EVALUATION OF NATURAL MARKERS TO ASSESS CROSS-SHELF CONNECTIVITY OF MESORAMERICAN REEF FISH POPULATIONS IN BELIZE

A Thesis

by

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ABSTRACT

Quantitative evaluations of early-life connectivity in reef fish populations are critical to the effective identification and management of productive nearshore nurseries. The present study evaluates the use of natural markers in assessing both trophic and population connectivity for three species of snappers (Lutjanidae) utilizing back-reef nurseries in southern Belize: dog snapper (*Lutjanus jocu*), gray snapper (*L. griseus*), and schoolmaster (*L. apodus*). Juvenile snappers were collected from inner- and outer-shelf nurseries across two latitudinal regions in the Belize coastal lagoon in order to: (a) utilize tissue stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) as dietary tracers to characterize organic matter production across the continental shelf, and (b) evaluate the utility of otolith stable isotopes ($\delta^{13}C$ and $\delta^{18}O$) as a marker of nursery origin.

Isosource models (based on tissue $\delta^{13}C$ and $\delta^{15}N$) revealed distinct differences in food web dynamics between pristine nurseries and those influenced by heavily impacted watersheds. Juvenile snappers at pristine sites were supported by organic matter derived from both benthic sources (seagrass/benthic diatoms) and the water column (phytoplankton), while sites impacted by anthropogenic runoff displayed significant decreases in water column-based production during the rainy season, accompanied by significantly decreased juvenile snapper condition (muscle lipid content) for all three species. These results emphasize the high land-sea

connectivity in this system and indicate that runoff from impacted watersheds has the potential to disrupt trophic production in nurseries across the continental shelf.

Otolith stable isotopes ($\delta^{13}C$ and $\delta^{18}O$) appeared to be strongly related to salinity gradients within the Belize coastal lagoon and, consequently, showed considerable promise in identifying juvenile snappers to shelf position. Both isotopes were both consistently enriched in snappers from outer-shelf nurseries, where freshwater influence was minimal, and $\delta^{18}O$ was enriched in the northern sampling region, which receives lower amounts of freshwater input compared to the south. Although individuals of each species were classified to specific study sites with varying success (58-81%), discrimination to shelf position was consistently high for all species (74-92%), indicating that otolith $\delta^{13}C$ and $\delta^{18}O$ may be useful in determining relative contribution rates of juvenile snappers produced at inner- and outer-shelf nurseries within tropical back-reef systems.

DEDICATION

This thesis is dedicated to my fiancé Alphonse Covas, as well as my parents, Lulu and Bob Wetmore, for their limitless support and encouragement throughout the course of this research.

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CHAPTER I

INTRODUCTION

Nearshore habitats such as back-reef lagoons and estuaries often represent important nurseries for marine fishes and invertebrates, and as human populations continue to grow, it is inevitable that these nursery habitats will be increasingly impacted by coastal development and anthropogenic effects (Botsford et al. 2003; Able 2005). In the tropics, many commercially and ecologically important coral reef fishes require multiple back-reef habitats (i.e. mangroves, seagrass beds, patch reefs) to complete juvenile development, and for these species, the preservation of productive nurseries within back-reef systems is critical to population replenishment (Mumby et al. 2004; Adams et al. 2006). Nursery productivity can be attributed to a number of ecological factors, including high juvenile densities, increased growth, decreased mortality, and enhanced movement of juveniles to adult populations (Beck et al. 2001). Thus, production within a given nursery is influenced not only by local habitat quality but also ecosystem context - environmental conditions, hydrographic features, trophic production, and connectivity with adult habitats (Anderson et al. 1995). Because of this, back-reef nurseries that are comparable in habitat attributes (e.g. seagrass beds) may not be functionally equivalent in terms of ecological value or nursery production.

Despite the clear importance of nearshore habitats in sustaining reef fish populations, the functional significance of putative nurseries within back-reef systems has rarely been quantified (Beck et al. 2001; Able 2005; Adams et al. 2006). It is

unlikely that all habitats occupied during early life are equal in nursery value, and identifying the most productive nearshore nurseries is becoming increasingly critical as coastal ecosystems worldwide are affected by habitat loss, degradation, and over-fishing (Ariola 2003; Botsford et al. 2003; Sale et al. 2005). Because resources are limited, it is unavoidable that only a subset of nearshore habitats can be selected for intensive management and protection (Dahlgren et al. 2006), and determining which nurseries are contributing the greatest numbers of individuals to adult populations (i.e. quantifying nursery production) will allow management efforts to be focused on those areas that are most essential to population replenishment (Beck et al. 2001; Mumby 2006).

The purpose of this research was to use stable isotopes in tissues and otoliths as natural markers to evaluate cross-shelf nursery production for juvenile snappers utilizing back-reef habitats in southern Belize. Tissue stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were used as dietary markers to identify the source(s) of organic matter supporting juvenile snappers across the continental shelf (i.e. characterize trophic production within nurseries), while otolith stable isotopes ($\delta^{13}C$ and $\delta^{18}O$) were evaluated as a marker of nursery origin for juvenile snappers, which could facilitate quantitative contribution estimates of nursery-associated juveniles from various back-reef habitats to adult populations.

CHAPTER II

TISSUE STABLE ISOTOPES AS A NATURAL MARKER CHARACTERIZING FOOD WEB DYNAMICS AND TROPHIC PRODUCTIVITY IN TROPICAL BACK-REEF NURSERIES

Introduction

Trophic productivity is an important component of nursery production because the availability of organic matter at the base of local food webs can determine the density of consumers (i.e. juvenile fishes) that a given nursery can support (Malone et al. 1988; Polis et al. 1996, 1997), and as well as the relative fitness and survival of nursery occupants (Lloret and Planes 2003). Multiple laboratory experiments have demonstrated that condition and growth of juvenile reef fishes are strongly influenced by food availability (Kerrigan 1994; Booth and Hixon 1999; Booth and Beretta 2004), and in field studies, increased nutritional condition and faster somatic growth have been observed in nurseries characterized by productive hydrographic features (e.g. upwelling, riverine input) and high prey abundance (Anderson et al. 1995; Lloret and Planes 2003). Nutritional condition is directly related to competitive ability and survivorship in newly settled reef fishes, and individuals in good nutritional condition (exposed to favorable feeding regimes) can experience substantially lower predator-induced mortality compared to individuals in poor condition (subjected to food limitation) (Booth and Hixon 1999; Booth and Beretta 2004; Hoey and McCormick 2004; Figueira et al. 2008). Even among surviving fishes, physiological condition during early life larval and

juvenile development is known to directly influence individual fitness during later life stages (McCormick and Moloney 1992; Booth and Hixon 1999; Hoey and McCormick 2004). Consequently, trophic productivity within back-reef nurseries has the potential to directly impact subsequent recruitment success of nursery-associated juveniles into adult reef fish populations (i.e. nursery production).

Unlike terrestrial or lacustrine environments where primary productivity is isolated and the export of nutrients is constrained over fairly short distances (Tilman 1982; Persson and Johansson 1992), estuarine and marine ecosystems are hydrologically interconnected, allowing organic matter and nutrients to be transported over relatively large spatial scales (Chelton et al.1982). Coastal water masses often differ substantially in organic content, and the vertical and horizontal transport of allochthonous, or externally derived, organic matter within the water column is frequently a key determinant of habitat productivity and trophic structure in back-reef systems (Angel 1984; Barry and Dayton 1991). Subsidies of nutrients derived from the water column can benefit nursery-associated consumers indirectly by stimulating in situ production from local (autochthonous) sources of organic matter (i.e. bottom-up effects, Menge 1992; Roesemond et al. 1993; Hillebrand 2002), while allochthonous prey items and suspended organic matter delivered through riverine plumes can broaden foraging opportunities and increase the pool of terrestrial carbon available to consumers (i.e. top-down effects, Whitfield 1985; Thresher et al. 1989; Nemerson and Able 2004). Consequently, productivity within a given nursery can be strongly influenced by local hydrographic features. Riverine input in particular can substantially increase the

contribution of terrestrial nutrients to nearshore waters, and the role of riverine organic material in subsidizing estuarine food webs has been well documented (Mayer et al. 1998; Mannino and Harvey 2000; Gordon and Goni 2002).

Because estuaries are dynamic systems, the relative contribution of allochthonous inputs to nursery-associated consumers can be expected to show considerable spatiotemporal plasticity in response to variable climatic and hydrological conditions (Jennings et al. 1997). This is particularly true in the tropics, where pronounced wet-dry seasonality often leads to substantial differences in the volume of freshwater runoff and riverine discharge (i.e. terrestrial carbon and nutrients) entering back-reef systems (Bouillon et al. 2004). Fluctuations in river flow have been linked to seasonal shifts in dietary composition for consumers occupying back-reef nurseries, as juvenile fishes and invertebrates opportunistically exploit allochthonous prey items and organic material with limited seasonal availability (Connolly et al. 2009; Vinagre et al. 2011). In some cases, back-reef nurseries that are supported almost exclusively by locally produced organic matter during periods of low river discharge (i.e. dry season) may receive the majority of production from riverine detritus during periods of flooding (i.e. rainy season), potentially leading to seasonal variability in nursery function (Chanton and Lewis 2002; Le Pape et al. 2003). Significant increases in the density, condition, and survivorship of nursery-associated juveniles during times of high freshwater input have been well documented in both tropical and temperate estuaries, and these have been widely attributed to increased allochthonous nutrient delivery from riverine sources (Grimes 2001; Darnaude et al. 2004; Dolbeth et al. 2008).

Although freshwater inflow has the potential to enhance nursery productivity, back-reef nurseries that are strongly influenced by coastal runoff (including riverine discharge) are inherently vulnerable to anthropogenic disturbance, as trophic production within these nurseries will be strongly influenced by land use in adjacent watersheds (Douglas et al. 2005; Finlay 2011). Reductions in freshwater discharge due to river diversion (e.g. dams, water reclamation) can greatly decrease the allochthonous nutrient subsidy supporting consumers and may also create sub-optimal temperature and salinity conditions within back-reef systems, resulting in osmoregulatory stress and increased energy requirements for nursery occupants (Aleem 1972; Nichols et al. 1986). Additionally, trophic production from both allochthonous and local sources within back-reef nurseries can be negatively impacted by sedimentation and anthropogenic nutrient loading in coastal runoff, as elevated nutrient levels and decreased light penetration in the water column have the potential to alter the composition and/or productivity of primary producers (Loneragan and Bunn 1999; Tewfik et al. 2005). Because of the high land-sea connectivity in coastal waters, effective management strategies designed to preserve nursery production must keep intact not only the physical range of back-reef habitats utilized by juvenile invertebrates and fishes but also the essential nutrient cycles and sources of organic matter supporting nursery-associated consumers, and this requires a spatiotemporally explicit understanding of food web dynamics within back-reef systems.

The goals of the current study were to a) identify the main source(s) of production supporting three species of juvenile snappers at inner-shelf and outer-shelf

nurseries throughout the back-reef lagoon of southern Belize using tissue stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), b) quantify the relative contribution of producers during both dry season (i.e. low freshwater input) and rainy season (i.e. high freshwater input) conditions, and c) evaluate spatial and seasonal variability in the nutritional condition of juvenile snappers based on muscle lipid content. Belize is unique among Central American countries in that a large percentage of its coastline and a majority of keys throughout its back-reef lagoon still display substantial mangrove and seagrass coverage (Murray et al. 2003). This widespread distribution of mangroves and seagrass nursery habitat across the continental shelf makes the Belize coastal lagoon an ideal model system in which to evaluate the offshore extent of terrestrial nutrient subsidy to nursery-associated food webs because cross-shelf variability in organic matter contribution can be directly assessed. The Belize coastal lagoon also provides an ideal natural set-up to evaluate the effects of watershed impacts on food web dynamics within back-reef nurseries, as a large portion of the Toledo watershed in southern Belize is managed under the Maya Mountain Marine Corridor (MMMC), while land use and coastal development in northern watersheds are largely unregulated.

Methods

Study Site

The back-reef lagoon enclosed by the Meso-American Barrier Reef (MBR) in southern Belize is characterized by a complex reef system and an extensive network of small keys, which are arranged in lines along both the inner-shelf (< 10 km from

coastline) and outer-shelf (> 40 km from coastline), both running roughly parallel to the coast (Figure 1). Study sites were located in two latitudinal regions; North (offshore from Placencia) and South (offshore from Punta Gorda, approximately 50 km south of Placencia). Each region contained inner and outer study sites spanning the coastal-marine ecotone, for a total of four sites in the study: north inner, north outer, south inner, and south outer. Each of the four study sites was comprised of three replicate mangrove cays located within a 5-km radius. The two study regions (north, south) were selected to evaluate the effect of watershed characteristics (i.e. land usage, freshwater input) on trophic production across the continental shelf. The majority of the Toledo watershed (adjacent to the south study region) is managed under the MMMC, a network of terrestrial and aquatic reserves designed to mitigate anthropogenic runoff to the Belize lagoon. As a result, coastal development within this watershed is minimal and the majority of land area consists of natural vegetation. In comparison, land use within the Stann Creek watershed (adjacent to the north sampling region) is largely unregulated, and riverine systems influenced by this watershed have been increasingly impacted by anthropogenic nutrient input (runoff from agriculture and aquaculture, sewage discharge), as well as increased sedimentation due to bank reclamation and deforestation. The two sampling regions also differ markedly in precipitation; annual rainfall averages 370-400 cm in Punta Gorda (south), compared with only 200-300 cm in Placencia (north) (Ariola 2003; Thattai et al. 2003). As a result, the volume of freshwater discharge entering the coastal lagoon is substantially higher in the south sampling region.

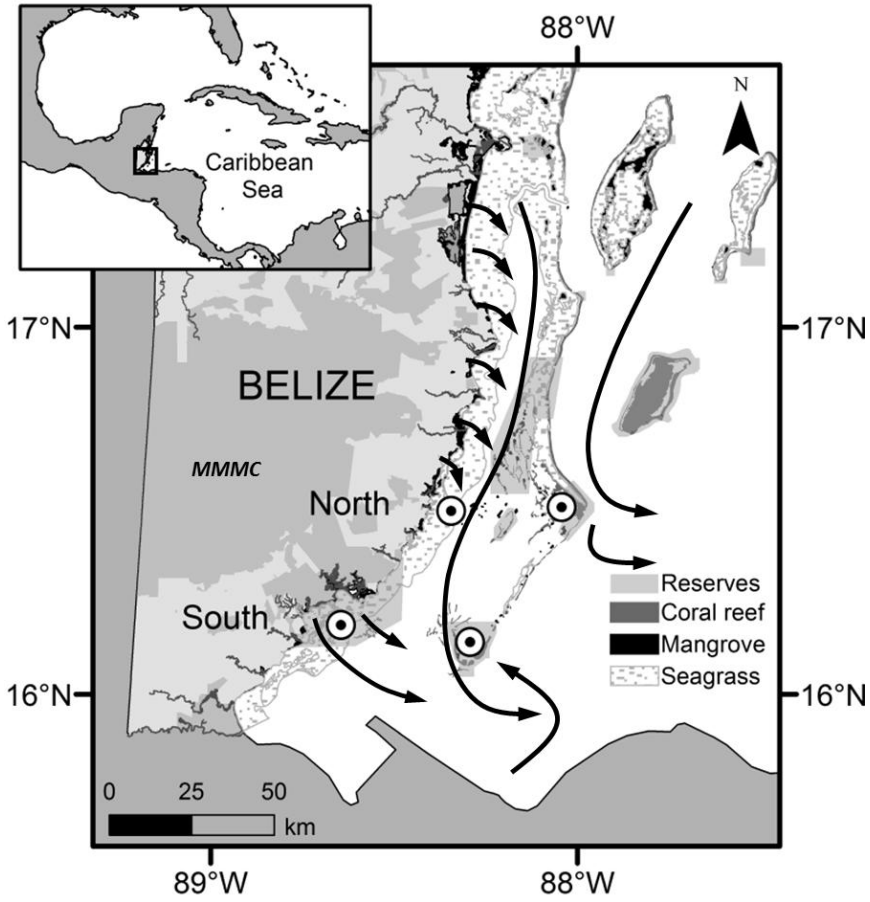

Figure 1. Map of study sites in southern Belize. Latitudinal regions are labeled (north, south) and study sites within each region (inner shelf, outer shelf) are indicated by circles. Shaded regions denote terrestrial and marine reserves (including the Maya Mountain Marine Corridor [MMMC]), as well as the spatial distribution of various habitat types across the continental shelf (see legend). Inset illustrates the regional context of the study location in the eastern Caribbean.

Sample Collections

Two sampling trips were conducted during March and July of 2009 to coincide with the dry season (February-April) and the rainy season (June-September) in the region. Four primary producers (phytoplankton, benthic diatoms, mangrove leaves, seagrass blades) and three species of juvenile snappers (dog snapper *Lutjanus jocu*, gray

snapper *L. griseus*, and schoolmaster *L. apodus*) were collected from four study sites (north inner, north outer, south inner, south outer) during each trip. For consistency, all samples were taken from keys with fringing mangroves and associated seagrass beds, and collections of primary producers and consumers (i.e., snappers) were limited to the mangrove prop-roots and the seagrass-mangrove ecotone (typically located within several meters of the prop-roots).

At each of the replicate mangrove keys, three representative samples of seagrass blades (turtle grass *Thalassia testudinum*) and fallen (i.e. detrital) red mangrove leaves (*Rhizophora mangle*) were collected manually, and three sediment collections were taken using a grab sampler in order to obtain benthic diatoms. Additionally, three surface tows using a plankton net (30-cm frame diameter, 333 μm mesh size) were conducted around the mangrove perimeter in order to collect samples of suspended particulate organic matter (POM). Phytoplankton is typically the major component of POM in many offshore marine systems (e.g. Hama 1999; Savoye et al. 2003), and isotopic values from POM samples are commonly used in food web studies as a proxy for the phytoplankton signature. However, this assumption is not necessarily valid in areas subject to coastal runoff, where POM may also contain a large fraction of terrestrial material (e.g. Savoye et al. 2003), or in shallow nearshore systems where large amounts of suspended plant or algal detritus are consistently present in the water column (Bouillon and Dehairs 2000; Bouillon et al. 2000; Miller and Page 2012). Thus, POM in the current study was not considered to be a direct proxy for phytoplankton, and isotopic values from samples

were later adjusted based on estimated phytoplankton content prior to interpretation of food web dynamics (Appendix A).

The three species of juvenile snappers (*Lutjanus jocu*, *L. griseus*, and *L. apodus*) were selected as model organisms for the study based on their ecological and commercial importance, as well as their strong association with mangrove-seagrass nurseries during the juvenile stage. All juvenile snappers were collected by microspear, and 15 individuals of each species were targeted at each study site, although *L. griseus* and *L. jocu* were less abundant than *L. apodus* and fewer than 15 juveniles of these two species were taken at some sites. To avoid possible discrepancies in isotopic signatures caused by ontogenetic shifts in feeding behavior, all juvenile fishes included in analyses were 70-130 mm total length (TL), or less than one year of age, as dietary shifts for juvenile snappers (*Lutjanus spp.)* typically occur at larger size classes (Cocheret de Moriniere et al. 2003; Faunce and Serafy 2007, Hammerschlag-Peyer and Layman 2012).

Sample Preparation

In the laboratory, POM samples were pre-filtered through a 118 μm sieve in order to minimize contamination from larger suspended particles (i.e. macrodetritus) and zooplankton, and then concentrated onto separate precombusted 0.7-micron Whatman filters using a vacuum system. Benthic diatoms were isolated from sediment collections using the vertical migration technique adapted from Couch (1989) and Wells et al (2008), and these were also stored on precombusted Whatman filters. All filtered

samples were immediately frozen and stored prior to stable isotope analysis. Juvenile fishes were measured to the nearest millimeter (total length), and two small sections of white trunk muscle tissue were removed from each specimen for lipid content and stable isotope analysis.

Although acid washing tissue samples in dilute HCl is commonly used to remove inorganic (i.e. non-dietary) carbonates, more recent research has suggested that the technique may also remove organic (i.e. dietary) compounds from algal, plant and animal tissue, and appears to disproportionately and unpredictably alter $\delta^{15}N$ values even though carbonates do not contain nitrogen (Bunn et al. 1995; Ng et al. 2007; Serrano et al. 2008). Consequently, in food web research involving multiple isotopes (i.e. carbon and nitrogen), it is widely recommended that acid washing be avoided and that muscle and/or plant tissue be isolated by manual dissection whenever possible. Multiple studies have demonstrated that after carbonate-rich parts have been removed, the effects of acid washing on $\delta^{13}C$ values in plant and animal tissue are ecologically negligible (e.g. Bunn et al. 1995; Serrano et al. 2008). In the current study, mangrove leaves and seagrass blades were carefully scraped with a spatula to remove all encrusting organisms and epiphytic algae, while samples of fish tissue were visually confirmed under a dissecting microscope to contain only white trunk muscle free of skin, scales, and bony fragments.

For obvious reasons, manual removal of carbonate components from benthic diatom and POM samples was not possible, and several previous studies have noted depletions (generally 0-1‰) in $\delta^{13}C$ for benthic microalgae (including diatoms) and phytoplankton-based POM following acidification. However, significant alterations in

$\delta^{15}N$ were also observed in these studies which were similar to or greater than the adjustments in $\delta^{13}C$, and the magnitude and variability of these effects were increased in filtered samples that were frozen prior to analysis (Lorrain et al. 2003; Ng et al. 2007). Given the relatively consistent observed effects of HCl treatment on $\delta^{13}C$ in marine primary producers (Ng et al. 2007), we elected not to acid-wash our filtered POM and benthic diatom samples to avoid introducing unnecessary (and unpredictable) error to our $\delta^{15}N$ values.

Stable Isotope Analysis

Tissue samples from all primary producers and juvenile fishes were thawed and then dried at 60°C for 24h. Dried tissue samples were powdered using a mortar and pestle, and packaged in tin capsules. For POM and benthic diatom samples on Whatman filters, samples were visually inspected under a dissecting microscope to confirm contents before six small (6 mm) hole punches were made from each filter paper and placed in a tin capsule. Scrapings from selected filters were also examined under light microscopy. Analysis of tissue stable isotopes was conducted at the Texas A & M University Stable Isotope Geosciences Facility, where a Finnigan MAT 252 stable isotope mass spectrometer attached to CosTech ECS 4010 Elemental Analyzer was used to determine the isotopic ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in each sample (Rooker et al. 2006; Wells et al. 2008). Results were compared to a Vienna Pee Dee Belemnite (VPDB) standard for carbon and atmospheric N_2 for nitrogen.

Because $\delta^{13}C$ in plant and animal tissue can vary with lipid content, physical extraction of lipids is sometimes performed in dietary studies prior to stable isotope analysis in order to minimize the effects of this variability among samples. However, processes used for lipid extraction can also significantly alter $\delta^{15}N$ values in fish muscle (Sotiropoulos et al. 2004; Logan and Lutcavage 2008), and previous studies have found the effects of lipid extraction on $\delta^{13}C$ to be negligible in tissue samples where lipid content is low (approximately 5% lipid, corresponding to a carbon content of < 40% for plants or a C:N ratio of < 3.5 for animal samples) (Post et al. 2007). All primary producers in the current study contained < 40% carbon with the exception of mangroves (46%), and while the mean C:N ratio of fish tissues was marginally higher (3.7; corresponding to an estimated lipid content of 6.1%) than the recommended 3.5 minimum for lipid extraction, applying the mathematical normalization recommended in Post et al (2007) would have resulted in a mean $\delta^{13}C$ enrichment of < 0.3‰ per sample across all study groups. Thus, similar to other dual-isotope food web studies where correction for lipids was considered unlikely to result in ecologically meaningful adjustments to $\delta^{13}C$ (e.g. Wyatt et al. 2012), we elected not to perform lipid extraction or mathematical adjustment on samples used in food web models.

Lipid Content Analysis of Fish Tissue

Small (~ one gram wet weight) samples of white trunk muscle tissue from juvenile snappers were also analyzed for lipid content in order to assess fish condition. Samples were thawed and dried at 60°C for 24h, then weighed to the nearest 0.0001

gram using a microbalance. Lipids were extracted from dried muscle tissue with petroleum ether using a Dionex ASE300 accelerated solvent extractor, and samples were re-weighed on the microbalance following lipid extraction. The lipid content (mg/g wet weight) in each tissue sample was calculated using the following equation:

$$\text{Lipid content} = \frac{\text{Pre extraction dry weight (mg)} - \text{Post extraction dry weight (mg)}}{\text{Pre extraction dry weight (g)}}$$

Characterization of Phytoplankton Content in Samples of POM

Given the high amount of plant-based detritus in samples of particulate organic matter (POM) in coastal systems (Miller and Page 2012), $\delta^{13}C$ and $\delta^{15}N$ values of POM samples were not used as a direct proxy for the phytoplankton signature. Instead, the proportion phytoplankton content in POM samples was estimated based on C:N ratio, and $\delta^{13}C$ and $\delta^{15}N$ values for phytoplankton were calculated based on methods adapted from Bouillon and Dehairs (2000; Appendix A). These estimated values are used hereafter to represent the phytoplankton signature (i.e. planktonic microalgae [PMA]).

Spatiotemporal Variability in Isotopic Signatures and Consumer Lipid Content

Three-way multivariate analysis of variance (MANOVA) was used to evaluate spatiotemporal variability in $\delta^{13}C$ and $\delta^{15}N$ for each of the four primary producers (phytoplankton, benthic diatoms, mangrove, seagrass), with study region (north, south), shelf position (inner, outer), and sampling season (dry, rainy) as the main effects. Spatiotemporal variability in isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of juvenile snappers

was evaluated using four-way MANOVA, with species, region, shelf position, and sampling season as the main effects. To evaluate potential variability in muscle lipid content, we used three-way analysis of variance (ANOVA), with species, study site, and season as the main effects. Because significant interactions were observed between study site and season, independent sample T-tests were used to compare mean lipid content between the dry season and rainy season for each species within a given site.

Since isotopic fractionation of $\delta^{15}N$ (and to a lesser extent, $\delta^{13}C$) in juvenile fishes is known to increase with size, linear regressions were used to examine the relationship between fish total length and isotopic values of $\delta^{13}C$ and $\delta^{15}N$ in tissue samples. No significant relationships between total length and isotopic signatures were detected for any of the three snapper species ($P > 0.05$), likely due to the small sizes (and narrowly constrained size range) of juvenile fishes included in the study. Simple linear regression was also used to test the relationship between muscle lipid content and total length, as lipid content will sometimes vary as a function of size in juvenile fishes. However, no significant relationships between total length and muscle lipid content were detected for any of the three species ($P > 0.05$) Thus, we determined that no size-correction factor was necessary. All data analyses were conducted in SPSS v. 19.

Evaluating Contribution from Primary Producers Using Isosource Models

Relative organic matter contributions from primary producers (phytoplankton, benthic diatoms, mangroves, seagrass) to juvenile snappers were estimated using a four-source dual-isotope $\delta^{13}C/\delta^{15}N$ model in the Isosource program (methods described in

Philips and Greg 2003). Estimates of producer source contribution were calculated individually for all three snapper species at each of the four study sites and for each species, two separate models were run using dry season and rainy season isotopic values, respectively. In Isosource models, producer signatures for phytoplankton, benthic diatoms and seagrass were calculated separately for each of the four study sites, while producer signatures for mangroves were pooled across the inner- and outer-shelf sites within each region. Source increments for all models were set at 1% and initial tolerance levels were set at 0.1‰ and increased incrementally up to a maximum of 0.5‰ if no feasible solutions were returned (i.e. consumer isotopic values fell outside the mixture polygon). For all but three models, tolerance levels of 0.1‰ returned feasible results.

$\delta^{15}N$ values in Isosource models were adjusted for isotopic fractionation using a trophic enrichment factor of 3.2‰, based on a recent meta-analysis of $\delta^{15}N$ fractionation rates in coastal marine food webs (Michener and Kaufman 2007). $\delta^{13}C$ values were adjusted using a trophic enrichment factor of 0.5‰, which was selected based on the average $\delta^{13}C$ fractionation rate reported in McCutchan et al. 2003, and also because it represents an intermediate value in the range of trophic enrichment factors (0-1‰) generally applied to carbon in food web models. In order to estimate the number of trophic levels separating producers from juvenile snappers (i.e. consumers), baseline $\delta^{15}N$ values (using the average $\delta^{15}N$ of all four primary producers) were calculated for each of the four study sites during the dry season and the rainy season, respectively (see Zeug and Winemiller 2008). For each study site and season, the differences in trophic

position between primary producers and each of the three snapper species were then estimated using the following equation adapted from Jepsen and Winemiller (2002):

$$\Delta \text{ Trophic position} = \frac{\delta 15\text{N}consumer \; - \; \delta 15\text{N}baseline}{3.2‰}$$

where $\delta^{15}N_{baseline}$ represents the baseline nitrogen value of producers (see above) for a given study site and season, $\delta^{15}N_{consumer}$ represents the mean nitrogen value of juvenile snapper tissue, and 3.2‰ represents expected the $\delta^{15}N$ enrichment per trophic level (i.e. trophic enrichment factor).

Due to high overlap in the isotopic signatures of seagrass and benthic diatoms, estimated organic matter contributions from these two primary producers were combined into a single source contribution using the a posteriori aggregation method described in Phillips et al. (2005). Combining these two sources was the most appropriate and conservative approach to use in estimating organic matter contribution given the high variability (and large associated error) observed in the isotopic signatures of benthic diatoms at most study sites. Interpreting data in this way was also reasonable from an ecological standpoint, as seagrass and diatoms both represent benthic sources of production, and would likely be utilized by consumers practicing a similar feeding mode. Thus, final Isosource models provide contribution estimates from three sources: phytoplankton, mangroves, and seagrass/benthic diatoms.

Results

Characterization of Phytoplankton Content in Samples of POM

During the dry season, estimated proportion phytoplankton content in POM (X_{phyto}) was highest at the south inner site (0.64) and lowest at the south outer site (0.05; Table 1). POM samples at both sites in the north (inner, outer) were similar in estimated phytoplankton content (0.32 and 0.33). In the rainy season, estimated phytoplankton content was highest at the north inner (0.47) and south inner (0.70) sites, and substantially lower at the north outer (0.17) and south outer (0.14). Estimated $\delta^{13}C$ values for phytoplankton (PMA) ranged from -17.9 to -27.1‰ in the dry season, and -20.1 to -31.3‰ during the rainy season. Estimated $\delta^{15}N$ values ranged from 0.9 to 5.2‰ during the dry season and 1.5 to 4.5‰ during the rainy season, with higher values generally observed at the inner-shelf sites.

Table 1: Estimates of phytoplankton content (X_{PMA}) and isotopic signature ($\delta^{13}C_{PMA}$, $\delta^{15}N_{PMA}$) in samples of suspended particulate organic matter (POM) collected from all four study sites during the dry season and rainy season (see APPENDIX A). These estimated values (in bold) are used in all subsequent analyses to represent the isotopic signature of phytoplankton (i.e. planktonic microalgae [PMA]).

			X_{PMA}	$\delta^{13}C_{POM}$	$\delta^{13}C_{SG}$	$\delta^{13}C_{PMA}$	$\delta^{15}N_{POM}$	$\delta^{15}N_{SG}$	$\delta^{15}N_{PMA}$
Dry	**North**	*Inner*	**0.33**	-13.45	-7.50	**-27.07**	2.12	2.40	**1.48**
		Outer	**0.32**	-13.21	-8.30	**-23.79**	2.49	3.30	**0.89**
	South	*Inner*	**0.64**	-14.08	-6.80	**-18.33**	4.14	2.30	**5.23**
		Outer	**0.05**	-8.64	-8.10	**-17.91**	1.19	1.20	**0.96**
Rainy	**North**	*Inner*	**0.47**	-19.14	-11.70	**-30.53**	2.46	0.90	**4.51**
		Outer	**0.17**	-11.63	-8.30	**-24.32**	1.48	1.40	**1.66**
	South	*Inner*	**0.70**	-24.17	-7.60	**-31.34**	3.98	3.00	**4.43**
		Outer	**0.14**	-9.87	-8.30	**-20.09**	2.25	2.40	**1.51**

Isotopic Signatures of Producers

Of the four primary producers (Table 2), only phytoplankton (PMA) and seagrass (SG) displayed significant spatial or seasonal variability in isotopic ($\delta^{13}C$ and $\delta^{15}N$) signatures. $\delta^{13}C$ values in PMA were significantly more depleted in the north study region (MANOVA; $P < 0.05$) and inner shelf position ($P < 0.05$), as well as during the rainy sampling season ($P < 0.05$). There was a significant interaction between shelf position and season, and observed dry-to-rainy season depletions in PMA $\delta^{13}C$ were more pronounced at inner-shelf ($\Delta \approx 12‰$) vs. outer-shelf ($\Delta \approx 0\text{-}3‰$) locations. During the dry season, mean $\delta^{13}C$ values of PMA at most sites (-17.9 to -23.8‰) resembled typical marine phytoplankton signatures in the tropics (≈ 20-22‰; Bouillon et al. 2008), while PMA at the north inner site was relatively depleted in $\delta^{13}C$ (-27.1‰) and likely influenced by a freshwater phytoplankton signature (≈ 32‰ in riverine systems; France 1995). During the rainy season, mean $\delta^{13}C$ values at the north and south inner sites (-30.5 and -31.3‰) both strongly resembled freshwater phytoplankton signatures, while mean $\delta^{13}C$ values at the north and south outer sites (- 24.3 and -20-9‰) remained similar to marine values.

PMA $\delta^{15}N$ values displayed significant regional, cross-shelf, and seasonal effects, with significant enrichment observed in the south study region (MANOVA; $P < 0.05$) and inner shelf position ($P < 0.05$), as well as during the rainy sampling season ($P < 0.05$). For $\delta^{15}N$, there was a significant interaction between season and region, and while overall $\delta^{15}N$ values were more enriched in the south (1.0-5.2‰ and 2.0-4.4‰

during the dry and rainy seasons, respectively), observed seasonal enrichment in $\delta^{15}N$ was most pronounced in the north, with isotopic values increasing from 0.9-1.5‰ during the dry season to 1.9- 4.6‰ during the rainy season. Outside of PMA, seagrass was the only other primary producer included in the study to show significant variability in $\delta^{13}C$ and $\delta^{15}N$. Seagrass $\delta^{13}C$ signatures displayed both cross-shelf and seasonal effects, with significantly depleted values observed at the outer shelf position (MANOVA; $P < 0.05$) and during the rainy sampling season ($P < 0.05$), Similar to PMA, dry-to-rainy-season depletions in $\delta^{13}C$ were more pronounced at inner-shelf ($\Delta \approx$ 2-4‰) vs. outer-shelf ($\Delta \approx$ 0-0.2‰) sites. Seagrass $\delta^{15}N$ signatures varied significantly only by season (MANOVA; $P < 0.05$); however, seasonal effects differed between study regions. In the north, seagrass $\delta^{15}N$ showed significant depletions from the dry season (2.4-3.3‰) to the rainy season (0.9- 1.4‰), while seagrass $\delta^{15}N$ in the south displayed an opposite trend, showing dry-to-rainy season enrichment (1.3 - 2.3‰ to 2.4 – 3.0‰). No significant regional, cross-shelf, or seasonal differences in $\delta^{13}C$ or $\delta^{15}N$ were observed for benthic diatoms (BMA) or mangroves (MG).

Isotopic Signatures of Consumers

$\delta^{13}C$ and $\delta^{15}N$ signatures of juvenile snappers differed significantly by region (MANOVA, $P < 0.05$), shelf position ($P < 0.05$), season ($P < 0.05$), and species ($P < 0.05$). However, despite inter-specific variability in $\delta^{13}C$ and $\delta^{15}N$ values, observed cross-shelf and seasonal trends in both isotopes were consistent among all three juvenile

snappers examined, with no significant interactions detected between species and either shelf position or season.

$\delta^{13}C$ values for juvenile snapper tissue throughout the study ranged from -11.4 to -16.8‰ (Table 2) and showed significant enrichment in fishes collected from the outer shelf position (MANOVA, $P < 0.05$) and during the rainy sampling season ($P < 0.05$), although no significant differences in $\delta^{13}C$ were detected between regions ($P > 0.05$). Seasonal effects were greatest at the south outer and north inner study sites, with observed dry-to-rainy season enrichments of 1.3-5.1‰ and 0.6-1.7‰ respectively, while seasonal enrichments in snapper tissue at the other two sites were less pronounced (0-0.2%). $\delta^{13}C$ also differed significantly among species (MANOVA, $P < 0.05$), with *Lutjanus jocu* displaying significantly depleted values compared to *L. apodus*, the species most enriched in $\delta^{13}C$ (Tukey HSD, $P < 0.05$).

$\delta^{15}N$ values for juvenile snappers in the study ranged from 9.3 to 11.7‰ (Table 2) and did not differ significantly by species (MANOVA, $P > 0.05$). However, $\delta^{15}N$ signatures did show regional, cross-shelf, and seasonal effects, with significantly enriched values observed in fishes from the south study region ($P < 0.05$) and inner shelf position ($P < 0.05$) and significant depletions observed in fishes collected during the rainy sampling season ($P < 0.05$). As we observed for $\delta^{13}C$, seasonal effects on juvenile snapper $\delta^{15}N$ were greatest at the south outer and north inner study sites, with observed dry-to-rainy season depletions of 0.9-1.5‰ and 0.6-1.1‰, respectively. Seasonal depletion in $\delta^{15}N$ was slightly less pronounced in fishes from the south inner site (0.5-

1.0‰), and juvenile snappers collected from the north outer site showed slight enrichment (+ 0.2-1.0‰), rather than depletion, in $\delta^{15}N$ during the rainy season.

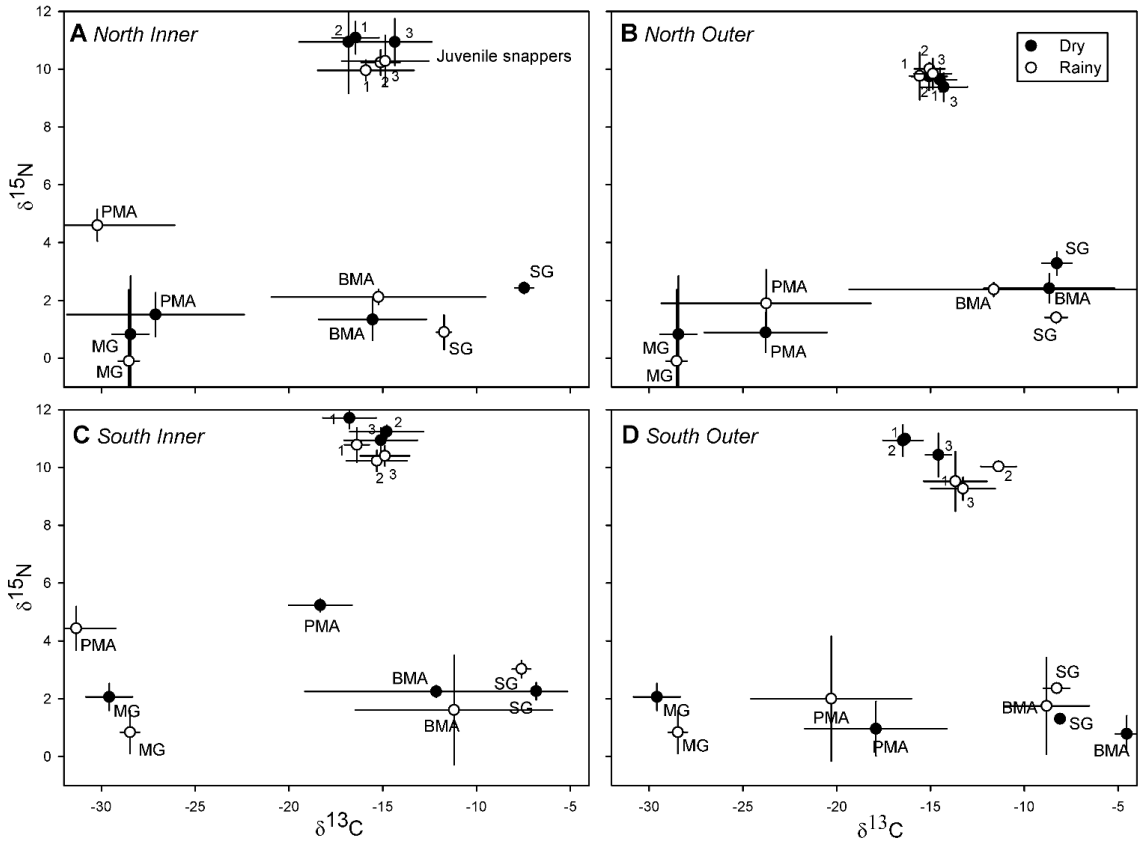

Figure 2. Mean tissue $\delta^{13}C$ and $\delta^{15}N$ values for primary producers (phytoplankton [PMA], benthic microalgae [BMA], mangrove [MG], seagrass [SG]) and juvenile snappers (dog snapper *Lutjanus jocu* [1], gray snapper *L. griseus* [2], schoolmaster *L. apodus* [3]) collected during the dry season (closed symbols) and rainy season (open symbols). Error bars represent one standard deviation from the mean.

Table 2: Tissue $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) for all primary producers (phytoplankton [PMA], benthic microalgae [BMA], mangrove [MG], and seagrass [SG]) and juvenile fishes (dog snapper *Lutjanus jocu*, gray snapper *L. griseus*, and schoolmaster *L. apodus*).

Region	Shelf	Sample	$\delta^{13}C$		$\delta^{15}N$	
			DRY	RAINY	DRY	RAINY
Producers						
North	Inner	PMA	-27.1 ± 4.7	-30.2 ± 4.1	1.5 ± 0.8	4.6 ± 0.6
		BMA	-15.5 ± 2.9	-15.2 ± 5.7	1.3 ± 0.7	2.1 ± 0.3
		MG	-28.4 ± 1.0	-28.5 ± 0.5	0.8 ± 2.0	-0.1 ± 2.5
		SG	-7.5 ± 0.5	-11.7 ± 0.4	2.4 ± 0.2	0.9 ± 0.6
	Outer	PMA	-23.8 ± 3.3	-23.8 ± 5.6	0.9 ± 0.7	1.9 ± 1.2
		BMA	-8.7 ± 3.5	-11.6 ± 7.7	2.4 ± 0.5	2.4 ± 0.2
		MG	-28.4 ± 1.0	-28.5 ± 0.5	0.8 ± 2.0	-0.1 ± 2.5
		SG	-8.3 ± 0.8	-8.3 ± 0.6	3.3 ± 0.4	1.4 ± 0.2
South	Inner	PMA	-18.3 ± 1.7	-31.3 ± 2.1	5.2 ± 0.2	4.4 ± 0.8
		BMA	-12.2 ± 7.0	-11.2 ± 5.3	2.3 ± 0.2	1.5 ± 1.9
		MG	-29.6 ± 1.3	-28.5 ± 0.5	2.1 ± 0.5	0.8 ± 0.7
		SG	-6.8 ± 0.3	-7.6 ± 0.5	2.3 ± 0.3	3.0 ± 0.3
	Outer	PMA	-17.9 ± 3.8	-20.1 ± 7.9	1.0 ± 0.9	2.0 ± 2.1
		BMA	-4.5 ± 0.6	-8.8 ± 2.3	0.8 ± 0.6	1.8 ± 1.7
		MG	-29.6 ± 1.3	-28.5 ± 0.5	2.1 ± 0.5	0.8 ± 0.7
		SG	-8.1 ± 0.1	-8.3 ± 0.7	1.3 ± 0.1	2.4 ± 0.1
Juvenile Snappers						
North	Inner	*L. jocu*	-16.5 ± 1.3	-15.9 ± 2.6	11.1 ± 0.6	10.0 ± 0.4
		L. griseus	-16.8 ± 2.6	-15.1 ± 1.0	10.9 ± 1.8	10.2 ± 0.4
		L. apodus	-14.4 ± 2.0	-14.9 ± 2.3	10.9 ± 0.8	10.3 ± 0.9
	Outer	*L. jocu*	-14.5 ± 0.9	-14.4 ± 1.4	9.6 ± 0.4	10.6 ± 1.1
		L. griseus	-15.1 ± 1.0	-15.0 ± 0.8	9.8 ± 0.5	10.0 ± 0.2
		L. apodus	-14.3 ± 1.3	-14.9 ± 1.0	9.4 ± 0.5	9.8 ± 0.5
South	Inner	*L. jocu*	-16.8 ± 1.4	-16.8 ± 0.7	11.7 ± 0.4	10.8 ± 0.6
		L. griseus	-14.8 ± 2.0	-15.3 ± 1.6	11.2 ± 0.2	10.2 ± 0.4
		L. apodus	-15.1 ± 2.0	-14.9 ± 1.3	10.9 ± 0.4	10.4 ± 0.4
	Outer	*L. jocu*	-16.4 ± 0.1	-13.7 ± 1.7	11.0 ± 0.2	9.5 ± 1.0
		L. griseus	-16.5 ± 1.1	-11.4 ± 0.9	10.9 ± 0.5	10.0 ± 0.1
		L. apodus	-14.6 ± 0.7	-13.3 ± 1.7	10.4 ± 0.7	9.3 ± 0.4

Organic Matter Contribution from Primary Producers

Isosource models consisted of two producers with enriched $\delta^{13}C$ values relative to juvenile snappers (i.e. seagrass [SG], benthic diatoms [BMA]) and two producers with $\delta^{13}C$ values that were depleted relative to snapper signatures (i.e. phytoplankton [PMA], mangroves [MG]; Figure 2). For the most part, Isosource estimates of producer contribution were similar for all three snapper species within a given study site and season, although observed seasonal variability in production was slightly less pronounced for schoolmaster *Lutjanus apodus* compared to *L. griseus* and *L. jocu*.

During the dry season, results from Isosource models clearly identified seagrass and benthic diatoms (SG/BMA) as the most important source of organic matter supporting juvenile snappers at three of the four study sites, with estimated minimum-maximum source contributions to each of the three snapper species ranging from 36 to 81% (median contribution estimates: 44 to 70%; Table 3). However, model results also indicate that fishes at these three sites during the dry season may receive considerable organic matter from phytoplankton-based sources in the water column (PMA), particularly at the north outer study site, where up to 52% of organic carbon supporting juvenile snappers is potentially derived from PMA (median contribution estimate: 31 to 33%). At the south outer site, Isosource models identified phytoplankton (PMA) as the most important source of organic matter supporting two of the three snapper species (*L. jocu, L. griseus;* estimated contribution 19 to 69%, median: 45%).

For two study sites (north outer, south inner), the primary sources of organic matter supporting juvenile snappers did not differ markedly from the dry season to the

rainy season (Figure 3b, 4a). In contrast, all three snapper species at the north inner and south outer sites (Figure 3a, 4b) displayed distinct seasonal shifts in producer source contribution; Isosource models for both of these locations indicated marked reductions in PMA production during the rainy season, accompanied by increased contribution from SG/BMA. At the north inner site, juvenile snappers received an estimated 48 to 81% (median: 52 to 70%) of organic matter from SG/BMA during the dry season and 70-91% (median: 78-83%) during the rainy season. Meanwhile, median contribution estimates for PMA among the three snapper species decreased by 2 to 20% from the dry to the rainy season. Shifts in organic matter production were even more pronounced at the south outer site, where contribution estimates for SG/BMA increased from 17 to 54% (median: 27 to 43%) during the dry season to 49 to 80% (median: 61 to 75%) during the rainy season, and median contribution estimates for PMA displayed a 14 to 33% seasonal decrease. Isosource did not identify mangrove detritus as a principle source of organic material supporting juvenile snappers at any of the four study sites in either dry season or rainy season models.

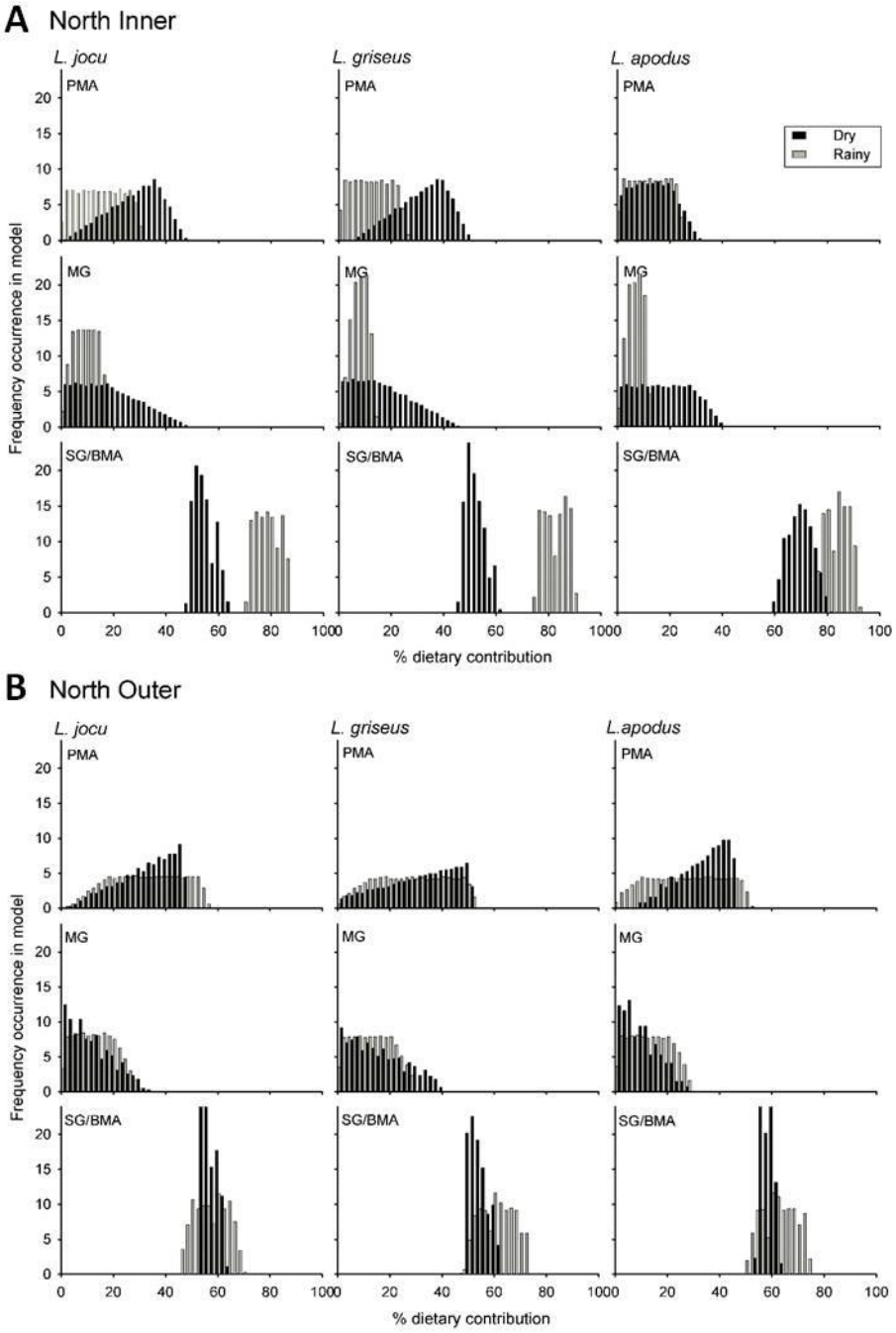

Figure 3. Results of Isosource models showing feasible percent source contributions from each primary producer (phytoplankton [PMA], mangrove [MG], seagrass/benthic microalgae [SG/BMA]) to juvenile snappers collected from the north inner (A) and north outer (B) study sites during the dry season (black) and rainy season (gray).

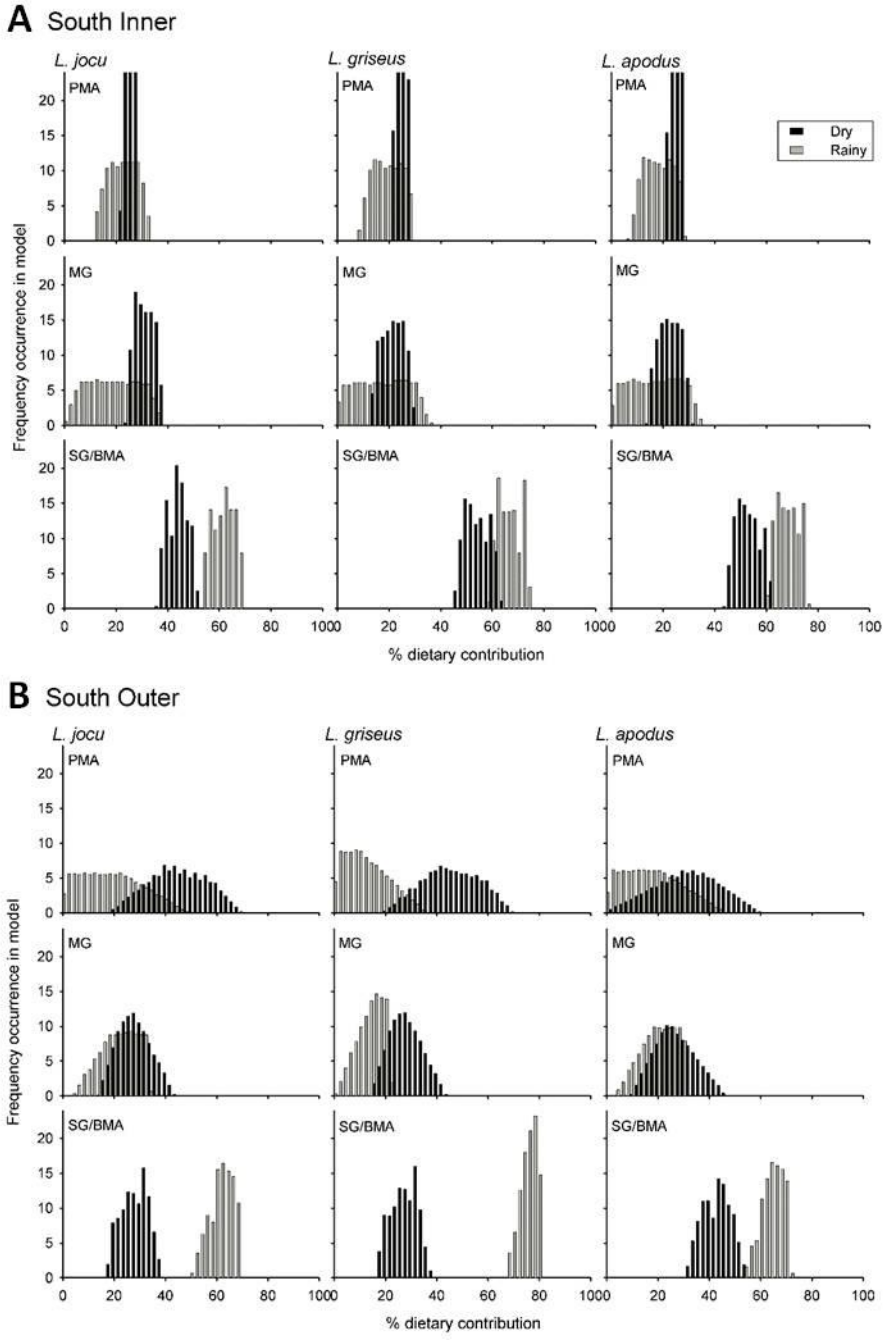

Figure 4. Results of Isosource models showing feasible percent source contributions from each primary producer (phytoplankton [PMA], mangrove [MG], seagrass/benthic microalgae [SG/BMA]) to juvenile snappers collected from the south inner (A) and south outer (B) study sites during the dry season (black) and rainy season (gray).

Table 3: Feasible organic matter contributions (range and mean ± SD) of primary producers for each species of juvenile snapper by region (north, south), shelf position (inner, outer), and sampling season (dry, rainy) based on Isosource models. Phytoplankton (PMA) and mangrove (MG) were considered as individual sources, while potential contributions from seagrass (SG) and benthic microalgae (BMA) were combined a posteriori into a single source category because of their similar isotopic values (Phillips et al. 2005; see METHODS). Primary producers likely to contribute a disproportionate amount of organic matter (mean > 33%) for a given species within a study site or season are highlighted in bold.

NORTH	**INNER**		**OUTER**	
L. jocu	***DRY***	***RAINY***	***DRY***	***RAINY***
PMA	3-47% (28 ± 10)	0-30% (14 ± 8)	4-47% (33 ± 11)	2-55% (31 ± 13)
MG	0-48% (18 ± 12)	0-16% (8 ± 4)	0-33% (11 ± 8)	0-29% (12 ± 7)
SG/BMA	**48-64% (54 ± 4)**	**70-86% (78 ± 4)**	**53-63% (57 ± 3)**	**45-69% (56 ± 6)**
L. griseus				
PMA	7-50% (32 ± 10)	0-25% (12 ± 7)	0-52% (31 ± 14)	0-52% (27 ± 14)
MG	0-45% (16 ± 11)	0-13% (7 ± 3)	0-39% (15 ± 11)	0-28% (12 ± 8)
SG/BMA	**46-61% (52 ± 3)**	**74-90% (81 ± 4)**	**48-61% (54 ± 3)**	**48-72% (60 ± 6)**
L. apodus				
PMA	0-31% (13 ± 8)	0-24% (11 ± 7)	9-46% (33 ± 9)	0-51% (26 ± 14)
MG	0-40% (17 ± 10)	0-12% (6 ± 3)	0-28% (9 ± 7)	0-28% (12 ± 8)
SG/BMA	**60-81% (70 ± 5)**	**75-91% (83 ± 4)**	**54-63% (58 ± 2)**	**49-73% (62 ± 6)**
SOUTH	**INNER**		**OUTER**	
L. jocu	***DRY***	***RAINY***	***DRY***	***RAINY***
PMA	22-28% (25 ± 2)	11-32% (22 ± 5)	**19-69% (45 ± 11)**	0-47% (18 ± 12)
MG	24-38% (31 ± 4)	0-36% (18 ± 10)	14-43% (28 ± 6)	4-33% (21 ± 7)
SG/BMA	**36-52% (44 ± 4)**	**53-68% (61 ± 4)**	17-38% (28 ± 5)	**49-68% (61 ± 4)**
L. griseus				
PMA	22-28% (25 ± 2)	7-28% (18 ± 5)	**19-69% (45 ± 11)**	0-34% (12 ± 8)
MG	13-30% (21 ± 4)	0-35% (16 ± 10)	15-43% (28 ± 6)	0-21% (13 ± 5)
SG/BMA	**45-63% (54 ± 4)**	**58-73% (66 ± 4)**	16-38% (27 ± 5)	**66-80% (75 ± 3)**
L. apodus				
PMA	22-28% (25 ± 2)	6-27% (13 ± 8)	0-59% (31 ± 13)	0-44% (17 ± 11)
MG	14-31% (23 ± 4)	0-26% (23 ± 8)	10-46% (27 ± 8)	3-30% (19 ± 7)
SG/BMA	**44-62% (53 ± 4)**	**62-75% (64 ± 1)**	**31-54% (43 ± 5)**	**53-71% (64 ± 4)**

Nutritional Condition of Consumers

Mean lipid content in juvenile snapper muscle tissue was similar among species (ANOVA, $P > 0.05$) but differed significantly by study site ($P < 0.05$) and season ($P < 0.05$). A significant interaction was also detected between study site and season. For all three species during the dry season, mean lipid content was highest at the north inner (range: 30.9-41.1 mg/g) and south outer (range: 25.2-42.5 mg/g) sites (Figure 5). Mean lipid content across the three species at the north outer site was slightly lower (range: 22.0-32.0 mg/g), while snappers collected from the south inner site displayed significantly lower lipid content (*L. jocu* = 16.3 mg/g, *L. griseus* = 15.2 mg/g, *L. apodus* = 13.7 mg/g) than fishes collected from any of the other three locations (Tukey HSD, $P < 0.05$).

During the rainy season, mean lipid content of each species was significantly higher at the north outer site than at any of the other locations (*L. jocu* = 34.1 mg/g, *L. griseus* = 22.3 mg/g, *L. apodus* = 27.6 mg/g), while mean lipid content across the three species at the two inner-shelf sites was similar: north inner (16.6-18.5 mg/g), south inner (13.7-18.7 mg/g). Mean lipid content of all three species from the south outer site was significantly lower during this season (*L. jocu* = 3.7 mg/g, *L. griseus* = 4.0 mg/g , *L. apodus* = 6.0 mg/g) than at any of the other sites in the study, which is notable because lipid content at this site during the dry season was the second highest among the four locations (Tukey HSD, $P < 0.05$).

At the two sites where significant seasonal shifts in producer contribution were observed in Isosource models (north inner, south outer), all three species of snapper

displayed significant decreases in muscle lipid content from the dry season to the rainy season (Student's T-test; $P < 0.05$). Conversely, at the two sites where Isosource producer contribution was relatively consistent between the dry and rainy season (north outer, south inner), muscle lipid content did not differ significantly between seasons for any of the three species (Student's T-test; $P > 0.05$).

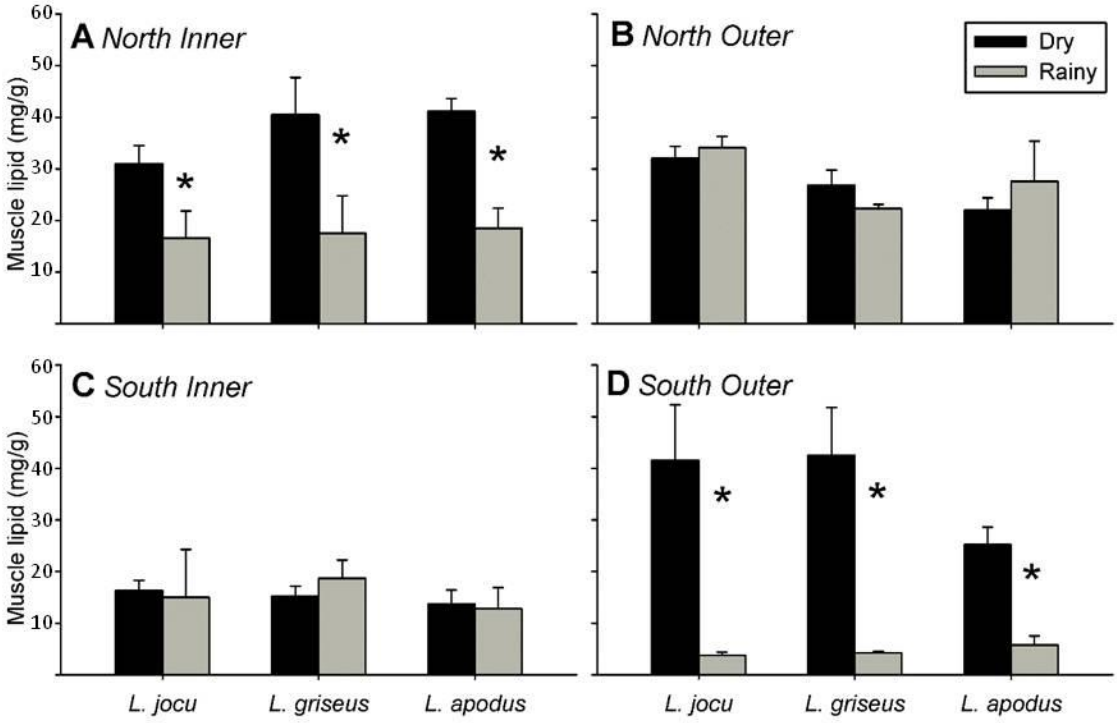

Figure 5. Seasonal comparison of muscle lipid content (mean ± SE) at each of the four study sites for dog snapper (*Lutjanus jocu*), gray snapper (*L. griseus*), and schoolmaster (*L. apodus*). Within a given site, asterisks denote species that displayed significant decreases in mean lipid content from the dry season to the rainy season.

Discussion

Similar to other estuarine systems (see Fry 1999), observed spatiotemporal variability in producer signatures in the current study appeared to be strongly related to

freshwater inflow to the Belize coastal lagoon. Depleted phytoplankton $\delta^{13}C$ signatures observed at inner-shelf study sites indicate that freshwater influence is strongest in back-reef nurseries directly adjacent to the coast, while depletions in $\delta^{13}C$ observed during the rainy sampling season indicate a shift in dominance from marine to freshwater phytoplankton during periods of high freshwater input (France 1995). The concurrent enrichments in phytoplankton $\delta^{15}N$ signatures that were also observed from the inner shelf position and rainy sampling season suggest that anthropogenic nutrient loading within back-reef food webs may be strongly related to freshwater input to the lagoon (McClelland and Valiela 1998). Enriched $\delta^{15}N$ signatures of aquatic organisms have been well established as a reliable biogenic indicator of anthropogenic eutrophication in estuarine systems (Hannson et al. 1997; McClelland and Valiela 1998; Fry 2013), and previous research characterizing riverine food webs in southern Belize has demonstrated that $\delta^{15}N$ signatures of consumers and producers from river basins influenced by agricultural runoff are significantly enriched relative to individuals collected from pristine watersheds (Winemiller et al. 2011). However, assessing anthropogenic influence based on variability in the $\delta^{15}N$ isotope can be complicated because the effects of nutrient loading on producer signatures can vary based on the source of nutrients in coastal runoff. For example, artificial fertilizers are isotopically light in $\delta^{15}N$ (≈ 0 ‰) relative to most organic nutrients, and thus often cause substantial depletion, rather than the expected enrichment, of $\delta^{15}N$ values in coastal producers (Derse et al. 2007). This may explain the opposite seasonal shifts observed for seagrass $\delta^{15}N$ between study regions, with dry-to-rainy season enrichment observed in the south (where sewage

discharge and organic fertilizers are the main sources of nutrient runoff), and dry-to-rainy season depletions observed in the north, where agriculture in adjacent watersheds is commercially developed and the use of chemical fertilizers is more widespread (Gibson et al. 1998; Winemiller et al. 2011).

Spatial variability in juvenile snapper $\delta^{13}C$ and $\delta^{15}N$ values also appeared to reflect gradients in freshwater input throughout the study area, with consistently enriched $\delta^{13}C$ values observed at outer-shelf locations, where marine influence is strongest, and consistently enriched $\delta^{15}N$ values observed at inner-shelf sites, where impacts of anthropogenic nutrient runoff are most direct. However, contrary to our initial predictions (i.e. that seasonal shifts in consumer signatures would be greatest at inner-shelf nurseries influenced by extensive coastal runoff), observed seasonal shifts in snapper $\delta^{13}C$ and $\delta^{15}N$ values did not appear to be directly related to the magnitude of freshwater input influencing a given location. For all three species, the largest dry-to-rainy season shifts in both $\delta^{13}C$ and $\delta^{15}N$ were consistently observed at the south outer and north inner sites, while minimal seasonal effects were observed at the other two sites (including the south inner, which received the largest volume of riverine discharge among collection locations). These results were unexpected given that seasonal variability in trophic production within coastal waters has been widely linked to seasonally pulsed riverine nutrient delivery (Darnaude et al. 2004; Fonseca et al. 2006; Dolbeth et al. 2008); however, the observed spatial patterns are likely linked to watershed dynamics and directional current flow within the study area (Figure 1). The north inner and south outer study sites are hydrologically connected by the prevalent

southward flowing current in the Belize lagoon, and perhaps more significantly, were the only sites in the study to be directly influenced by coastal runoff from multiple watersheds in the north, where urban development, commercial agriculture, and aquaculture are most expansive (Gibson et al. 1998; Heyman and Kjferve 1999). In contrast, the two sites where snapper isotopic signatures were relatively consistent (indicating similar sources of production across seasons) either received limited freshwater input (north outer) or received extensive freshwater input from pristine watersheds (south inner) managed under the Maya Mountain Marine Corridor. Thus, while seasonal food web dynamics in back-reef systems have been largely attributed to variable freshwater input, our results indicate that seasonality in producer contribution to nursery-associated consumers may be determined primarily by the source and quality of freshwater runoff (i.e. watershed impacts) rather than the volume of freshwater discharge delivered to a given location. Here, the two sites receiving substantial anthropogenic runoff from impacted watersheds represented the only two nurseries to display significant rainy season shifts in the dietary signatures of associated consumers.

The net effects of anthropogenic nutrient loading on coastal food webs are often unpredictable and can vary substantially across ecosystems (Loneragan and Bunn 1999; Marcarelli et al. 2011). When food is limiting, terrestrial nutrients delivered via coastal runoff can increase the pool of organic matter available to consumers by stimulating primary and secondary productivity in the water column (Finlay 2011), and in estuaries, elevated phytoplankton and zooplankton densities have been widely associated with riverine plumes (Grimes 2001). Still, increased nutrient availability will not result in a

net increase in nursery production unless organic matter derived from anthropogenic sources is efficiently assimilated into upper trophic levels and utilized by nursery-associated consumers (Grimes 2001; Davis et al. 2010; Finlay 2011). In estuarine systems where anthropogenic nutrients do contribute substantially to coastal food webs, enriched $\delta^{15}N$ signatures typically occur throughout all trophic levels (Hannson et al. 1997), and similar spatiotemporal patterns in both $\delta^{13}C$ and ^{15}N are generally observed between producers and consumers, reflecting seasonal and spatial variability in freshwater input (Winemiller et al. 2011). This was not the case in the current study; although PMA showed the expected rainy-season enrichment in $\delta^{15}N$ at the two study sites heavily influenced by anthropogenic nutrient runoff (north inner, south outer), all three juvenile snapper species at these two sites displayed significant rainy-season depletions in $\delta^{15}N$, suggesting that anthropogenic nutrients utilized by phytoplankton are not being well assimilated into the diets of consumers. Opposite directional shifts were also observed for $\delta^{13}C$ at these sites (rainy-season depletion in PMA but enrichment in juvenile snappers), further indicating that observed shifts in snapper dietary signatures in nurseries heavily influenced by anthropogenic runoff are driven by a seasonal shift in the source of primary production, rather than a case of juvenile snapper signatures simply tracking spatiotemporal variability in producer signatures (Melville and Connoly 2003) or assimilating terrestrial organic matter depleted in carbon (Bouillon et al. 2004).

Seasonal shifts in the source of organic matter supporting juvenile snappers at heavily impacted nurseries were further substantiated by the results of our Isosource models. Given that enhancement of primary and secondary production in the water

column is the chief mechanism by which riverine nutrient subsidies are thought to enhance trophic production for upper level consumers (Loneragan and Bunn 1999; Grimes 2001), we expected that juvenile snappers collected from nurseries influenced by high nutrient runoff would receive increased contribution from water-column based sources (i.e. PMA) during the rainy sampling season. However, results of Isosource models at both study sites where anthropogenic runoff is pronounced (north inner, south outer) reveal that observed seasonal shifts in snapper signatures at these two nurseries are driven by a seasonal decrease in PMA contribution during periods of high freshwater input, with most snapper species during the rainy season receiving a large majority of organic matter from benthic sources (SG/BMA). In freshwater systems, similar rainy season decreases in water column production have been widely attributed to shifts in phytoplankton community composition due to nutrient loading (Wootton and Power 1993; Davis et al. 2010; Marcarelli et al. 2011). Because elevated nutrient levels typically favor fast-growing, opportunistic producers, anthropogenic nutrient input may effectively decrease the pool of organic matter available to upper-level consumers by replacing producers that contribute to nursery food webs (e.g. diatoms) with high densities of undesirable prey species that are unpalatable or low in nutrient value (e.g. dinoflagellates, cyanobacteria) (Tewfik et al. 2005; Finlay 2011). While we did not directly measure phytoplankton biomass in our samples, estimated phytoplankton content (X_{PMA}) in suspended POM showed a 50-300% increase during the rainy season at both sites influenced by anthropogenic runoff (north inner, south outer), even though percent contribution estimates of phytoplankton-based organic matter to juvenile

snappers utilizing these nurseries during the rainy season was markedly reduced (Table 1). In contrast phytoplankton content at both the north outer and south inner sites, where phytoplankton production was relatively consistent across seasons, remained relatively constant or decreased during the rainy season. Recent food web research in freshwater systems has increasingly recognized the importance of nutrient quality, as well as nutrient quantity, in quantifying the net effects of spatial organic matter subsidies on ecosystem production (Marcarelli et al. 2011). Our results suggest that this approach may also be useful if applied in other estuarine and marine systems. Much of the existing research attempting to link riverine discharge to trophic production within back-reef nurseries has used phytoplankton content in the water column as an indicator of increased primary production within nursery food webs. However, based on our results, quantifying net production from allochthonous nutrient input based solely on producer abundance may be misleading, as decreased productivity from "less-desirable" producer species appears to offset or exceed any ecosystem benefits resulting from increased producer biomass in the water column.

Although increased allochthonous subsidy delivered through riverine input did not appear to enhance trophic productivity within nursery food webs at any of our four study sites, results from this study indicate that anthropogenic nutrient input from impacted watersheds has the potential to substantially decrease trophic productivity, and possibly nursery production, by disrupting the sources of organic matter supporting nursery-associated consumers. Similar to previous studies in freshwater systems demonstrating that even minor (< 5%) reductions in nutrient availability can result in

disproportionate decreases in fish growth (31%) and biomass (50%) (Nakano and Nakamuri 2001; Baxter et al. 2007), seasonal decreases in PMA contribution at both the north inner and south outer sites were accompanied by significant decreases in nutritional condition (based on muscle lipid content) for all three species of juvenile snappers. During the dry season, mean lipid content in juvenile snapper tissue at both of these sites (≈ 30-50 mg/g) was similar to published values for fully fed tropical reef fish juveniles in laboratory feeding experiments conducted at similar temperature and salinity conditions (Kerrigan 1994), while lipid content during the rainy season (≈ 3-18 mg/g) closely resembled the lipid content observed in starved fishes. Decreases in muscle lipid content appeared to be directly proportional to the magnitude decrease in estimated phytoplankton contribution, and the most pronounced seasonal decreases in juvenile snapper condition for all three species were observed at the south outer study site, corresponding to the largest seasonal shift in the sources of organic matter supporting nursery food webs. It is also notable that the two impacted nurseries (north inner, south outer) during the dry season displayed the highest muscle lipid content for most snapper species, suggesting that potential high quality nursery habitats in the region may be most negatively impacted by anthropogenic pollution.

CHAPTER III

OTOLITH STABLES ISOTOPES AS A NATURAL MARKER OF NURSERY ORIGIN FOR JUVENILE SNAPPERS IN BACK-REEF SYSTEMS

Introduction

Quantitative evaluations of nursery productivity require a reliable method of determining the origin of adult fishes. However, identifying suitable natural markers with the resolution to discriminate individuals from different nearshore nurseries has proved challenging (Gillanders 2005; McMahon et al. 2011). Chemical tags in biogenic hard parts such as otoliths (ear stones) have been used successfully to evaluate stock structure (e.g. Edmonds et al. 1989; Campana et al. 1994), large-scale movement (Rooker et al. 2008), and population connectivity (Yamashita et al. 2000; Rooker et al. 2010) of marine fishes. However, the spatial resolution at which otolith chemical signatures can be used as site-specific markers is limited by the amount of environmental variation in water chemistry among locations (Gillanders and Kingsford 1996; Thorrold et al. 1998), and the majority of studies that have successfully used chemical tags in otoliths to determine nursery origin have done so for nurseries that are separated by hundreds to thousands of kilometers (e.g. Leakey et al. 2008; Rooker et al. 2008; Ashford et al. 2011). Few studies have attempted to evaluate these chemical tags as a marker of nursery origin for habitats in close proximity (e.g. back-reef habitats in the tropics), and the effectiveness of these markers at smaller spatial scales has been variable among ecosystems (e.g. Hamer et al. 2003; Chittaro et al. 2005; McMahon et al. 2011).

The present study evaluates the utility of stable isotopes in otoliths as natural markers of nursery origin for three snappers in the family Lutjanidae (dog snapper *Lutjanus jocu*, gray snapper *L. griseus*, and schoolmaster *L. apodus*) that inhabit back-reef nurseries along the Mesoamerican Barrier Reef (MBR) in southern Belize. These three reef fishes were chosen as model species for analysis based on their ecological and commercial importance (Polunin and Roberts 1993; Nagelkerken et al. 2000), well-documented juvenile association with mangrove and seagrass habitat (Nagelkerken et al. 2001; Mumby et al. 2004), and widespread distribution throughout the Belize coastal lagoon, thus accommodating a sampling design that is spatially explicit yet consistent among habitat types. In the study, we evaluated the extent of regional (north vs. south) and ecotonal (inshore vs. offshore) variability in otolith stable isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) of these juvenile snappers, and examined whether isotopic signatures in otoliths can be used to reliably identify juvenile fishes collected from different back-reef nurseries within the coastal lagoon.

Methods

Study Area and Sampling Design

The study was conducted in the southern portion of the coastal lagoon enclosed by the Mesoamerican Barrier Reef (MBR) in Belize (Figure 1). The lagoon is characterized by a complex reef system and an extensive network of small keys, which are arranged in lines roughly parallel to the coast along the inner (5-10 km from coastline) and outer (> 40 km from coastline) continental shelf. Belize is unique among

Central American countries in that a large percentage of its 384 km coastline is still lined with fringing mangroves (primarily red mangrove *Rhizophora mangle*), and approximately 60 to 80% of keys throughout the lagoon (including areas of the inner to outer shelf) also have substantial mangrove and seagrass coverage (Murray et al. 2003). This unique habitat configuration makes the Belize coastal lagoon an ideal model system in which to evaluate the utility of otolith stable isotope ratios as a marker of nursery origin in reef fishes, as the widespread distribution of back-reef nursery habitats (mangroves and seagrass beds) across the continental shelf facilitates the assessment of large-scale ecotonal differences in these natural markers.

Sampling was conducted across two latitudinal regions in southern Belize. The reef system offshore from Port Honduras at the far southern edge of the MBR was designated as our south sampling region while the reef system off Placencia approximately 50 km to the north represented the north sampling region (Figure 1). Each region contained one inner-shelf study site (located within 10 km of the coast) and one outer-shelf study site (located at the edge of the MBR, ≈ 40 km offshore) for a total of four sites in the study (north inner, north outer, south inner, south outer). Each study site was comprised of three replicate mangrove keys situated within a 5-km radius.

There is a pronounced latitudinal gradient in precipitation in Belize (Heyman and Kjerfve 1999), and our two sampling regions were selected to evaluate the effects of differential freshwater input on otolith isotopic signatures across the continental shelf. Rainfall in Port Honduras (adjacent to the south sampling region) averages 3700-4000 mm annually (Thattai et al. 2003), compared to only 2000-3000 mm in Placencia

(adjacent to the north sampling region; Ariola 2003), and freshwater input resulting from terrestrial runoff and riverine discharge strongly influence the temperature, salinity, dissolved oxygen (DO), and dissolved inorganic carbon (DIC) content of coastal waters in the lagoon (Heyman and Kjerfve 1999; Thattai et al. 2003).

Field Collections

Juvenile snappers were collected during two sampling trips conducted in March and July of 2009, and all four study sites were sampled during each trip. All fishes were collected by microspear, and specimens included in the study were generally 60-120 mm total length (ca. one year of age or younger; Jones et al. 2010). This size was chosen as the experimental cut-off based on previous studies indicating that ontogenetic habitat shifts from mangrove and seagrass nurseries for *L. apodus* (Verweij et al. 2007; MacDonald et al. 2009), *L. griseus* (Faunce and Serafy 2007), and *L. jocu* (Moura et al. 2011), typically occur at larger sizes. From each of the four study sites, a subsample of 10 individuals for each species was targeted for otolith stable isotope analysis, although sample sizes were less than 10 for some species in the southern study sites (Table 4). During snapper collection trips, three replicate temperature and salinity measurements were taken at each study site using a Sonde 6920 Environmental Monitoring System (YSI Inc.).

Table 4: Summary of juvenile samples collected in 2009 from each study site, with results of otolith stable isotope analysis for dog snapper *Lutjanus jocu*, gray snapper *L. griseus*, schoolmaster *L. apodus*, and all species pooled (Total). Errors are reported as one standard deviation from the mean.

Site	***Species***		***Total length (mm)***		***Mean isotopic ratios (‰)***	
		n	**Mean**	**Range**	**$\delta^{13}C$**	**$\delta^{18}O$**
North Inner	***L. jocu***	10	73.1 ± 11.0	58-91	-4.57 ± 0.88	-1.78 ± 0.21
	L. griseus	10	83.2 ± 14.6	59-105	-5.55 ± 0.95	-1.55 ± 0.23
	L. apodus	10	74.9 ± 10.6	62-90	-4.30 ± 1.33	-1.90 ± 0.37
	Total	**30**	**77.1 ± 12.6**	**58-105**	**-4.81 ± 1.17**	**-1.74 ± 0.31**
North Outer	***L. jocu***	10	82.4 ± 14.0	58-104	-4.16 ± 0.98	-1.49 ± 0.25
	L. griseus	10	82.7 ± 11.1	60-96	-4.79 ± 0.66	-1.27 ± 0.16
	L. apodus	10	78.0 ± 6.3	70-86	-4.39 ± 0.90	-1.26 ± 0.17
	Total	**30**	**81.0 ± 10.8**	**58-104**	**-4.45 ± 0.87**	**-1.34 ± 0.22**
South Inner	***L. jocu***	7	101.0 ± 23.0	70-125	-5.96 ± 0.90	-1.93 ± 0.47
	L. griseus	10	95.9 ± 12.2	75-115	-5.46 ± 0.87	-1.98 ± 0.46
	L. apodus	7	76.7 ± 8.0	68-87	-6.43 ± 0.89	-2.20 ± 0.57
	Total	**24**	**90.9 ± 17.3**	**68-125**	**-5.89 ± 0.94**	**-2.03 ± 0.49**
South Outer	***L. jocu***	8	88.5 ± 29.1	53-132	-4.16 ± 1.34	-1.66 ± 0.23
	L. griseus	5	106.0 ± 10.8	88-116	-2.29 ± 1.40	-1.61 ± 0.35
	L. apodus	9	72.2 ± 8.5	60-85	-3.08 ± 0.86	-1.42 ± 0.25
	Total	**22**	**85.8 ± 22.6**	**53-132**	**-3.29 ± 1.34**	**-1.55 ± 0.28**

Otolith Stable Isotope Analysis

In the laboratory, fishes were measured to the nearest millimeter (total length) before sagittal otoliths were extracted. A single otolith (left or right) was selected randomly from each snapper and carefully cleaned to remove any residual tissue. In order to obtain the isotopic signature of the nursery period without including the signature from the pelagic larval phase, the portion of the otolith corresponding to the first six months of life following settlement was isolated using a drill path programmed into a New Wave MicroMill System (Figure 6). The template for this drill path was

based on measurements taken from sectioned otoliths of juvenile snappers ≈ 90 mm TL, which were determined to be approximately 210 days of age, and patterns were created separately for each of the three snapper species. Powdered otolith material was sent to the Stable Isotope Geosciences Facility at Texas A&M University, where $\delta^{13}C$ and $\delta^{18}O$ were measured using a KIEL-IV automated carbonate preparation device coupled to a Thermo Scientific MAT 253 Isotope Ratio Mass Spectrometer (IRMS). Ratios of $\delta^{13}C$ and $\delta^{18}O$ were reported relative to the Pee Dee Belemnite (PDB) following calibration against the international carbonate standard, NBS-19, and analytical uncertainties were reported as 0.04 ‰ for $\delta^{13}C_{PDB}$ and 0.06 ‰ for $\delta^{18}O_{PDB}$.

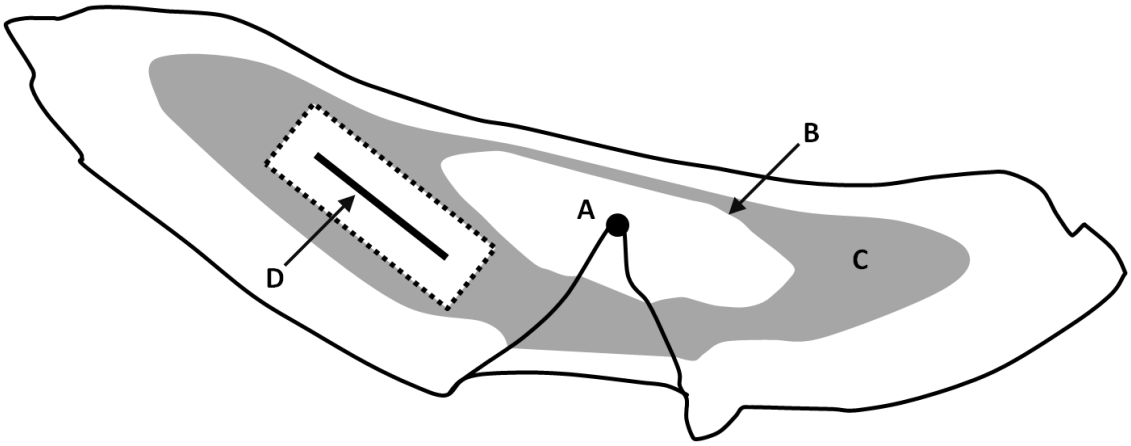

Figure 6. Cross section of juvenile snapper otolith showing the otolith core (A), settlement mark (B), nursery period (C) and Micromill drill pattern (D). Programmed drill pattern (D) was 150 µm in length, and samples were milled using a 500-µm diameter carbide drill bit. Dotted lines denote the actual area sampled from the milling pattern. Nursery period (C; shaded in gray) represents the area of the otolith corresponding to the first 30-210 days of life, or the six-month period immediately following settlement to the nursery.

Data Analysis

Spatiotemporal variability in water temperature and salinity was evaluated using three-way analysis of variance (ANOVA), with region (north, south), shelf position (inner, outer), and season (dry, rainy) as the main effects.

For each of the three snapper species (dog snapper, gray snapper, schoolmaster), two-way ANOVAs were conducted to evaluate regional and cross-shelf differences in otolith $\delta^{13}C$ and $\delta^{18}O$. Potential inter-specific differences in otolith signatures ($\delta^{13}C$, $\delta^{18}O$) were evaluated using multivariate analysis of variance (MANOVA), with species, region, and shelf position as the main effects. Quadratic discriminant function analysis (QDFA) was then used to determine the percent classification success (cross-validated) of juvenile fishes originating from different regions and shelf positions to their respective nursery areas. All data analyses were conducted in SPSS v. 19 and SYSTAT 13.1.

Results

Physicochemical Characterization of Study Sites

Seasonality in the climate of Belize is driven primarily by intra-annual variability in precipitation, and temperatures in most parts of the country remain relatively constant year round (Heyman and Kjferve 1999). This was strongly reflected in the environmental measurements taken during field collections (Table 5). Mean water temperature showed only minimal variability (~ 1°C) across all four study sites over the course of the study, and no significant differences in temperature were detected between

regions, seasons, or shelf positions (ANOVA, $F = 1.21$, $P > 0.05$). In contrast, salinity varied significantly by both region and shelf position, with higher salinity values observed at the outer shelf position (ANOVA, $F = 75.88$, $P < 0.001$) and in the north sampling region (ANOVA, $F = 30.01$, $P < 0.001$). Significant differences in salinity were also detected between the dry and rainy season, and this seasonal effect was most pronounced in the south (ANOVA, $F = 5.88$, $P < 0.05$).

Table 5. Environmental parameters measured at study sites during the dry season (March-April) and rainy season (July) field collections. Values are given as the mean (± standard deviation) of all three station measurements taken within each study site.

Temperature (C°)			
	Mar-Apr (Dry)	*July (Rainy)*	***Overall***
North Inner	31.3 ± 0.5	30.3 ± 0.4	**30.8 ± 0.7**
North Outer	29.3 ± 0.2	30.4 ± 1.0	**29.9 ± 0.9**
South Inner	30.6 ± 0.8	31.4 ± 0.9	**31.0 ± 0.9**
South Outer	30.1 ± 2.3	30.7 ± 0.6	**30.4 ± 1.5**
Salinity (ppt)			
	Mar-Apr (Dry)	*July (Rainy)*	***Overall***
North Inner	30.7 ± 1.2	30.7 ± 2.3	**30.7 ± 1.6**
North Outer	35.7 ± 0.6	36.0 ± 0.5	**35.8 ± 0.5**
South Inner	30.2 ± 0.3	28.3 ± 0.6	**29.3 ± 1.1**
South Outer	33.7 ± 1.2	30.7 ± 1.2	**32.2 ± 1.9**

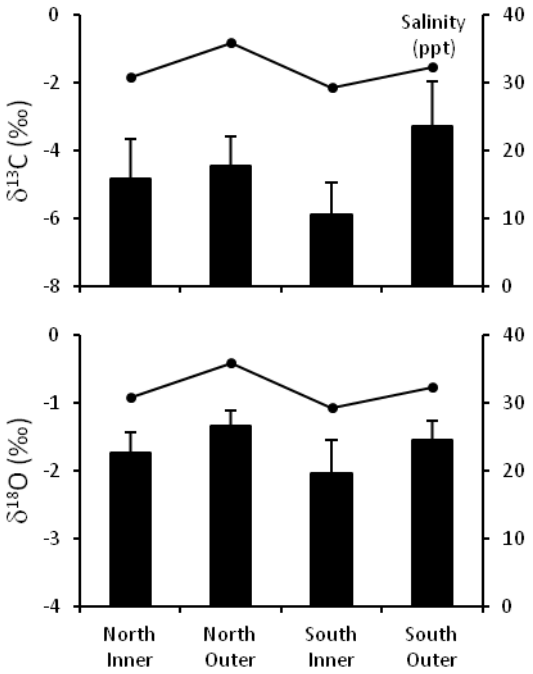

Figure 7. Mean otolith isotopic values (pooled across dog snapper *Lutjanus jocu*, gray snapper *L. griseus* and schoolmaster *L. apodus*) of $\delta^{13}C$ (A) and $\delta^{18}O$ (B) in relation to salinity. Error bars represent one standard deviation from the mean. Salinity values (solid line) represent the average of all salinity measurements taken at each study site during both the dry and rainy seasons.

Spatial Variability in Otolith Isotopic Signatures

For all three snapper species, both otolith isotopes ($\delta^{13}C$ and $\delta^{18}O$) showed significant enrichment in snappers collected from outer-shelf sites (Figure 8; ANOVA, $P < 0.05$). Otolith $\delta^{13}C$ signatures at inner-shelf sites ranged from -5.14 to -5.51‰ for each species, while values from the outer shelf were consistently higher (-3.77 to -4.04‰). Similar cross-shelf effects were observed for $\delta^{18}O$, and again, isotopic values at the inner shelf position (-1.76 to -2.02‰) showed consistent depletion relative to the outer shelf signatures (-1.34 to -1.54 ‰).

No significant region effect was detected for otolith $\delta^{13}C$ in any of the species investigated. However, otolith $\delta^{18}O$ did appear to be influenced by region, and all three species displayed significant enrichment in $\delta^{18}O$ in the northern sampling region (-1.26 to -1.90‰) relative to the south (-1.42 to -2.20‰). In general, spatial variability in isotopic signatures was relatively consistent across snapper species, and no significant differences in $\delta^{13}C$ or $\delta^{18}O$ values were detected (MANOVA; $P < 0.05$), suggesting that all three congeners included in the study reflected ambient water chemistry in a similar manner.

Classification of Juvenile Snappers to Nursery of Origin

Despite the similarities in otolith $\delta^{13}C$ and $\delta^{18}O$ values observed for juvenile snappers, discrimination among the four study sites based on otolith signatures varied by species. Overall cross-validated classification success was highest for schoolmaster (81%) followed by gray snapper (69%) and dog snapper (58%). Classification success for all three species was highest at the north outer study site: schoolmaster (100%), dog snapper (90%), gray snapper (80%) (Table 6). Cross-validated classification success to shelf position (i.e. inner vs. outer; pooled across sampling regions) was relatively high for both schoolmaster (92%) and gray snapper (89%), and slightly lower for dog snapper (74%). For all three species, classification success to shelf position was highest on the outer shelf: schoolmaster (100%), gray snapper (93%), dog snapper (83%).

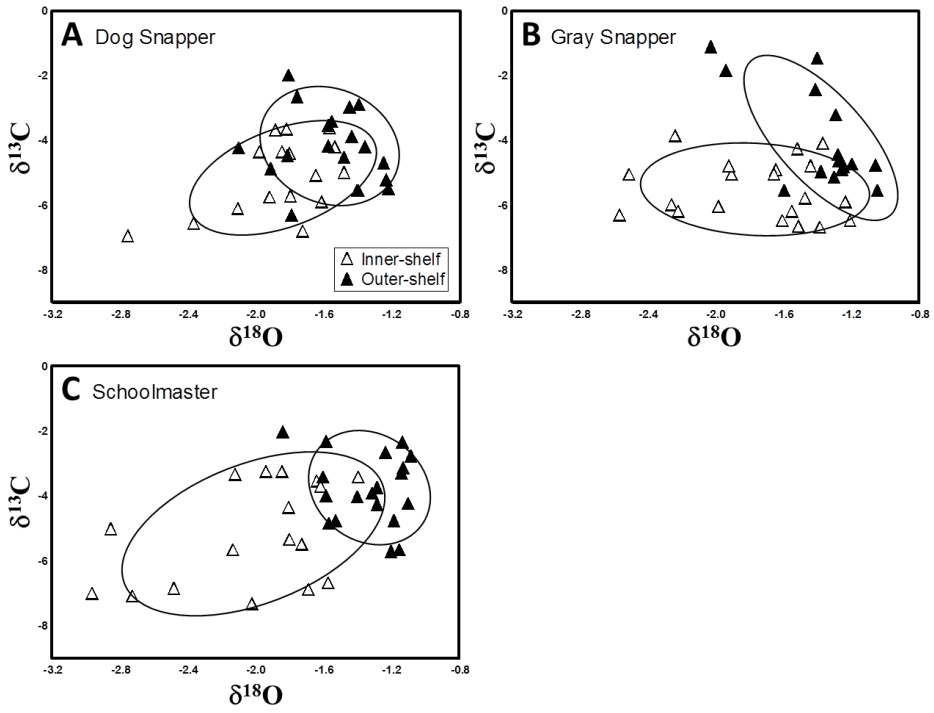

Figure 8. Otolith $\delta^{13}C$ and $\delta^{18}O$ for dog snapper *Lutjanus jocu* (A), gray snapper *L. griseus* (B), and schoolmaster *L. apodus* (C) by shelf position (Inner, Outer), with samples from the north and south sampling regions pooled. Ellipses represent one standard deviation from the mean for each shelf position.

Table 6: Classification success of juvenile snappers to study site and shelf position based on quadratic discriminant function analysis (QDFA) of otolith $\delta^{13}C$ and $\delta^{18}O$.

Classification success by study site				
	Species pooled	***L. jocu***	***L. griseus***	***L. apodus***
North Inner	43%	55%	60%	60%
North Outer	70%	90%	80%	100%
South Inner	58%	57%	60%	86%
South Outer	55%	25%	80%	78%
Total	**57%**	**58%**	**69%**	**81%**
Classification success by shelf position				
	Species pooled	***L. jocu***	***L. griseus***	***L. apodus***
Inner	76%	65%	85%	82%
Outer	90%	83%	93%	100%
Total	**83%**	**74%**	**89%**	**92%**

Discussion

Spatial variability in otolith $\delta^{13}C$ was similar for all three species of juvenile snappers examined, and appeared to be related to coastal hydrology and salinity gradients within the Belize coastal lagoon. Similar to previous laboratory and field experiments that have documented strong positive relationships between salinity and otolith $\delta^{13}C$ (e.g. Dufour et al. 1998; Elsdon and Gillanders 2002; Rooker et al. 2010), $\delta^{13}C$ values in the current study were consistently higher (more enriched) in juvenile snappers taken from outer-shelf nurseries, where salinity generally resembled marine conditions (32-36‰), and more depleted in snappers taken from inner-shelf nurseries, where lower salinity values (29-31‰) indicate stronger freshwater influence. Otolith $\delta^{13}C$ is determined primarily by dissolved inorganic carbon (DIC) content in the water column (Campana 1999), and here, significantly lower $\delta^{13}C$ signatures observed at inner-shelf nurseries likely reflect an influx of terrestrial DIC from riverine input and coastal runoff, which is typically depleted in $\delta^{13}C$ compared to marine sources (Boutton 1991; Chanton and Lewis 2002). Because a small proportion (~10-30%) of otolith carbon is metabolically derived (Campana 1999; Tohse and Mugiya 2008), it is also possible that observed variability in $\delta^{13}C$ may be influenced by dietary differences among study sites. However, outer-shelf food webs in back-reef systems are typically depleted in $\delta^{13}C$ due to increased contribution of coral-based organic matter (Verweij et al. 2008; Huijbers et al. 2013), and considering that otolith $\delta^{13}C$ signatures of snappers showed significant enrichment (rather than depletion) at the outer shelf position, it

appears that cross-shelf differences in otolith $\delta^{13}C$ were driven primarily by salinity gradients as opposed to metabolic (i.e. dietary) effects.

Similar enrichments in otolith $\delta^{18}O$ signatures were observed for all three juvenile snapper species at the outer shelf position, and it is likely that these were also strongly influenced by coastal hydrology and physicochemical water properties (i.e. salinity) across the Belize continental shelf. Unlike $\delta^{13}C$, $\delta^{18}O$ in biogenic carbonates (e.g. otoliths) is generally accreted at near equilibrium with ambient water conditions and is not significantly affected by diet (Thorrold et al. 1997). Temperature and salinity are the primary water column parameters influencing otolith $\delta^{18}O$ signatures in most systems (Campana 1999). However, given the low variability in water temperature occurring throughout our study area (Heyman and Kjferve 1999; also see Table 5), it is likely that, similar to $\delta^{13}C$, depleted $\delta^{18}O$ signatures observed at inner-shelf nurseries are driven primarily by offshore gradients in salinity; specifically, increased freshwater runoff and input of $\delta^{18}O$ depleted rainwater closer to the coast (Avery et al. 2006).

Variable salinity conditions may also explain the significant depletion in otolith $\delta^{18}O$ observed in the southern sampling region. Due to the strong latitudinal gradient in precipitation in Belize, annual rainfall in the Toledo watershed (south sampling region) is markedly higher than in the Stann Creek watershed (north sampling region), resulting in increased freshwater input to the lower portion of the lagoon (Heyman and Kjferve 1999). Over the course of the study, average salinity measurements were ~1.5-3.5‰ lower in the southern sampling region, while mean otolith $\delta^{18}O$ for each species showed an average depletion of ~ 0.1-0.4‰ from the north to the south. These results are

consistent with previous studies conducted in tropical systems that have reported a 0.11-0.14‰ enrichment in otolith $\delta^{18}O$ for each 1‰ increase in salinity (Craig and Gordon 1965; Dufour et al. 1998), indicating that regional (north to south) variability in otolith $\delta^{18}O$ for juvenile snappers in Belize is likely driven largely by a latitudinal gradient in salinity.

Despite the significant regional and cross-shelf differences in otolith $\delta^{13}C$ and $\delta^{18}O$ observed for all three species, the relatively low classification success to study site for dog snapper (58%) and gray snapper (69%) indicates that differences in ambient water chemistry among collection locations may not be distinct enough for stable isotopes to serve as reliable site-specific nursery markers in this system (Gillanders 2005). Still, consistently high classification success to shelf position for individual species (74-92%) suggests that otolith $\delta^{13}C$ and $\delta^{18}O$ are effective in discriminating between fishes from inner-shelf and outer-shelf nurseries in the Belize coastal lagoon. Classification success for all three species was highest to outer-shelf nurseries (83-100%), and this could reflect the more stable environmental conditions in the outer portion of the lagoon. Previous studies conducted in estuarine nurseries have found that variability in otolith isotopic signatures tends to be greater at sites where freshwater input is highly variable (e.g. Rooker et al. 2010), and in Belize, strong seasonal variability in rainfall is reflected by a two- to five-fold increase in riverine discharge during the rainy season (June-September) (Heyman and Kjerfve 1999; Cherubin et al. 2008). Because the milling patterns developed for each species reflect ~ 6 months of life following settlement, isotopic signatures are likely to include material accreted during

both dry season and rainy season conditions, potentially leading to increased isotopic variability and decreased classification success for fishes at inner-shelf sites, where the influence of coastal runoff is greatest.

Classification success also differed among species, with the highest discrimination observed for schoolmaster (92%), and this may be related to differences in habitat utilization during the juvenile stage. Schoolmaster are highly associated with mangroves during the first year of life and generally feed within the prop-roots (MacDonald et al. 2009; Hammerschlag-Peyer and Layman 2012), while gray snapper and dog snapper within the experimental size range are commonly found in mangroves but also frequently utilize and forage in other back-reef habitats (Bartels and Ferguson 2006; Faunce and Serafy 2007; Moura et al. 2011), which may increase variability in isotopic signatures. Regardless, moderate to high classification success for all three species suggests that otolith $\delta^{13}C$ and $\delta^{18}O$ represent useful markers for in quantifying the relative productivity of inner- and outer-shelf back-reef nurseries in Belize.

To date, few studies have provided empirical evidence linking putative nearshore nursery habitats supporting high densities of juvenile fishes to adult populations on the fore-reef (Beck et al. 2001; reviewed in McMahon et al. 2011). Given that otolith $\delta^{13}C$ and $\delta^{18}O$ is useful for distinguishing juvenile fishes originating from inner-shelf and outer-shelf nurseries, the technique has promise for evaluating cross-shelf contribution rates of different nurseries in the Belize coastal lagoon, and potentially other tropical back-reef systems. Most studies attempting to utilize otolith chemistry to determine nursery origin for tropical reef fishes have focused primarily on discriminating among

different types of nursery habitats (e.g. Gillanders and Kingsford 1996; Chittaro et al. 2004; McMahon et al. 2011), which has proved useful in reconstructing ontogenetic habitat use (e.g. Nakamura et al. 2008; Verweij et al. 2008; McMahon et al. 2011) as well as characterizing the aggregate nursery contributions of different habitat types (e.g. coral reefs vs. mangroves) on a regional or island-wide scale (Gillanders and Kingsford 1996; Mateo et al. 2010; Huijbers et al. 2013). However, while the majority of these studies identify back-reef habitats as important nurseries for reef fishes, efforts to identify the most productive nursery areas within a given habitat type have been largely unsuccessful (Chittaro et al. 2005, 2006). In back-reef systems such as Belize, where mangroves and seagrass beds are widely distributed across the continental shelf, the development of a natural marker with the resolution to quantify relative productivity between inner- and outer-shelf nurseries that are similar in habitat could have important implications for coastal management. The majority of conservation efforts in the Belize coastal lagoon are currently focused on outer-shelf mangrove keys based on their proximity to the Mesoamerican Barrier Reef (MBR), while coastal mangroves and inner-shelf keys have been increasingly threatened by deforestation, sedimentation, and anthropogenic runoff (Gibson et al. 1998; Cho 2005). The contribution of juvenile fishes from inner-shelf keys to adult reef fish populations on the MBR is poorly understood, and in the current study, it is notable that all three sub-adult fishes captured at outer-shelf study sites had isotopic nursery signatures (corresponding to the first year of life) that grouped strongly with inner-shelf sites, suggesting that movement offshore is likely occurring and the importance of inner-shelf nurseries may be overlooked.

CHAPTER IV

CONCLUSIONS

Production within coastal nurseries is determined by the density of juvenile fishes that a given habitat can support, the growth and survivorship of individuals within the habitat, and the successful movement of individuals from the habitat to adult populations. The present study evaluated nursery production for juvenile snappers across the Belize back-reef lagoon by (a) utilizing tissue stable isotopes to characterize trophic production within nurseries (which can strongly influence the density, growth, and survivorship of juvenile fishes) and (b) evaluating the utility of otolith stable isotopes as a marker of nursery origin (which can eventually be used to characterize the movement of nursery-associated juveniles to adult populations).

Results from the trophic component of this research revealed that primary production and food web dynamics within back-reef nurseries are strongly influenced by the hydrology of the Belize coastal lagoon, and particularly by the degree of anthropogenic impact in upstream watersheds. In other estuarine systems, allochthonous nutrient subsidies from coastal runoff and riverine discharge have been considered to increase productivity within back-reef nurseries by stimulating primary and secondary production in coastal waters and increasing the biomass of juveniles that can be supported in nursery food webs (Darnaude et al. 2004; Dolbeth et al. 2008). However, our results indicate that the net effects of freshwater nutrient subsidy may not be homogenous across all estuarine systems, and that coastal runoff from impacted

watersheds has the potential to significantly decrease production within back-reef nurseries by altering community composition of primary producers in the water column, resulting in decreased phytoplankton contribution to consumers during periods of high freshwater inflow. The pronounced seasonal shift in food web dynamics and juvenile snapper condition observed at our offshore (south outer) study site emphasized that the spatial extent of anthropogenic nutrient export can be unpredictable, and watershed impacts on downstream nursery production in Belize may extend across the continental shelf and throughout adjacent watersheds. Thus, effective efforts to conserve coastal nursery production in this region must preserve not only the physical nursery habitat but also the ecological processes supporting nursery-associated food webs, which may require mitigation of anthropogenic runoff to the back-reef lagoon.

Although the successful movement of nursery-associated individuals into adult populations is considered the most important component of nursery production (Beck et al. 2001), attempts to quantify nursery contribution within back-reef systems have been limited by the resolution of natural markers in discriminating individuals from different nearshore nurseries (Gillanders and Kingsford 1996). Results from the current research indicate that stable isotopes ($\delta^{13}C$ and $\delta^{18}O$) in otoliths of juvenile dog snapper, gray snapper, and schoolmaster are useful for quantifying productivity of inner- and outer-shelf nurseries in the Belize coastal lagoon, and may also be effective in other tropical back-reef systems with similar cross-shelf gradients in salinity. Although we found that otolith $\delta^{13}C$ and $\delta^{18}O$ did not consistently discriminate between our northern and southern study sites, using these isotopic signatures in conjunction with a

complementary natural markers (e.g. trace elements) in the future may improve the resolution at which this approach can be used to distinguish among nurseries (see Mateo et al. 2010). Otolith trace elements have shown to be an effective site-specific marker for juvenile and sub-adult fishes in Belize (68-85%) (Chittaro et al. 2004), and recent research has reported distinct differences in the elemental concentrations of coral cores taken from northern and southern portions of the Belize lagoon (Carilli et al. 2009), suggesting that elemental concentrations in otoliths may be useful in discriminating across latitudinal regions and may warrant future consideration.

As coastal development expands, characterizing productive back-reef nurseries and developing natural markers with the resolution to identify critical nursery areas is becoming increasingly important in Belize, where the commercial fishery is comprised primarily of species that utilize back-reef habitats as juveniles. Snappers (and other aggregation spawners) are particularly vulnerable to exploitation, and recent assessments of Belize fishery stocks have found that even smaller species not generally considered to be heavily targeted (e.g., schoolmaster) show indicators of overfishing (Babcock et al. 2013). If otolith stable isotopes or other natural markers can be used to reliably identify juvenile snappers to their nursery of origin, subsequent sampling of adult populations on the fore-reef, as well as individuals in spawning aggregations, will allow for the identification of nurseries most critical in replenishing adult stocks in Belize, which may aid in the management of sustainable fisheries in the region.

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APPENDIX A

CHARACTERIZATION OF PHYTOPLANKTON IN SAMPLES OF POM

A.1 Rationale

The isotopic signature of phytoplankton in field studies has been notoriously difficult to constrain, due to the technical difficulty involved with isolating phytoplanktonic organisms from other small particles (e.g. plant-based detritus) in samples of suspended particulate organic matter (POM) (summarized Miller and Page 2012). While commonly practiced in other aquatic systems, the direct substitution of POM isotope values as a proxy for the phytoplankton signature is not always valid in nearshore environments, where samples influenced by riverine input or coastal runoff often contain large amounts of terrestrial material (Bouillon and Dehairs 2000; Bouillon et al. 2000; Savoye et al. 2003), and samples taken near shallow, vegetated habitats (e.g. seagrass beds, mangrove creeks, kelp forests) are unavoidably contaminated with plant or algal detritus (Bouillon et al. 2008; Miller and Page 2012). Because reliable estimates of source contribution to upper-trophic level consumers are contingent on the availability of correct isotopic signatures for producers, the inability to obtain accurate isotopic values for coastal phytoplankton is considered to be one of the major barriers in the ecological interpretation of food web dynamics, primary production and nutrient cycling in mangroves and other nearshore ecosystems (reviewed in Bouillon et al. 2008; Miller and Page 2012).

The majority of studies using stable isotopes to characterize nearshore food webs have used $\delta^{13}C$ values of offshore phytoplankton (typically -18‰ − -22‰) taken from the literature. However, this can result in systematic overestimations of carbon contribution from autochthonous producers (e.g. kelp, mangroves) (Bouillon et al. 2008; Miller and Page 2012) and may obscure important seasonal and spatial trends in coastal productivity (Savoye et al. 2003), as well as potential variability in the importance of phytoplankton-based carbon to upper-level consumers (Bouillon et al. 2000, 2004). The use of a single $\delta^{13}C$ value to characterize food web dynamics is particularly problematic in estuarine systems (such as the back-reef lagoon in the current study). Because freshwater phytoplankton is generally depleted in $\delta^{13}C$ (≈ -32‰) relative to the marine signature (≈ -22‰) (France 1995), inshore-offshore gradients in the isotopic ratios of consumers in estuaries may be mistakenly attributed to increased contribution from $\delta^{13}C$-depleted primary producers (e.g. mangroves) at inshore sites, rather than the expected gradient in phytoplankton $\delta^{13}C$ which is usually not measured (see Chong et al. 2001; Bouillon et al. 2008). Despite this, due to the high (and variable) detrital content in collections of suspended POM, most food web studies in estuarine mangrove systems continue to use a single tropical offshore $\delta^{13}C$ value (-20‰ − -22‰) for the phytoplankton signature (Bouillon et al 2008), while others have omitted phytoplankton contribution from mixing models altogether (e.g. Melville and Connolly 2005).

The content of suspended POM samples is often qualitatively assessed using C:N molar ratios, which are typically around 6.6 for phytoplankton (i.e. Redfield ratio), but consistently higher (mean 20 - 22) for plants and algae (Atkinson and Smith 1983; Eyre

and Ferguson 2002). Although C:N values in plant material are variable among taxa, as well as by season, region and state of decay, POM samples with C:N values ≥ 12 are generally indicative of samples containing mainly terrestrial or plant-based detritus (Cifuentes et al 1996), while lower C:N values (≈ 6 - 8) are considered indicative of samples containing primarily phytoplankton (Bouillon et al. 2000; Savoye et al. 2003). Bouillon and Dehairs (2000) also proposed a quantitative method of estimating phytoplankton content in POM based on C:N values, and this was demonstrated to be relatively successful in deriving the isotopic signature of phytoplankton from the $\delta^{13}C$ values of bulk POM samples contaminated with variable amounts of terrestrial/mangrove detritus. However, because estimates of phytoplankton content and $\delta^{13}C$ using this method are subject to uncertainty in the C:N ratio and isotopic signature of detritus within a POM sample, the authors of the original paper submitted this technique as a tool useful in characterizing seasonal and spatial variability in the phytoplankton isotopic signature and "correcting for" the presence of detritus, but did not consider the estimated $\delta^{13}C$ values suitable for use in mixing models attempting to determine the exact contributions of phytoplankton-based production to upper-level consumers (Bouillon and Dehairs 2000). As a result, this method has been largely overlooked in food web research.

In the current study, mean $\delta^{13}C$ values of suspended POM samples collected during the dry season ranged from -13.5‰ to -14.0‰ at inshore sites and -8.6‰ to -13.2‰ at offshore sites, while the mean values of samples collected during the rainy season ranged from -19.1‰ to -24.1‰ inshore and -9.9‰ to -11.6‰ offshore (Table

A1). In general, these values are markedly enriched compared to the mean published $\delta^{13}C$ values for both marine (-22‰, range: -16‰ to -32‰) and freshwater (-32‰, range: -20‰ to -44‰) phytoplankton in the literature (France 1995), particularly considering the substantial riverine (i.e. freshwater) influence at our inshore study sites. Similar to studies conducted in other back-reef systems, discrepancies between $\delta^{13}C$ values in our POM samples and the expected phytoplankton $\delta^{13}C$ signature appear to be driven by large amounts of plant-based material in the water column. None of our POM samples had C:N ratios < 8, and only seven individual samples had C:N ratios < 10 (all of which were collected from inshore locations). Additionally, the highest C:N ratios (≈14 – 18) were consistently observed in samples with the most enriched $\delta^{13}C$ values, and these were almost exclusively collections taken at offshore sites where oligotrophic conditions would ostensibly result in lower densities of phytoplankton.

Unlike many other studies where high detritus content in suspended POM results in a depletion of $\delta^{13}C$ (relative to phytoplankton) and can be attributed to a number of potential sources (terrestrial, mangrove, algal, etc) (see Bouillon and Dehairs 2000), we can be reasonably confident here that the bulk of plant-based material in our samples originates from the dominant seagrass (*T. testudinium*). First, seagrasses are typically enriched in $\delta^{13}C$ (typically 12‰ - 16‰) relative to almost all other primary producers in mangrove systems (Bouillon et al. 2008), and in the current study, seagrass blades were the only producers with consistently more-enriched $\delta^{13}C$ values (-7.4‰ to -11.7‰) than those found in POM samples. In addition, visual inspection of filtered samples under dissecting and light microscopy revealed high densities of green and brown particles

which were identified as seagrass fragments, and the exceptionally low $\delta^{13}C$ values and high C:N ratios of POM samples collected at offshore study sites were nearly identical to the $\delta^{13}C$ and C:N of seagrass blades taken from the same locations (Table 1). Thus, because much of the potential error in estimated phytoplankton content (and $\delta^{13}C$) using the conversion equations in Bouillon and Dehairs (2000) stems from uncertainty in the origin of plant-based detritus in the water column, we felt that the use of this technique (with minor modification) was appropriate in the current study, where we are able to identify with relative certainty the primary source of suspended detritus in our samples.

A.2 Calculations

A.2.1 Estimating proportion phytoplankton content X_{Phyto} in samples of suspended POM

First, the phytoplankton content of each POM sample was estimated using the following nonlinear equation (adapted from Bouillon and Dehairs 2000):

$$X_{PMA} = \frac{(C_{SG} - C{:}N_{POM} * N_{SG})}{(C{:}N_{POM} * N_{PMA} - C{:}N_{POM} * N_{SG} + C_{SG} - C_{PMA})}$$

(Equation A1)

where $C{:}N_{POM}$ represents the observed carbon to nitrogen ratio of the POM sample, C_{SG} and N_{SG} represent the carbon and nitrogen content (g / g dry weight) of seagrass blades collected from the same study site (and during the same sampling season) as that POM sample, and C_{PMA} and N_{PMA} represent the theoretical carbon and nitrogen content (g / g

dry weight) of pure phytoplankton. For the sake of simplicity, carbon content (C) for all samples was standardized at 0.45 (see Bouillon and Dehairs 2000), and nitrogen content (N) was calculated to reflect either the observed (for seagrass) or theoretical (for phytoplankton) C: N ratio of each producer. In the current study, seagrass C:N ratios ranged from 19.3 – 21.5 (resulting in an N_{SG} of 0.021 - 0.024), while N_{PMA} for all calculations was set at 0.068, derived from the typical phytoplanktonic C:N ratio of 6.6 (i.e. Redfield ratio; see Atkinson and Smith 1983, Eyre and Ferguson 2002 and citations therein). X_{PMA} is expressed as the proportion of a POM sample comprised of phytoplankton, with a value of 1.0 denoting pure phytoplankton and a value of 0.0 denoting pure seagrass detritus.

A.2.2 Adjustment of POM $\delta^{13}C$ and $\delta^{15}N$ values to approximate phytoplankon isotopic signature

Based on estimated phytoplankton content, the isotopic signature ($\delta^{13}C_{PMA}$ and $\delta^{15}N_{PMA}$) of phytoplankton in each POM sample was then calculated using the following equation (originally developed for $\delta^{13}C$ by Bouillon and Dehairs 2000, but adapted here to estimate $\delta^{15}N$ as well):

$$\delta^{13}C_{PMA} = \frac{\delta^{13}C_{POM} - (1 - X_{PMA}) * \delta^{13}C_{SG}}{X_{PMA}}$$

(Equation A2)

$$\delta^{15}N_{PMA} = \frac{\delta^{15}N_{POM} - (1 - X_{PMA}) * \delta^{15}N_{SG}}{X_{PMA}}$$

(Equation A3)

where $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$ represent the actual isotopic values of carbon and nitrogen from the POM sample, $\delta^{13}C_{SG}$ and $\delta^{15}N_{SG}$ represent the actual isotopic values of carbon and nitrogen for seagrass blades collected from the same site/season as that POM sample, and X_{Phyto} represents the estimated proportion of the POM sample comprised of phytoplankton (calculated in Eq. A1). Input parameters used in all calculations of $X_{PMA,}$ $\delta^{13}C_{PMA}$ and $\delta^{15}N_{PMA}$ are summarized in Table A1. The estimated $\delta^{13}C_{PMA}$ and $\delta^{15}N_{PMA}$ values reported in Table A1 are used in all food web models to represent the phytoplankton isotopic signature (referred to as "phytoplankton" rather than "POM" from here on).

A.3 Evaluation of results and validity of estimated phytoplankton signatures

As emphasized by the original authors, the main drawback in using the above method to estimate phytoplankton content and isotopic signature in POM samples is uncertainty in assigning the C:N ratio and $\delta^{13}C$ values (and here, $\delta^{15}N$ values) of suspended detritus. However, we feel that in systems such as ours where the bulk of detrital material originates from a single source (i.e. *T. testudinium*), it is possible to remove much of this potential error through seasonally and spatially explicit sampling for both suspended POM and the primary detrital source. The sampling regime in the

current study allowed us to use season- and site-specific measured values for the C:N, $\delta^{13}C$ and $\delta^{15}N$ of *T. testudinium* in place of the single, generic estimates for detrital C:N and $\delta^{13}C$ used by Bouillon and Dehairs (2000), which were calculated based on the published average values for mangrove and terrestrial detritus in the region. The reluctance of the original authors to recommend $\delta^{13}C$ values calculated from these estimates for use in mixing models is understandable; in addition to showing substantial variability among taxa, C:N ratios in marine plants are known to show considerable intra-specific variability based on nutrient availability and ambient environmental conditions (Atkinson and Smith 1983), and even after identifying seagrass as the primary source of detritus in the current study, a single collection of *T. testudinium* would have been insufficient to capture spatiotemporal variability in the chemical composition of this producer. Consistent with the expectation that high-nutrient regimes should result in decreased C:N values in seagrasses (Atkinson and Smith 1983; Fourqurean and Zieman 2002), we found lower C:N ratios (as well as enriched $\delta^{15}N$ values) in seagrass samples collected from inshore study sites and during the rainy sampling season, and this variability was incorporated into our models (Table A1.1).

We were admittedly fortunate in the chemical properties of *T. testudinium*, which, similar to other seagrasses (see Harrison 1989), has been demonstrated in multiple studies to remain relatively unchanged in C:N ratio during the first 30-200 days of decay (Knauer and Ayers 1977; Rublee and Roman 1982; Fourqurean and Schrlau 2003), allowing us to use samples of fresh seagrass material to approximate C and N content in suspended detritus. In contrast, C:N ratios for many terrestrial plants are

greatly altered during bacterial decomposition, and uncertainty in the elemental C:N for suspended mangrove detritus (which commonly ranges from 12 - 100 and may be altered by > 50% during the first 45-60 days of decay) (e.g. Cifuentes et al. 1996; Dehairs et al. 2000; Fourqurean and Schrlau 2003) was another area of major concern for Bouillon and Dehairs (2000).

With spatiotemporally explicit sampling, $C:N_{PMA}$ is the only remaining input parameter used in our calculations that is estimated based on published values, and because the range of C:N ratios typically observed in both marine and freshwater phytoplankton is relatively small (≈ 6 – 8), the use of the Redfield ratio (6.6) is generally accepted in ecological models (Bouillon and Dehairs 2000). In the current data set, substituting a "maximum" planktonic C:N ratio of 8 in place of 6.6 would have resulted in an enrichment of 2 – 4‰ for estimated phytoplankton $\delta^{13}C$ and an enrichment of 0 – 0.7‰ for $\delta^{15}N$, but because these effects were relatively uniform among study sites and sampling seasons (and because the isotopic signatures of other primary producers in the study were so widely separated), the substitution of these isotopic values in mixing models did not substantially alter estimates of source contribution to juvenile fishes. Thus, we feel confident in our use of 6.6 as an approximation for $C:N_{PMA}$. Additionally, despite the minor assumptions required in assigning $C:N_{PMA,}$ one important advantage of using the above method is that because C:N ratios are similar between marine and freshwater phytoplankton (see Kendall et al. 2001, Savoye et al. 2003), calculations of $\delta^{13}C_{PMA}$ and $\delta^{15}N_{PMA}$ can be made without any assumption of phytoplankton composition or origin, allowing us to use estimated isotopic values to characterize spatial

and seasonal variability in riverine (i.e. freshwater) influence. In what is perhaps the most important test of model validity, estimated values for both $\delta^{13}C_{PMA}$ and $\delta^{15}N_{PMA}$ in all POM samples were reasonable from an ecological standpoint, falling well within the published ranges for marine and/or freshwater phytoplankton (France 1995, see above) and showing the expected depletion in $\delta^{13}C$ and enrichment in $\delta^{15}N$ (indicating freshwater influence/nutrient runoff) at inshore study sites, particularly during the rainy sampling season (Table A1.2).

After the above modifications in experimental design (reducing the number of assumptions required to generate input parameters), we feel that the potential error introduced by using estimated phytoplankton $\delta^{13}C$ and $\delta^{15}N$ values in our mixing models is minimal in comparison to the near-certain error that would have resulted from the more-common method of omitting $\delta^{15}N$ and using a single offshore phytoplankton signature for $\delta^{13}C$, particularly given the seasonally and spatially dynamic influence of riverine input and nutrient runoff within our study system (see Heyman and Kjerfve 1999; Bouillon et al. 2008). The two alternative approaches frequently taken in food web studies (i.e. using raw isotopic values from bulk POM as a proxy for phytoplankton, or omitting phytoplankton from mixing models altogether) would also have been highly likely to introduce substantial error if applied here, as both methods would leave mangroves as the only primary producer with more-depleted $\delta^{13}C$ values than those found in juvenile fish tissue, necessarily resulting in a substantial overestimation of mangrove source contribution (see Bouillon et al. 2008; Miller and Page 2012). Considering the limitations of the above options, we felt that mathematical correction for

detrital content in suspended POM samples, while not without fault, was the best available approach for approximating phytoplankton isotopic signature in the current study.

Table A1. Input parameters used in (1) the estimation of proportion phytoplankton (PMA) content in samples of suspended POM and (2) the estimation of phytoplankton (PMA) isotopic signatures.

1. Estimation of phytoplankton content in POM

			$C{:}N_{POM}$	C_{PMA}	N_{PMA}	C_{SG}	N_{SG}	X_{PMA}
Dry	**North**	*Inner*	12.22	0.45	0.068	0.45	0.022	**0.33**
		Outer	12.28	0.45	0.068	0.45	0.022	**0.32**
	South	*Inner*	8.80	0.45	0.068	0.45	0.023	**0.64**
		Outer	18.52	0.45	0.068	.045	0.022	**0.05**
Rainy	**North**	*Inner*	10.63	0.45	0.068	0.45	0.024	**0.47**
		Outer	14.79	0.45	0.068	0.45	0.023	**0.17**
	South	*Inner*	8.26	0.45	0.068	0.45	0.023	**0.70**
		Outer	16.71	0.45	0.068	0.45	0.021	**0.14**

2. Adjustment of phytoplankton isotopic signature from POM

			$\delta^{13}C_{POM}$	$\delta^{13}C_{SG}$	$\delta^{13}C_{PMA}$	$\delta^{15}N_{POM}$	$\delta^{15}N_{SG}$	$\delta^{15}N_{PMA}$
Dry	**North**	*Inner*	-13.45	-7.50	**-27.07**	2.12	2.40	**1.48**
		Outer	-13.21	-8.30	**-23.79**	2.49	3.30	**0.89**
	South	*Inner*	-14.08	-6.80	**-18.33**	4.14	2.30	**5.23**
		Outer	-8.64	-8.10	**-17.91**	1.19	1.20	**0.96**
Rainy	**North**	*Inner*	-19.14	-11.70	**-30.53**	2.46	0.90	**4.51**
		Outer	-11.63	-8.30	**-24.32**	1.48	1.40	**1.66**
	South	*Inner*	-24.17	-7.60	**-31.34**	3.98	3.00	**4.43**
		Outer	-9.87	-8.30	**-20.09**	2.25	2.40	**1.51**

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