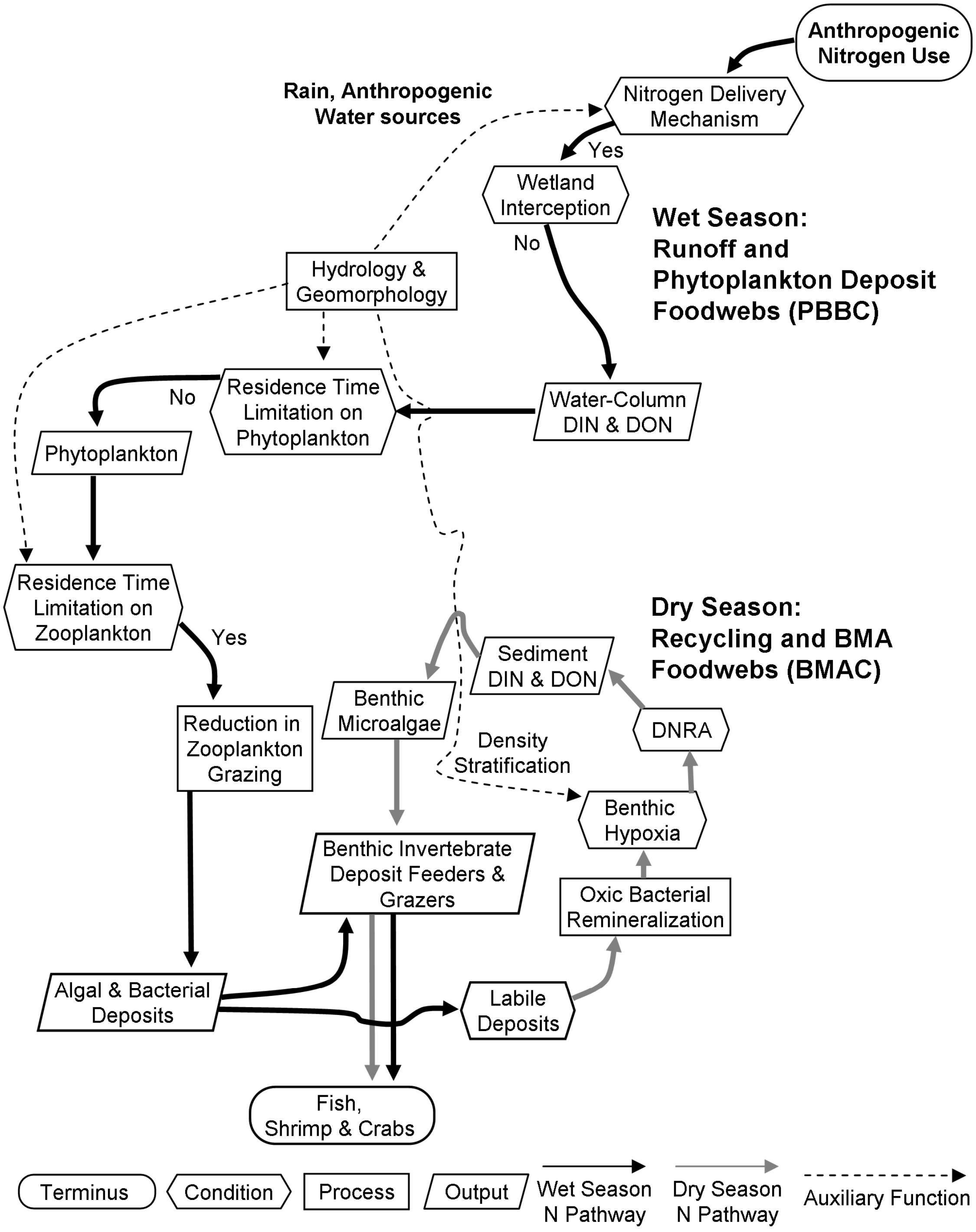

Figure 7.1: Synthesis of Nitrogen Pathways in Tidal Fish Nursery Habitats. Thick black arrows indicate the dominant wet season pathway leading from land use derived nutrients through phytoplankton based benthic foodwebs as well as some phytoplankton deposit burial. Thick grey arrows indicate the recycling of buried nitrogen during the dry season which support foodwebs via benthic microalgal based benthic communities.

Implications for Ecosystem Management

Based on previous discussions about nitrogen origins and delivery mechanisms, the following recommendations can be made for ecosystem managers. Managers must first recognize that microalgae is the dominant supporter of tidal nursery foodwebs and should consider modeling the processes of light attenuation and residence times to determine zones of high benthic microalgae survivability and phytoplankton deposition (Chapter 2). Since microalgae support fish, the second step is to define the relationship between nitrogen loads and fish abundance. If this relationship is unknown, the results of increasing nitrogen loads on fish production will remain uncertain; foodwebs in nursery habitats may collapse due to eutrophication or fish abundance may increase due to an increase in food supply.

Once the relationship between nitrogen loads and fish abundance is established, the connectivity factors derived from stable isotope mechanistic mass-balance models can be used as measurable targets for groups of watersheds. In cases where nitrogen remediation is required, ecosystem managers should consider policies that increase nitrogen uptake efficiency on cropland and policies that reduce fertilizer application in affluent residential landscapes.

The use of wetlands as nitrogen remediation tools may not be an effective way of removing nitrogen in these systems; nitrogen transformation processes such as dissimilatory nitrate reduction to ammonium, nitrification, and remineralization likely dominate removal processes such as uptake and denitrification in wetland soils (Chapter 3). The effectiveness of wetlands as nitrogen removal tools must be explored further. These transformative processes also seem to dominate in the sediments during the dry season (Chapter 5), and therefore, more testing on these sediments is required before they are characterized as zones of nitrogen removal.

Nitrogen Loads and Fish Production

To establish effective nitrogen loading polices that ensure maximum fish production, the relationship between nitrogen loads and total fish production must be well-defined. A clue to the true relationship between nutrient loads and fish abundance may be revealed by plotting mean annual chlorophyll a concentrations against fish abundance data with the assumption that chlorophyll concentrations are related to nutrient availability. Sparse chlorophyll data compiled from the EPA's *Storet* website and fish abundance data taken from Greenwood et al. (2009) and Tampa Bay (2008) were used to construct a nekton-abundance versus chlorophyll plot (Figure 7.2). Taking all watersheds into consideration, chlorophyll seems to have a positive relationship with

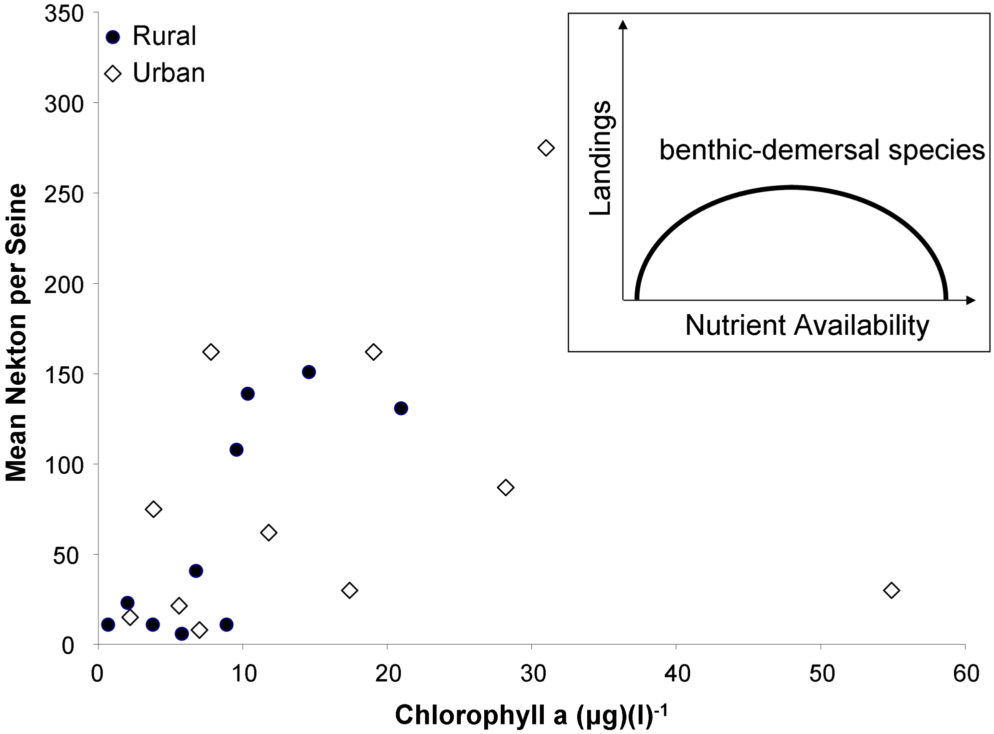

Figure 7.2: Nekton Abundance versus Chlorophyll. Geometric annual mean of nekton per seine plotted against annual mean chlorophyll a concentrations for all watersheds studied. Also included is a modification of Caddy's (1993) model that depicts the response of benthic demersal fish landings to nutrient availability.

nekton abundance up to a concentration of 30 $(\mu g)(l)^{-1}$. As chlorophyll concentrations increase beyond this point, nekton abundance drops significantly. Most watersheds studied seem to be on the left side of the Caddy (1993) curve.

Agricultural watersheds as a whole seem to be in better shape than urban watersheds; increasing nitrogen loads in urban watersheds seems more likely to cause a reduction in fish abundance in comparison to rural watersheds. In urban watershed the main nitrogen sources are septic tanks, leaky sewage pipes, and lawn fertilizer. Groundwater delivers septic tank nitrogen and nitrogen derived from leaky sewage pipes to nursery habitats. Unfortunately the most effective nitrogen remediation policy would likely be to target fertilizer application by affluent residents, since nitrogen policy is unlikely to reduce the loads from septic tanks and leaky sewage pipes present in poorer neighborhoods.

Connectivity Factors as Measurable Targets

In agricultural and rural watersheds, the connectivity factors discussed in Chapter 3 may be used as manageable targets. Connectivity factors represent the relative amount of nitrogen exported per unit land use area. Managers may aim to reduce the relative amount of nitrogen exported from specific agricultural land use types. If this is the case, managers should conduct the study described in chapter 3 before implementing policy to determine pre-policy connectivity factors. After conducting the study, managers should set targets for connectivity factors and establish policies to meet those targets. Once these targets are established and implemented, managers should repeat the study to determine post-policy connectivity factors. Since this study calculates connectivity using data from different watersheds, mangers should assign watersheds to groups before conducting this study.

Nitrogen Remediation in Wetlands and DNRA

In order to meet specific targets, managers may recommend the establishment of wetlands and riparian buffer zones to remediate nitrogen. Data from Chapter 3 and Chapter 5 indicate that dissimilatory nitrate reduction may be a major nitrogen transforming process in wetland soils during the wet season and in tidal nursery sediments during the dry season. Unlike denitrification that removes bioavailable nitrogen, DNRA transforms nitrogen from one bioavailable form to another. This process has been shown to dominate in sediments with high organic content and low nitrate concentrations (Nijburg et al. 1997). Data from Chapter 3 suggest that wetlands are major zones of nitrogen transformation. Based on the negative connectivity factors calculated for wetlands, it is unlikely that removal process outweigh transformative processes in these ostensible nitrogen remediation zones. Under circumstances of low flow wetlands may also be sources of nitrogen as opposed to sinks. Data presented in this study suggests that there is a need to determine whether wetlands and riparian zones are removing, transforming, or delivering nitrogen.

DNRA and Total Maximum Daily Loads

Managers should be aware that DNRA was found to be a major process in tidal nursery sediments (Chapter 5), and reducing nitrogen loads may actually increase nitrogen retention in these systems. Under low nitrate concentrations, DNRA is preferred over denitrification (Nijburg et al. 1997), and nitrogen is transformed from one labile state to another rather than being removed to the atmosphere via dentirifcaiton. In highly eutrophic systems with large amounts of microalgae deposits stored within anaerobic sediments, an increase in oxygen to the system may cause bacterial remineralization and the release of copious amounts of ammonium. Managers should therefore not be surprised if a successful TMDL policy leads to increased dissolved oxygen levels and a temporary increase in dissolved nitrogen concentrations. Increased macrofaunal biomass in recovering systems may trigger dominance by the more efficient electron transport system (remineralization through aerobic respiration) for consuming stored labile organic matter, thereby creating negative short-term feedback but ultimately resulting in improved ecosystem condition.

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