COASTAL AND MARINE NITROGEN SOURCES SHIFT ISOTOPIC BASELINES IN PELAGIC FOOD WEBS OF THE GULF OF MEXICO

A Thesis

by

SAMUEL DORADO

Submitted to the Office of Graduate Studies of Texas A&M University
and the Graduate Faculty of The Texas A&M University – Corpus Christi
in partial fulfillment of the requirements for the joint degree of

MASTER OF SCIENCE

May 2011

Major Subject: Marine Biology

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Approved by:

Chair of Committee,	Antonietta Quigg
Committee Members,	Jay R. Rooker
	Anja Schulze
Head of Department,	Christopher D. Marshall

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ABSTRACT

Coastal and Marine Nitrogen Sources Shift Isotopic Baselines in Pelagic Food Webs of the Gulf of Mexico. (May 2011)

Samuel Dorado, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Antonietta Quigg

Upwelling, atmospheric nitrogen (N_2) fixation by cyanobacteria, and freshwater inputs from the Mississippi River system have been shown to stimulate new production by alleviating nitrogen (N) limitation in the northern Gulf of Mexico (GoM). Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes were used to investigate whether these sources are utilized differentially by coastal and marine pelagic food webs. Particulate organic matter (POM), *Trichodesmium*, and zooplankton were collected from the Mississippi River plume and Loop Current (LC) which were detected using remote sensing data. Stable isotope values were used to separate coastal and marine water masses and environmental data (salinity, nutrient and pigment concentrations) allowed me to relate variability to the degree of freshwater influence. Published food web data from these two environments were then assessed to establish whether isotopic baseline shifts observed in our data occur at an ecosystem level.

Isotope values of the POM and zooplankton were found to be significantly different between coastal and marine water masses. This was not the case for *Trichodesmium* whose isotope values were not significantly different between the two

water masses. We found that marine water masses (sal > 35) exhibited silicate concentrations, cyanobacterial pigments and DIN: P that suggest an increased abundance of diazotrophs. In contrast, coastal water masses (sal < 35) exhibited increased diatom pigments and molar C:N indicating terrestrial sources fuel phytoplankton production. When published food web data were compared, we found producer and consumer $\delta^{15}N$ values were enriched in the coastal compared to the marine environments.

This work suggests that differences in $\delta^{15}N$ values within my data set and published data reflect a shift in the use of biologically available N where higher trophic levels are sustained by diazotrophic activity in marine environments versus those supported by terrestrial sources in coastal ones. Food webs that have been constructed without considering *Trichodesmium* as a significant source of organic matter in the GoM should be reconsidered. By re-evaluating published data, this research gives insight into the early life ecology of larval fishes and works to help answer questions about the structure and function of pelagic food webs.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Antonietta Quigg, and my committee members, Dr. Jay Rooker and Dr. Anja Schulze for their guidance and support throughout the course of this research. Thanks also go to the Marine Biology IDP and faculty and staff for making my time at Texas A&M University at Galveston a great experience. I also want to extend my gratitude to all members of the Phytoplankton Dynamics Laboratory and the Fisheries Ecology and Ecosystem Research Laboratory who helped in the collection and processing of samples. Appreciation goes to the McDaniel Foundation, who provided substantial funding and the captain and crew of the R/V Lady Bride. Finally, thanks to my mother for her encouragement and support of my educational pursuits.

NOMENCLATURE

$\delta^{13}C$	Stable Carbon Isotope Abundance
$\delta^{15}N$	Stable Nitrogen Isotope Abundance
DIN	Dissolved Inorganic Nitrogen
GoM	Gulf of Mexico
HPO_4^-	Phosphate
LC	Loop Current
N	Nitrogen
N_2	Atmospheric Nitrogen
NH_4^+	Ammonium
NO_3^-	Nitrate
NO_2^-	Nitrite
nMDS	Non-Metric Multidimensional Scaling
MANOVA	Multivariate Analysis of Variance
POM	Particulate Organic Matter
PSI	Photosystem I
PSII	Photosystem II
RuBisCo	Ribulose-1,5-Bisphosphate Carboxylase Oxygenase
SSC	Sea Surface Chlorophyll *a*
SSH	Sea Surface Height
SiO_2	Silicate

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1. INTRODUCTION

The northern Gulf of Mexico (GoM) has been shown to serve as spawning, nursery and foraging habitat for ecologically and economically important fish species (e.g. billfishes, tunas, swordfish - Arnold 1955, McGowan & Richards 1989, Block et al. 2005, Teo et al. 2007, Rooker et al. 2008). The loop-hole theory by Bakun and Broad (2003) suggests that large pelagic fishes utilize nutrient depleted waters to spawn because larvae benefit from decreased predation. Primary production sustaining higher trophic levels in many marine environments is limited by the biological and physical processes which introduce new combined nitrogen (N) to the photic zone (Dugdale & Goering 1967, Eppley & Peterson 1979). It is therefore important to determine whether primary production from differential N sources functions as the underlying mechanism contributing to the success of these fish species in the GoM.

This important subtropical basin is a natural experimental system to study the use of multiple N sources because upwelling, atmospheric nitrogen (N_2) fixation, and freshwater discharge have been shown to enhance primary and secondary production by alleviating N limitation (Walsh et al. 1989, Biggs & Ressler 2001, Dagg & Breed 2003, Holl et al. 2007). In addition, N_2 fixation has been shown to be important during summer months (Holl et al. 2007) when the export of river water into offshore regions has been documented to regularly occur (Walker et al. 2005). The GoM is also an ideal system because distinct water masses can be detected using sea surface height (SSH) and sea surface chlorophyll *a* (SSC) maps generated from remote sensing data (Fig. 1;

This thesis follows the style of Marine Ecology Progress Series.

Zimmerman & Biggs 1999, Biggs et al. 2008). Finally, multiple food web models have been established using the natural abundance of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes which describe the success of fish assemblages in coastal and marine environments.

In these studies, phytoplankton (measured as particulate organic matter - POM) and *Sargassum* spp. (hereafter *Sargassum*) were identified as important producers that supply organic matter to higher trophic levels (Rooker et al. 2006, Wells & Rooker 2009). Similar methods have been applied to show N_2 fixation by *Trichodesmium* spp. (hereafter *Trichodesmium*) support large zooplankton assemblages in offshore regions of the Gulf (Holl et al. 2007). Depleted $\delta^{15}N$ values measured in producers and consumers from Wells and Rooker (2009) suggests N_2 fixation plays a role, but the significance of this process has yet to be determined. Here, POM, *Trichodesmium*, and zooplankton $\delta^{13}C$ and $\delta^{15}N$ values were measured from areas of the northern GoM where important larval and juvenile fishes are known to occur to assess variability in N sources in those environments. Meta-analysis of data which describe food webs in coastal and marine areas of the GoM was also performed to assess whether isotopic shifts observed in our data function at an ecosystem level.

This research aims to evaluate the relative importance of marine and freshwater sources of N in sustaining secondary production needed to maintain larval and juvenile fish populations in the GoM. This study also illustrates how ecosystem function within this basin can be attributed to the utilization of different N sources by the planktonic community and provide an example of how the products of diazotrophy and terrestrially

derived N shift isotopic baselines within one basin. Results help gain further insight into the early life ecology of larval fish and answer questions relating to resource acquisition in coastal and marine ecosystems. This work will help direct future research aimed to understand the underlying mechanisms contributing to the success of fish populations that spawn in oligotrophic environments.

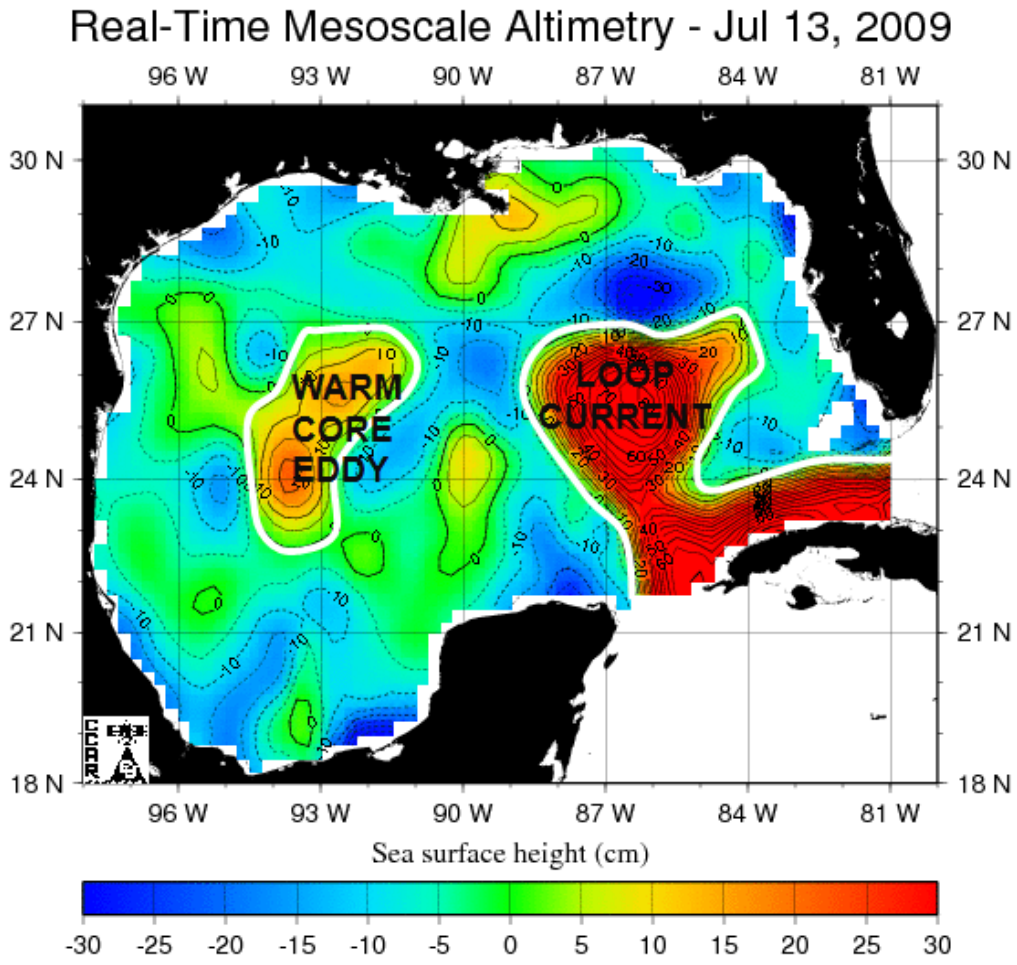

Fig. 1. Example of water mass detection. Positive sea surface height (warm colors) indicates the presence of the Loop Current and warm core eddies that are targeted during sampling efforts (modified from CCAR).

1.1 Nitrogen in the Gulf of Mexico

The major sources of new N to the GoM include advection of nitrate (NO_3^-) through the Yucatan Channel, N_2 fixation by cyanobacteria, and the discharge of sewage and fertilizer by rivers (Walsh et al. 1989). The Loop Current (LC) and Mississippi River discharge influence the physical structure and chemistry of surface waters (Nurnberg et al. 2008) which in turn have been shown to impact phytoplankton community composition and primary production (Qian et al. 2003, Rabalais et al. 1996, Lambert et al. 1999). The LC introduces subtropical marine waters from the Caribbean Sea to the GoM and acts as a biological conveyor belt, facilitating the exchange of pelagic species between basins. This feature dominates the eastern portion of the GoM, enters through the Yucatan Channel and exits through the Straits of Florida where it connects to the Gulf Stream and travels up the Atlantic coast of the continental United States. Surface waters to depths of 80-90 m are oligotrophic, where NO_3^- and phosphate (HPO_4^-) concentrations are below analytical detection (Walsh et al. 1989, Biggs & Ressler 2001) and the phytoplankton community is dominated by small sized phytoplankton and prokaryotic cyanobacteria such as *Trichodesmium* (Biggs & Ressler, 2001).

This genus has been shown to contribute up to 20% of the primary productivity in the Caribbean Sea (Carpenter & Price, 1977) and is considered globally important (Capone, 2001). This is because *Trichodesmium* are capable of enzymatically converting atmospheric N_2 to biologically available forms; a process termed diazotrophy. Enumeration of – and the measurement of N_2 fixation rates by

Trichodesmium have been recorded in virtually almost every major body of water where this genus occurs (Mulholland et al. 2006). *Trichodesmium* have been identified as organisms that alleviate N limitation in oligotrophic environments by converting N_2 to forms of N that are available to heterotrophic bacteria, other photoautotrophs and eventually higher trophic levels (Gilbert & Bronk, 1994). The N physiology of *Trichodesmium* and the effects it may have on other organisms were first described by Mulholland and Capone (2000) who reported that this cyanobacterium contributes to the overall N turnover in oceanic systems. This occurs directly through the release of amino acids, dissolved organic N, and ammonium (NH_4^+) and indirectly via the regeneration of dissolved inorganic and organic N by bacteria and grazers living in association with it.

Trichodesmium in the GoM is understudied, but has been documented during summer months when waters are warm, quiescent, and stratified (Holl et al. 2007). *Trichodesmium* abundance correlated with the distribution of warm core eddies across the North Atlantic Ocean (Davis & McGillicuddy, 2006), and eddies in the GoM may exhibit similar environmental conditions that favor their growth. *Trichodesmium* specific N_2 fixation rates measured by Mulholland et al. (2006), and Holl et al. (2007) in the GoM were similar to those measured by prior investigators in other oligotrophic gyres. This is supported by Brown et al. (2008) who concluded that capacities (based on the molar quantities of PSI, PSII, ATP synthase, RuBisCo, and nitrogenase) for carbon and N_2 fixation of natural populations of *Trichodesmium* in the GoM were comparable to populations in the North Atlantic Ocean and *Trichodesmium* strains grown in culture. *Trichodesmium* also has been shown to fuel blooms of *Karenia brevis* in Florida's

western coast (Jason et al. 2001, Walsh & Steidinger 2001, Mulholland et al. 2006) and documented to supplement organic matter to large zooplankton assemblages in the western GoM (Holl et al. 2007).

Finally, the hydrography of the GoM is influenced by freshwater discharge from the Mississippi and Atchafalaya rivers (Nurnberg et al. 2008) that introduce low salinity waters containing sediments, nutrients, and pollutants into the northern GoM (Walker et al. 1994). This freshwater plume has been categorized into three "fields" based on salinity and distance from shore that exhibit differences in production (Dagg & Breed 2003). Near-field waters are typically close to shore where salinities between 0 and 18 and primary production between 0 and 1 g-C m-2 d-1 have been measured. Here, there are adequate nutrients available for phytoplankton growth, but sediment originating in the Mississippi River limit light. Mid-field conditions occur when salinity ranges from 18 to 32; phytoplankton and bacterial growth rates approach their theoretical maximum (0.5 to 11.5 g-C m-2 d-1). This occurs because adequate nutrients are available and light limitation is relieved because highly turbid material falls out of the photic zone. Far-field environments are classified as having salinities greater than 32, low productivity (0.2 to 3 g-C m-2 d-1), and phytoplankton are thought to be limited by low nutrient concentrations.

The overall biological response in the Mississippi River plume driven by terrestrial N sources stimulates increased levels of phytoplankton production which in turn support high rates of bacterial production, protozoan and metazoan grazing, and fisheries production (Dagg & Breed 2003). Qian et al. (2003) found that phytoplankton

community composition changed in response to freshwater discharge where the relative abundance of groups such as diatoms and dinoflagellates increased in areas characterized by low salinity and increased NO_3^- concentrations. It has been shown that the increase in primary productivity near the Mississippi River is accompanied by an increase in zooplankton and fish abundances abundance (Ortner et al. 1989). The transport of these highly productive coastal waters to the oligotrophic environment is facilitated by the circulation patterns induced by the LC and presence of warm-core features. This can be visualized using SSC maps that depict fluvial waters exhibiting increased chlorophyll *a* biomass mixing with oligotrophic water masses lacking ocean color. Food webs between these seemingly different environments have been studied separately, but the impacts entrained coastal surface water has on the marine realm have yet to be established.

1.2 Current Food Web Models

$\delta^{13}C$ and $\delta^{15}N$ values have been used to describe food webs in many aquatic ecosystems. The assimilation of carbon dioxide during photosynthesis is subject to kinetic fractionation that causes distinct $\delta^{13}C$ values in plants (Post 2002). The $\delta^{13}C$ values of consumers show minimal fractionation during assimilation (0-1‰) and thus are often used to assess the relative importance of plant material or origin of organic matter sustaining various consumer groups. For consumers that utilize multiple sources of organic carbon, the relative importance of source(s) of organic matter can be calculated using mass balance equations (Post, 2002), and methods have been

developed to measure ranges of potential contributions if the system exhibits more than three sources (Phillips & Gregg 2003, Phillips et al. 2005).

In the GoM, important producers identified using the analysis of stable isotopes include marine phytoplankton and bacteria (measured as bulk POM), *Sargassum* and *Trichodesmium* (Macko et al. 1984, Rooker et al. 2006, Holl et al. 2007, Wells & Rooker 2009). Isotopes have also been measured from various consumers including zooplankton assemblages and isolated invertebrate spp. in addition to larval, juvenile, and adult fish species (Macko et al. 1984, Rooker et al. 2006, Holl et al. 2007, Wells & Rooker 2009). Cross-plots of $\delta^{13}C$ and $\delta^{15}N$ are generally used to describe food webs and several have been composed for the GoM (Fig. 2). Carbon source estimates derived from a two-source mixing model indicated that in a coastal offshore environment 78% of the organic matter supplied to consumers living amongst *Sargassum* communities is derived from the phytoplankton community in summer months (Rooker et al. 2006). The $\delta^{15}N$ values of the POM in this study ranged from approximately 6 to 8‰ which is enriched when compared to producer data from offshore marine environments where Wells & Rooker (2009) measured $\delta^{15}N$ values of the POM which ranged from approximately 1 to 3‰. Here it was estimated that POM and *Sargassum* contributed equally to the success of larval and juvenile fish species. This is also apparent in the consumer data, where $\delta^{15}N$ values of fishes from a coastal habitat (Rooker et al. 2006) range from approximately 11 to 16‰ compared to 2 to 9‰ measured in a marine environment (Wells & Rooker 2009).

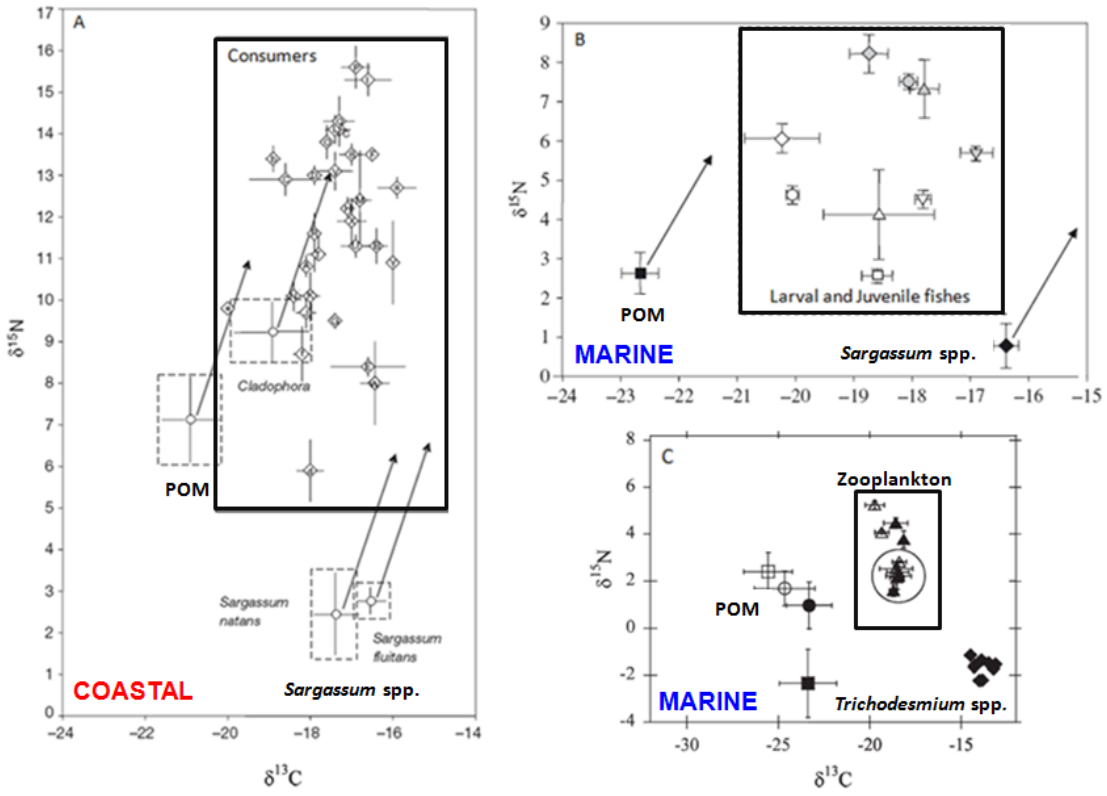

Fig. 2. Current food web models in the Gulf of Mexico. Producer and consumer data from (A) Rooker et al (2006) show juvenile and adult fishes are supported by phytoplankton (as POM) and *Sargassum*, (B) Wells & Rooker (2009) show POM & *Sargassum* supporting larval and juvenile fishes, and (C) Holl et al. (2007) show POM and *Trichodesmium* supporting zooplankton spp. (A) presents data from a coastal water mass while (B) and (C) present data from marine water masses. Note differences in $\delta^{15}N$ values of POM and *Sargassum* between the coastal and marine environments.

1.3 Ecosystem Wide Patterns of $\delta^{15}N$

Deep water NO_3^-, products of diazotrophy, and anthropogenic inorganic N each have been shown to exhibit unique $\delta^{15}N$ values (Fig. 3). The $\delta^{15}N$ value of deep water NO_3^- ranges between 3 and 6‰ with a global average of approximately 4.6‰ (Montoya, 2008). In the GoM, the $\delta^{15}N$ of deep water NO_3^- has been measured to be approximately 2‰ in the upper 200 to 600 m of the water column and approximately 4‰ below 600 m during summer months (Holl et al. 2007), which suggests that diazotrophy is important. This is because the $\delta^{15}N$ of marine N_2 fixing organisms such as *Trichodesmium* and diatom-diazotroph assemblages have a characteristic $\delta^{15}N$ values of -1 to -2‰ (Montoya 2008), and *Trichodesmium* collected in the GoM exhibits this depletion (Holl et al. 2007). Terrestrial $\delta^{15}N$ values of dissolved N in sewage, terrestrial runoff, and groundwater are typically enriched compared to that of the marine $\delta^{15}N$ signature, but vary due to differences in fractionation during the nitrification and volitization of NH_4^+ and denitrification of NO_3^- (Montoya, 2008). Data from a two year study described the POM from the Mississippi River as having an average $\delta^{15}N$ value around 7‰ (Wissel and Fry, 2005) which is enriched compared to that of POM from marine origins.

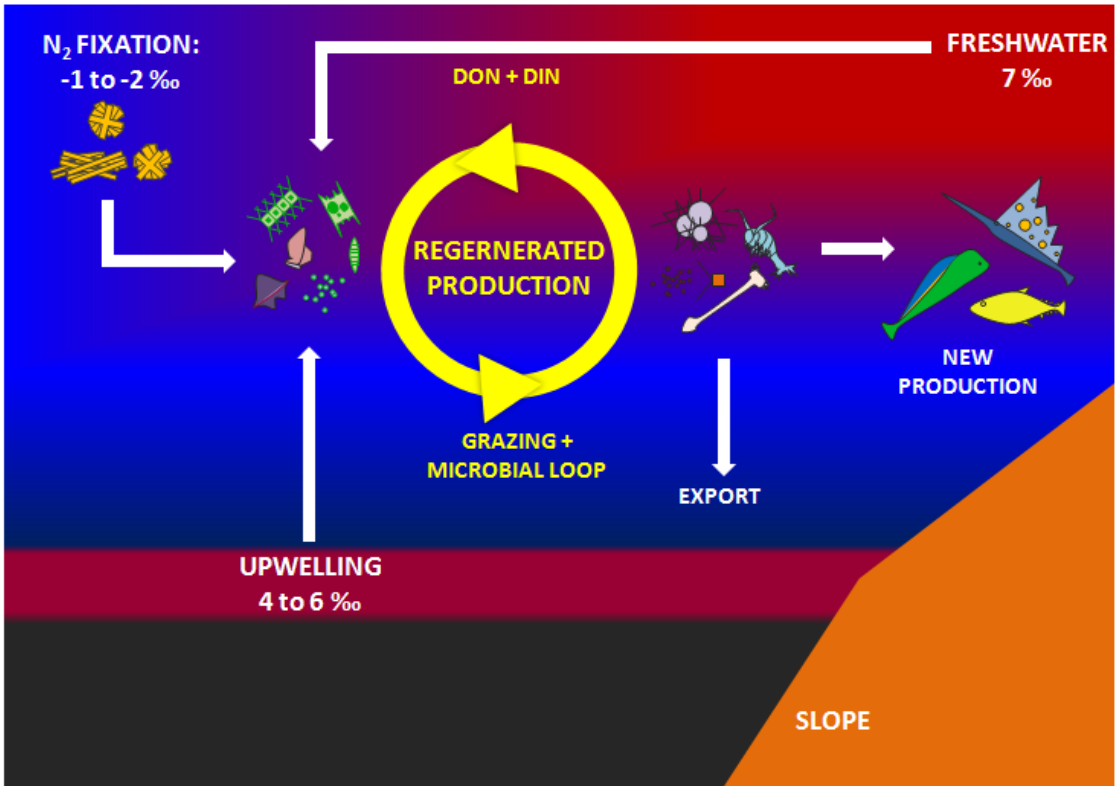

Fig. 3. $\delta^{15}N$ values of nitrogen sources in the Gulf of Mexico. Yellow arrows represent regenerated nitrogen that is recycled back to phytoplankton by grazing and the microbial loop and white arrows depict new sources of nitrogen that support new production and export.

Variation in $\delta^{15}N$ values of producers and consumers have been used to gain information on basin scale N cycling and have been used to trace biological N fixation (decrease) and anthropogenic activity (increase). For example, the $\delta^{15}N$ values of juvenile loggerhead sea turtles (*Caretta caretta*) were found to be depleted in the northwest Atlantic compared to the southwest Pacific Ocean (Pajuelo et al. 2010). Depleted $\delta^{15}N$ values measured in tissues of consumers belonging to the same trophic guild was attributed to higher N_2 fixation in the Atlantic compared to the Pacific. This type of "negative" isotopic baseline shift has been found in tissues of other organisms from areas where N_2 fixation is thought to contribute significantly to new production such as the East China, Sargasso and Mediterranean Seas, and the western Atlantic Ocean (Minagawa & Wada 1986, Gruber & Sarmiento 1997, Montoya et al. 2002). A positive isotopic baseline shift can occur where $\delta^{15}N$ values of producers and consumers are enriched in areas of increased anthropogenic activities. This pattern has been shown for coral reef organisms (Risk et al. 2009), and fish species (Borderelle et al. 2009) in addition to producers and consumers in seagrass (Olsen et al. 2010), salt marsh (McClelland et al. 1997), and mangrove (Fry et al. 2000) communities. I utilized these ecosystem-wide patterns to assess whether the plankton community was utilizing different N sources within coastal and marine water masses in the GoM. Figure 4 depicts how the stable isotope abundance of inorganic N dictates the isotopic abundance in producers that ultimately causes isotopic shifts in consumers grazing upon them.

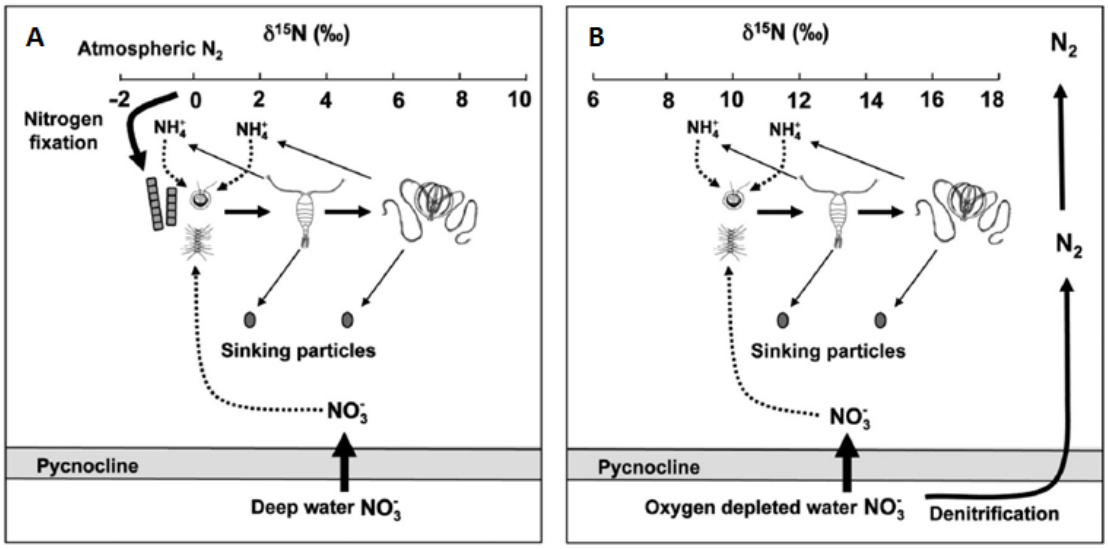

Fig. 4. Influence of N_2 fixation on $\delta^{15}N$ values. Differential sources of nitrogen shift $\delta^{15}N$ of producers and consumers in marine food webs where (A) products of biological N_2 fixation are depleted and (B) inorganic nitrogen originating from the pycnocline are enriched (Pajuelo et al. 2010). N source material can therefore be differentiated because shifts are reflected in producer and consumer $\delta^{15}N$ values.

2. OBJECTIVES AND HYPOTHESES

1. Identify differences in $\delta^{13}C$ and $\delta^{15}N$ values of POM, *Trichodesmium* and zooplankton collected from coastal and marine water masses in the GoM.

   H_1: POM, *Trichodesmium*, and zooplankton collected from coastal and marine water masses will exhibit unique isotope values.

2. Establish whether the degree of freshwater inflow affects $\delta^{13}C$ and $\delta^{15}N$ values of POM, *Trichodesmium*, and zooplankton collected from coastal and marine water masses.

   H_2: Variation in isotope values will be correlated to environmental parameters that distinguish coastal water masses from marine water masses.

3. Use published data to assess whether ecosystem level differences in stable isotope values exist between coastal and marine environments.

   $H_{3:}$ Published isotope values of producers and consumers between coastal and marine environments will parallel the trends in our data set.

Overall, I hypothesize that producers and consumers collected from coastal water masses will have enriched $\delta^{15}N$ values from the utilization of terrestrial and anthropogenic nitrogen sources. In addition, I predict that producers and consumers from marine water masses will exhibit depleted $\delta^{15}N$ values that result from N_2 fixation by *Trichodesmium*.

3. METHODS

3.1 Sampling Area

Samples were collected aboard the R/V Ladybride from slope and shelf regions of the northern GoM along transects between 26-28° N and 87-94° W (Fig. 5). This region of the GoM is influenced during summer months by the transport of coastal waters offshore from the Mississippi River system (Biggs et al. 2008, Walker et al. 2005). During the course of our study (June and July 2009), the eastern sections of our sampling transects were dominated by the western edge of the LC while the western sections transected a warm-core eddy. During July, we also transected water masses that exhibited decreased salinities and increased turbidity which we considered entrainments of coastal freshwater from the Mississippi River. Prior to sampling, the relative locations of water masses were identified remotely using SSH and SSC anomaly maps (Colorado Center for Astrodynamics Research; CCAR- http://argo.colorado.edu/ ~realtime/ welcome/). The LC and associated eddies were identified using SSH maps and the Mississippi River plume was detected from elevated levels of phytoplankton biomass using SSC maps.

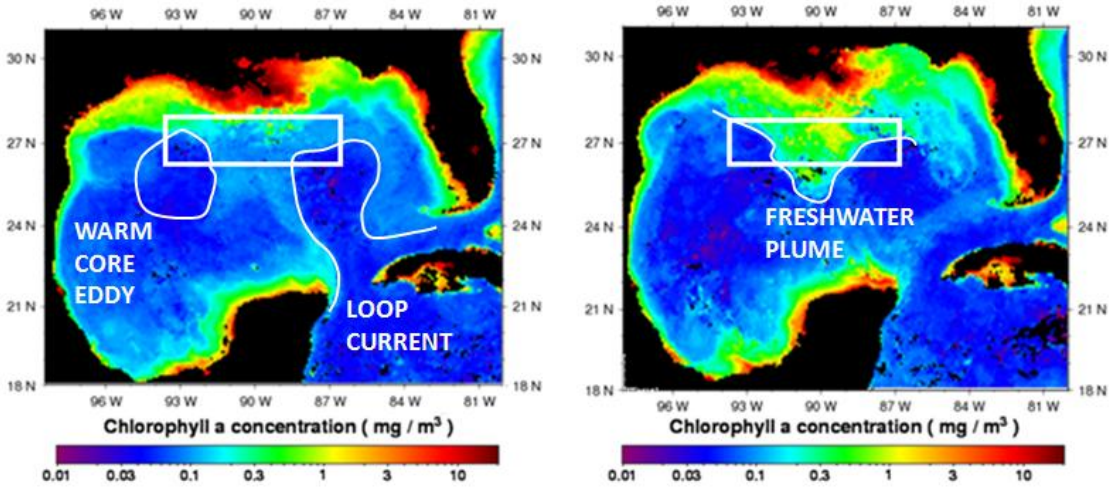

Fig. 5. Map of study area. Plots show sea surface chlorophyll *a* concentrations for June (left) and July (right) in 2009. Corridors where samples were taken are depicted by the box and targeted water masses include a warm core eddy, the Loop Current, and a coastal freshwater plume during the 2009 sampling period. SSC maps were obtained from Ocean Color Web (http://oceancolor.gsfc.nasa.gov /cgi/l3?sen=A).

3.2 Sample Collection

POM, *Trichodesmium*, and zooplankton were collected from surface waters (<1 m) by towing plankton nets (mesh size: 20 µm & 333 µm) for a maximum of 10 min. POM collections targeted net phytoplankton (>20µm) which are likely the food source of the macro-zooplankton collected in this study. Large colonies of *Trichodesmium* were then isolated using sterile metal inoculating loops and transferred to a nitex sieve (20 µm mesh). Zooplankton assemblages were isolated using sterilized forceps, and large zooplankton (chaetognaths, gelatinous masses, copepods, and other crustaceans) were transferred to a nitex sieve (120 µm mesh). After collection, all samples were rinsed three times with filtered sea water (0.2 µm) and then filtered onto pre-combusted (400°C for 5 hrs) Whatman GF/F (nominal pore size of 0.7 µm). Filters were subsequently folded, stored in foil packets, and immediately frozen (-20°C)

Salinity measurements were taken at all stations using a calibrated Hydrolab while samples for pigment and nutrient analysis were taken only at select stations. Pigment samples were collected after manually concentrating 40 L of water using a plankton net (mesh size: 20 µm). The concentrate was filtered onto a pre-combusted Whatman GF/F and immediately frozen until later analysis at the University of South Carolina HPLC facility. Nutrient samples were analyzed for combined N, HPO_4^-, silicate (SiO_2), and urea by collecting 45 mL of surface water in 50mL centrifuge tubes which had been acid-washed (1N HCl) prior to use. Samples were immediately frozen after collection and sent to the Geochemical and Environmental Research Group at Texas A&M University for analysis. Dissolved inorganic nitrogen (DIN) was calculated by

summing up the concentrations of NO_3^-, nitrite (NO_2^-) and NH_4^+ and used to calculate DIN: P ratios.

3.3 Stable Isotope Analysis

Filters were dried at 60°C and for POM, hole punches were taken from the filter and packed into tin cups. For zooplankton and *Trichodesmium* samples, 0.5 - 1.0 mg of dry material was packed into tin cups. Isotope ratios were determined using a Thermo Finnigan Delta plus isotope ratio mass spectrometer that was coupled to a Costech elemental analyzer in the Environmental Quality Analysis Laboratory (EQAL) at the University of Regina. Stable carbon and nitrogen abundance were calculated according to Equation 1 by comparing the ratios of the heavy to light isotopes of the sample to those of a known standard, and are reported as $\delta^{15}N$ or $\delta^{13}C$:

$$\delta^{15}N \text{ or } \delta^{13}C\ (‰) = [R_{sample}/R_{standard}] \text{ -1 x } 1000 \quad (1),$$

where R is $^{13}C:^{12}C$ or $^{15}N:^{14}N$. Values are relative to international standards, which are Pee Dee Belemnite and atmospheric nitrogen for carbon and nitrogen, respectively. The accuracy of the measurements was 0.2‰ for both $\delta^{15}N$ and $\delta^{13}C$.

3.4 Multivariate Analysis

The statistical software "R" (version 2.9.0: http://www.r-project.org/) and vegan package (version 1.15-3) were used to assess site assemblage and characterize water masses according to stable isotope, salinity, nutrient, pigment, and molar C:N data. Results from multivariate community analysis methods (Oksanen, 2011) were then

coupled with multivariate analysis of variance (MANOVA) tests to establish significant differences in the stable isotope values using SPSS (version 17.0). Normality and homogeneity of variance assumptions were tested using Kolmogorov-Smirnov and Levene's tests respectively. Stable isotope data were not distributed normally, therefore significance was established if Pillais trace values were less than or equal to 0.025 (Pallant 2010). Once significance was established, observed power was calculated to assess whether type II error influenced the insignificant results. Means of isotope and environmental data are presented with standard deviations unless otherwise noted.

For community analysis methods using R, POM stable carbon and nitrogen isotope data were $\log_{10}(|x|+1)$ transformed to remove negative $\delta^{13}C$ and $\delta^{15}N$ values and scale data appropriately. Transformed data were then used to calculate a triangular similarity matrix using the Bray-Curtis index, which was used as input for hierarchical cluster analysis. A dendrogram was created using the agglomerative average linkage method also known as Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and allowed us to visualize the similarity among sites. Stations were labeled prior to this analysis in order to determine whether separation during cluster analysis and ordination could be attributed to salinity or month of collection (June vs. July). Stations were considered marine (M) if their salinities were greater than 35 and considered coastal (C) if below 35.

We use MANOVA to test whether the POM, *Trichodesmium*, and zooplankton isotope values between June and July are significantly different and repeat the test to assess whether the isotope values between coastal and marine water masses are

significantly different. Based on dendrogram results and MANOVA testing, data from *Trichodesmium* and zooplankton isolates were then separated according to whether significant differences existed between June and July or the coastal and marine water masses. POM, *Trichodesmium* and zooplankton data were then averaged, and isotopic shifts were visualized using a cross plot of $\delta^{13}C$ and $\delta^{15}N$.

Non-metric multidimensional scaling (nMDS) was then used to visualize a sub set of stations in ordination space based on pigment and nutrient data. Results were coupled to environmental vector fitting which relates environmental parameters to the two dimensional nMDS solution (Oksanen, 2011). This approach allowed us to describe the water masses and attribute variation in the isotopic composition of POM to significant differences in salinity, nutrient concentrations, the relative abundance of accessory pigments and molar C:N. To calculate the relative abundance of accessory pigments, pigment concentration data were divided by respective chlorophyll *a* concentration (standardization to maximum: $xAP_{1,2,\ldots n}/xCHL_{1,2,\ldots n} = x'_{1,2,\ldots n}$) and then divided by the sum of all the accessory pigments (standardize to total: $x'_{1,2,\ldots n}/\Sigma x'_{1,2,\ldots n}$). The result is equal to the fraction of each accessory pigment in relation to one chlorophyll *a* molecule and is equivalent to Wisconsin double standardization commonly used in ecological community analysis methods (Oksanen, 2011). This pigment matrix was used to determine the relative importance of algal groups using environmental vector fitting. Finally, molar C:N of samples were compared to published molar C:N from the Mississippi River to further explain the isotopic shifts.

3.5 Meta-Analysis

Data from Rooker et al. (2006) and Wells & Rooker (2009) were collected from authors and the digitizing software Engauge was used to gather remaining data from figures presented in Holl et al (2007). Data from Macko et al. (1984) was taken from tables presented and we present means of collected data with standard deviation unless otherwise noted. Table 1 summarizes the stable isotope data from coastal producers and consumers, while Table 2 presents data used from marine producers and consumers. Data were then combined and a cross-plot of $\delta^{13}C$ and $\delta^{15}N$ was used to visualize the isotopic shifts between producers and consumers in the coastal and marine environments.

Table 1. Coastal producer and consumer $\delta^{13}C$ and $\delta^{15}N$ values.

Group , common name (*species*)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Source
Primary Producers			
POM	-20.0 ± 1.4	7.5 ± 0.8	Macko et al. (1984)
	-20.1 ± 1.8	8.0 ± 3.0	Rooker et al. (2006)
Sargassum spp.	-16.9 ± 0.9	2.6 ± 1.6	Rooker et al. (2006)
Secondary Consumers			
Zooplankton	-19.2 ± 0.7	8.9 ± 0.9	Macko et al. (1984)
Juvenile Fishes			Rooker et al. (2006)
Sargeant major (*Abudefduf saxatilis)*	-18.0 ± 0.3	10.7 ± 0.4	
Dotterel filefish (*Aluterus heudeloti)*	-18.6 ± 1.5	12.9 ± 0.7	
Scrawled filefish (*Aluterus scriptus)*	-17.9 ± 0.4	13.0 ± 0.5	
Least puffer (*Canthigaster rostrata)*	-17.4 ± 0.8	14.9 ± 0.8	
Yellow jack *(Caranx bartholomaei)*	-16.5 ± 0.3	13.5 ± 0.3	
Blue runner (*Caranx crysos)*	-17.3 ± 0.3	9.5 ± 0.9	
Sargassumfish *(Histrio histrio)*	-18.0 ± 0.8	10.1 ± 2.0	
Planehead filefish (*Monocanthus hispidus)*	-18.4 ± 0.7	10.1 ± 1.2	
Freckled driftfish *(Psenes cyanophrys)*	-17.6 ± 0.4	13.8 ± 0.9	
Greater amberjack (*Seriola dumerili)*	-18.3 ± 0.4	10.2 ± 1.3	
Almano Jack (*Seriola rivoliana)*	-17.0 ± 0.6	13.6 ± 0.5	
Chain pipefish (*Syngnathus louisianae)*	-17.8 ± 0.4	11.1 ± 0.9	
Sargassum pipefish (*Syngnathus pelagicus)*	-16.9 ± 0.8	11.3 ± 0.6	
Yellowfin tuna (*Thunnus albacares)*	-16.6 ± 0.5	7.8 ± 1.7	

Table 2. Marine producer and consumer $\delta^{13}C$ and $\delta^{15}N$ values.

Group , common name (*species*)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Source
Primary Producers			
Trichodesmium spp.	-13.8 ± 0.5	-1.7 ± 0.4	Holl et al. (2007)
POM	-22.2 ± 1.4	2.7 ± 1.9	Rooker & Wells (2009)
	-23 to -27	1 to 3.2	
Sargassum spp.	-17.3 ± 1.4	0.8 ± 1.5	Rooker & Wells (2009)
Secondary Consumers			
Zooplankton	-17.5 to -19.5	1 to 3	Holl et al. (2007)
Larval Fishes			Rooker & Wells (2009)
Blue marlin (*Makaira nigricans*)	-19.0 ± 1.0	2.2 ± 0.7	
Dolphinfish (*Coryphaena hippurus*)	-18.2 ± 0.9	4.9 ± 1.5	
Pompano dolphinfish (*Coryphaena equiselis*)	-19.3 ± 1.2	5.5 ± 1.0	
Sailfish (*Istiophorus platypterus*)	-20.1 ± 0.6	4.3 ± 1.2	
Swordfish (*Xiphias gladius*)	-18.5 ± 1.1	4.4 ± 0.8	
Juvenile Fishes			Rooker & Wells (2009)
Dolphinfish (*Coryphaena hippurus*)	-17.7 ± 0.5	6.7 ± 1.7	
Pompano dolphinfish (*Coryphaena equiselis*)	-18.6 ± 1.2	8.1 ± 1.7	
Sailfish (*Istiophorus platypterus*)	-18.2 ± 0.2	6.4 ± 1.1	
Swordfish (*Xiphias gladius*)	-18.2 ± 0.7	4.1 ± 1.3	

4. RESULTS AND DISCUSSION

4.1 Site Distribution

During June and July 2009, high salinities were recorded in water masses that appeared swimming pool blue, and lower salinities were recorded in water masses that exhibited increased turbidity during July only. *Trichodesmium* colonies were visible at all stations in June and were present at most of the stations in July except few that exhibited the lowest salinities. Similarity in POM $\delta^{13}C$ and $\delta^{15}N$ values was calculated using the Bray-Curtis index and site distributions were visualized using agglomerative clustering. This classification method was effective because $\delta^{13}C$ and $\delta^{15}N$ values were taken into consideration simultaneously when the triangular similarity matrix was constructed. By coding the stations with the month and water mass (coastal or marine), we were able to quickly assess whether isotope differences were attributed to spatial or temporal variation.

Cluster analysis identified two major site assemblages and this method proved useful in quickly attributing the separation of sites to month or water mass (Fig. 6). According to the analysis, sites did not group according to month, as cluster 1 exhibited stations from both June and July, while cluster 2 was composed of stations from July only. Instead, sites separated based on salinity, where cluster 1 was composed of 21 marine stations (salinity > 35) from both June (11) and July (10), and cluster 2 included 15 coastal stations (salinity <35) from July only. Using MANOVA we then tested whether isotope values of the POM, *Trichodesmium* and zooplankton were significantly

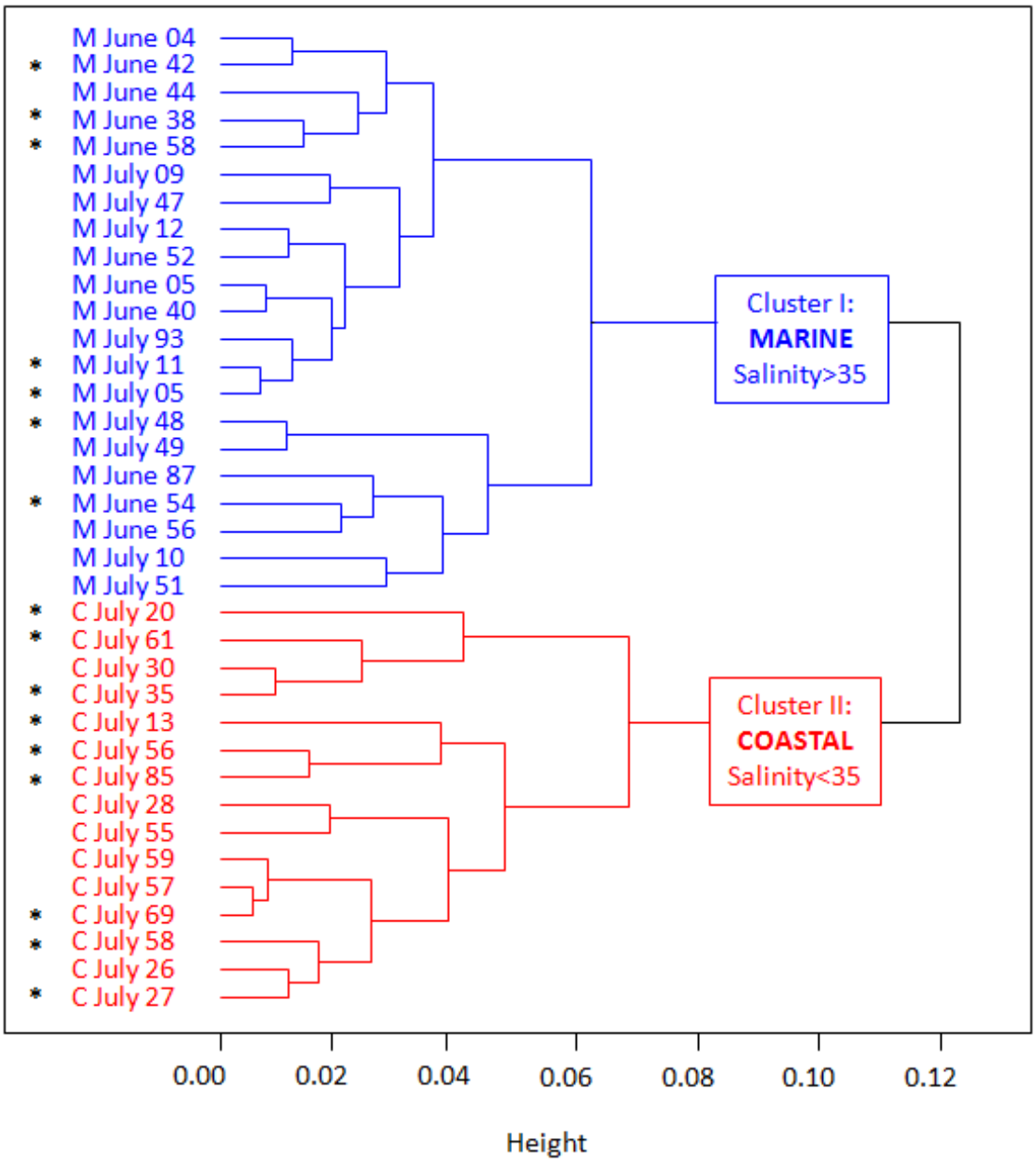

Fig. 6. Site dendrogram. Agglomerative clustering identified two major clusters based on the isotopic composition of the POM. Data were pre-labeled according to month (June or July) and salinity (M= marine; salinity > 35, C= coastal; salinity < 35), and stations used for nMDS are denoted by an asterisk (*).

different between June and July and repeated the analysis to assess significant differences between the two water masses.

4.2 Isotope Analysis

Stable isotope values of POM fell within the range of published values which have been shown to range from -18 to -25‰ for $\delta^{13}C$ and -2 to 8‰ for $\delta^{15}N$ in the GoM (Thayer et al. 1983, Macko et al. 1984, Rooker et al. 2006, Holl et al. 2007, Wells & Rooker 2009). There was an overall enrichment in POM $\delta^{13}C$ and depletion in $\delta^{15}N$ in June when compared to July (Table 3), but results of MANOVA indicated that the differences were not significant (Pillai's trace $\leq$ 0.025). This could be due to small sample size or other factors that increase type II error as observed power values were low for this test (Table 3). In contrast, both POM $\delta^{13}C$ and $\delta^{15}N$ were significantly different between coastal and marine water masses (Pillai's trace $\geq$ 0.025). POM $\delta^{13}C$ values were on average 4.7‰ enriched while $\delta^{15}N$ values were on average 3‰ depleted at marine stations compared to coastal ones (Table 3, Fig. 7).

POM from marine water masses are generally enriched in $\delta^{13}C$ compared to regions receiving terrestrial organic carbon (Thayer et al. 1983), and enriched $\delta^{13}C$ values in June suggest that POM samples were not influenced by riverine sources. Depleted $\delta^{13}C$ and enriched $\delta^{15}N$ values have been reported for POM originating from the Mississippi River which suggests that coastal water masses encountered in July were influenced by terrestrially derived organic matter. Also, depleted $\delta^{15}N$ values of the POM and zooplankton signify that combined N resulting from biological N_2 fixation

were more widespread in June and in marine water masses. Results are consistent with SSC maps which depict a freshwater plume being entrained into marine surface waters in July only.

Table 3. POM, *Trichodesmium*, and zooplankton $\delta^{13}C$ and $\delta^{15}N$ values. Range and Mean ± S.D. stable isotope values are given for June and July and coastal and marine stations. P values are in bold and indicate whether differences in isotope values are significant (*) between groups according to a Pillais trace value < 0.025. For insignificant results, observed power values are given and in italics.

	June	July	Marine	Coastal
	Range Mean ± S.D.		Range Mean ± S.D.	
POM	**0.082;** *0.239*		*** 0.000**	
$\delta^{13}C$ (‰)	-19.5 to -15.5 -17.6 ± 1.4	-26.7 to -14.6 -19.9 ± 3.5	-19.5 to -14.6‰ -17.0 ± 1.3‰	-26.7 to -18.5 -22.2 ± 2.3
$\delta^{15}N$ (‰)	-0.9 to 1.8‰ 0.9 ± 0.8	-1.3 to 6.3 2.5 ± 2.3	-1.3 to 1.8 0.5 ± 0.8	2.2 to 6.3 4.2 ± 1.3
Trichodesmium	**0.217;** *0.036*		**0.084;** *0.077*	
$\delta^{13}C$ (‰)	-15.1 to -13.9 -14.5 ± 0.6	-14.6 to -13.5 -13.9 ± .05	-15.1 to -13.9 -14.4 ± 0.6	-14.6 to -13.5 -13.9 ± 0.6
$\delta^{15}N$ (‰)	-1.7 to -0.4 -1.2 ± 0.7	-1.4 to 0.5 -0.4 ± 0.8	-1.7 to -0.4 -1.3 ± 0.6	-0.5 to 0.5 -0.06 ± 0.5
Zooplankton	*** 0.000**		*** 0.000**	
$\delta^{13}C$ (‰)	-21.4 to -18.8 -20.4 ± 0.7	-21.0 to -17.8 -19.6 ± 0.9	-21.4 to -17.8 -19.9 ± 0.9‰	-21.0 to -18.1 -19.8 ± 0.9‰
$\delta^{15}N$ (‰)	0.5 to 3.6 2.4 ± 1.0	1.2 to 7.8 4.6 ± 1.7	0.5 to 6.5 2.8 ± 1.4	2.6 to 7.8 5.4 ± 1.1

Trichodesmium isolates had the least apparent shifts in isotope values (Table 3, Fig. 7) and MANOVA indicated that isotope differences between months and water masses were not significant (Pillai's trace ≥ 0.025). Overall, *Trichodesmium* $\delta^{13}C$ values were enriched relative to POM and zooplankton samples, while $\delta^{15}N$ values were depleted relative to the other groups examined. Stable isotope values were similar to those previously published which shows this diazotroph has both the most enriched $\delta^{13}C$ and depleted $\delta^{15}N$ values compared to any other marine phytoplankton (Carpenter et al. 1997, Tchernov & Lipshultz, 2008). Observed power values for these tests were also low (Table 3) which indicated that the insignificant differences for this group could have resulted from a small sample size or other violations to MANOVA assumptions.

For zooplankton, MANOVA testing indicated that isotopic differences between both June and July and between coastal and marine water masses were significant (Table 3, Pillai's trace ≤ 0.025). $\delta^{13}C$ values of zooplankton were similar between all stations, but $\delta^{15}N$ values were more depleted at marine stations and during the June sampling period. Zooplankton isotope values measured in this study are comparable to previously documented ranges of -18 and -20‰ for $\delta^{13}C$ and 2 to 9‰ for $\delta^{15}N$ (Macko et al. 1984, Holl et al. 2007). The narrow range of $\delta^{13}C$ values points to the use of a similar carbon source by zooplankton (marine phytoplankton and bacteria), while a wide range of $\delta^{15}N$ values is likely attributed to shifts in the isotopic baseline between the coastal and marine environments (Fig. 7).

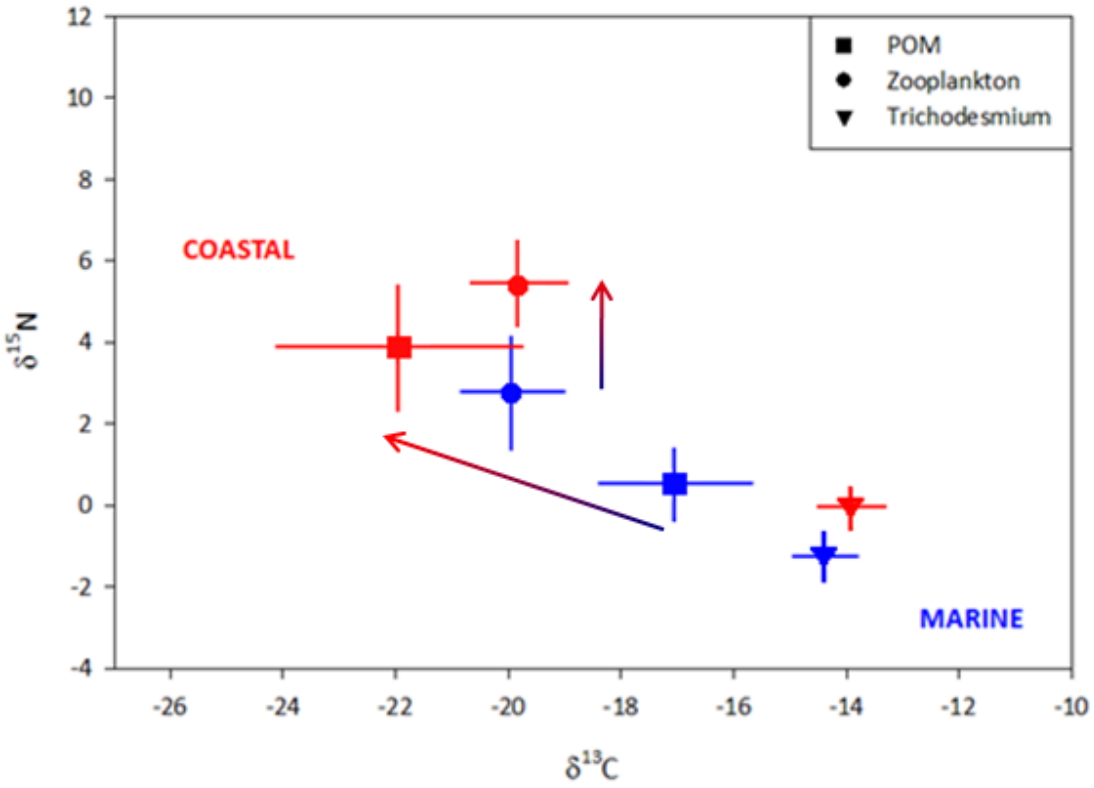

Fig. 7. Coastal and marine $\delta^{13}C$ and $\delta^{15}N$ values. Cross plot of mean ± S.D carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes of POM, *Trichodesmium*, and zooplankton between coastal and marine stations. Arrows highlight isotopic shifts that were significant according to MANOVA.

4.3 Freshwater Influence

Next, we investigated the environmental parameters that may have contributed to the separation of coastal and marine stations with unique POM $\delta^{13}C$ and $\delta^{15}N$ values. Nutrient and pigment data were collected from approximately 33% of the marine stations (7/21) and 53% of the coastal stations (9/15). These stations were visualized using nMDS, and environmental vector fitting was applied to relate salinity, nutrient,

pigment and molar C:N data to the nMDS solution (Fig. 8). This method allowed us to characterize the coastal and marine water masses in terms of phytoplankton community (relative abundance of accessory pigments) and the degree of freshwater influence (salinity, nutrient concentrations, and molar C:N) according to the strength and direction of vectors. Strength refers to the length of the vector and is related to the correlation between the environmental parameter and the nMDS solution while gradient refers to the direction of the vector and represents the direction of most rapid change.

Marine stations lie on the negative side of nMDS axis1 while coastal stations lie on the positive side (Fig 8). The environmental parameters which significantly correlated to the 2-dimensional nMDS solution included salinity, silicate, DIN:P, the accessory pigments zeaxanthin, chlorophyll *c1c2*, fucoxanthin, and diadinoxanthin, and molar C:N. The gradient and strength of the salinity, silicate, zeaxanthin, and DIN:P vectors indicated that values for these parameters were increased at marine stations where POM $\delta^{13}C$ was enriched and POM $\delta^{15}N$ was depleted. In contrast, the strength and gradient of the accessory pigments chlorophyll *c1c2*, fucoxanthin, and diadinoxanthin in addition to molar C:N indicated these parameters were increased in the coastal stations where POM $\delta^{13}C$ was depleted and POM $\delta^{15}N$ was enriched.

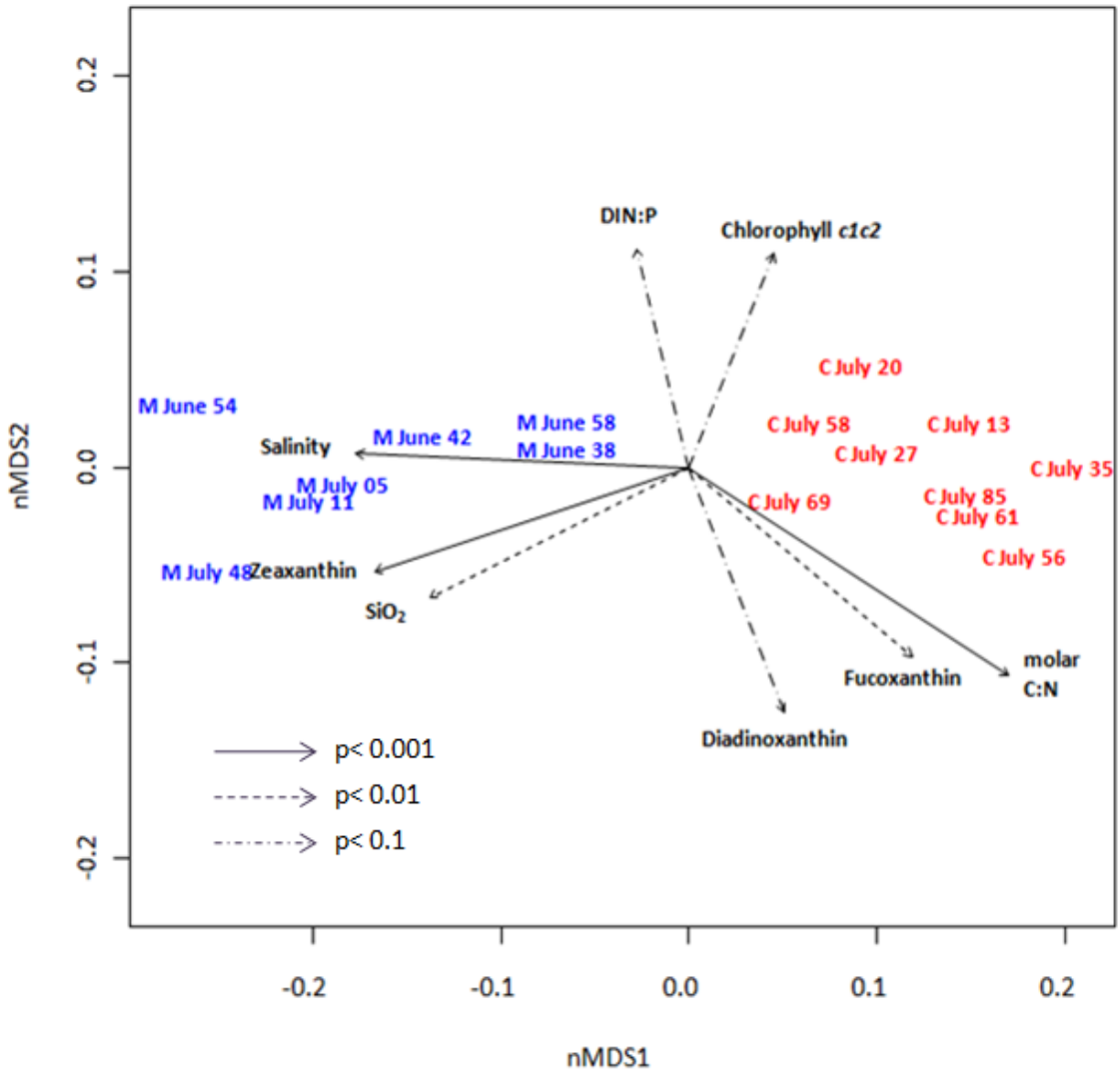

Fig. 8. nMDS solution and environmental vectors. Distribution of sites is visualized in ordination space using nMDS. Environmental vector fitting depicts the strength and gradient of variables relative to the nMDS axes.

Table 4. Environmental variable cluster averages. Significance between environmental parameters and NMDS solution (asterisk) with environmental variable cluster averages ± S.D.

	Sig.	MARINE Mean ± S.D.	COASTAL Mean ± S.D.
Salinity	***	36.3 ± 0.51	31.4 ± 1.59
Accessory Pigments (%/chl *a*)			
Chlorophyll *c1c2*	*	20.6 ± 5.5	29.6 ± 6.3
Fucoxanthin	**	6.2 ± 2.3	15.5 ± 4.8
Diadinoxanthin	*	3.2 ± 1.8	8.2 ± 4.6
Zeaxanthin	***	28.4 ± 8.6	9.6 ± 3.8
Other Pigments		41.7 ± 8.1	37.1 ± 5.5
Nutrients (µmol/L)			
NO_3^-		0.08 ± 0.06	0.07 ± 0.06
HPO_4^+		0.15 ± 0.08	0.16 ± 0.10
SiO_2	**	0.87 ± 0.30	0.32 ± 0.22
NH_4^+		1.55 ± 1.55	0.94 ± 0.69
NO_{2-}		0.07 ± 0.08	0.12 ± 0.07
Urea		0.32 ± 0.26	0.33 ± 0.10
DIN:P	*	11.9 ± 8.1	9.0 ± 6.0
Molar C:N	***	5.8 ± 0.6	7.9 ± 2.1
***p<0.001, **p<0.01, *p<0.1			

SiO_2 was the only nutrient to correlate well with the nMDS solution (Table 4), and values at coastal stations were decreased relative to marine stations. This may reflect a greater utilization of SiO_2 by the phytoplankton community in the coastal stations and this finding is consistent with pigment data (see below) which showed an increase in the abundance of diatoms at coastal stations. DIN:P also related to the nMDS solution (Table 4), and was also relatively decreased at coastal stations relative to marine stations. We found NO_2^-, NH_4^+, HPO_4^-, and urea did not vary much between coastal and marine stations and NO_3^-, concentrations were below the limit of detection in all samples. Combined DIN values were at the lower end of the typical range of values measured in

the GoM (Lambert, 1999) suggesting that both coastal and marine water masses sampled in this study were oligotrophic in nature.

In marine environments, phytoplankton are considered phosphorus limited when DIN:P values exceed 30 and phosphate concentrations are below 0.2μmol L^{-1} (Dortch & Whitledge 1992, Quigg et al. 2011). In this study, average phosphate concentrations were below this value and average DIN:P were low in both clusters indicating overall N limitation. DIN:P correlated to the nMDS solution (Table 4) and increased DIN:P were measured at marine stations only. This may suggest that phosphorus limitation plays a role in the marine stations, which has been reported previously for the northern GoM (Sylvan et al. 2007). However, the use of organic forms of phosphorus in the marine realm has been shown to occur in many phytoplankton taxa including *Trichodesmium* (Dyhrman et al. 2006). Dagg et al. (2007) suggested that organic phosphorus was an important secondary source of phosphorus to phytoplankton in the GoM but there are few actual measurements and not measured in our study. Using resource limitation assays, Quigg et al. (2011) revealed that organic forms of phosphorus were as important as traditional measured inorganic forms at alleviating phosphorus limitation. Therefore, I hypothesize that data from our present study reflect an alleviation in N limitation in the marine stations where inputs of N from diazotrophy increase the DIN:P.

Phytoplankton pigments have been used as chemical biomarkers to estimate phytoplankton biomass and identify dominant phytoplankton groups such as diatoms (fucoxanthin) and cyanobacteria (zeaxanthin) in the GoM (Lambert et al. 1999, Qian et al. 2003). To evaluate phytoplankton community structure between the coastal and

marine stations, accessory pigment contribution per chlorophyll *a* molecule was calculated as a percentage, and station averages were compared (Fig. 9). Zeaxanthin and chlorophyll *c1c2* were the most dominant accessory pigments and their relative abundances correlated with the nMDS solution (Table 4). For the marine stations, the accessory pigment contribution of zeaxanthin increased when compared to the coastal stations, indicating that cyanobacteria were dominant in these surface waters. This is in agreement with previous studies that describe the phytoplankton community of LC surface waters being dominated by small-sized phytoplankton and cyanobacteria such as *Trichodesmium* (Biggs & Ressler, 2001). In addition, depth integrated *Trichodesmium* abundance in the GoM was measured by Holl et al. (2007) to be 4.1 x 108 ± 3 x 108 trichomes m^{-2}, but when oligotrophic stations farthest offshore were isolated, values increased to 7.3 x 108 ± 5.4 x 108 trichomes m^{-2}.

In contrast, the contribution of chlorophyll *c1c2,* fucoxanthin and diadinoxanthin increased in the coastal stations, which reflects an increase in the relative abundance of diatoms, dinoflagellates and other eukaryotic phytoplankton. This agrees with previous studies in the GoM which show increased diatom abundance inshore (Lambert et al. 1999). Qian et al. (2003) found that the spatial distribution of chlorophyll *a* and the phytoplankton community composition were controlled by riverine inputs of nutrient enriched freshwater. More specifically, diatoms were increased in waters over the inner shelf, but occurred in significant abundance offshore during the summer extension of low salinity water over the shelf.

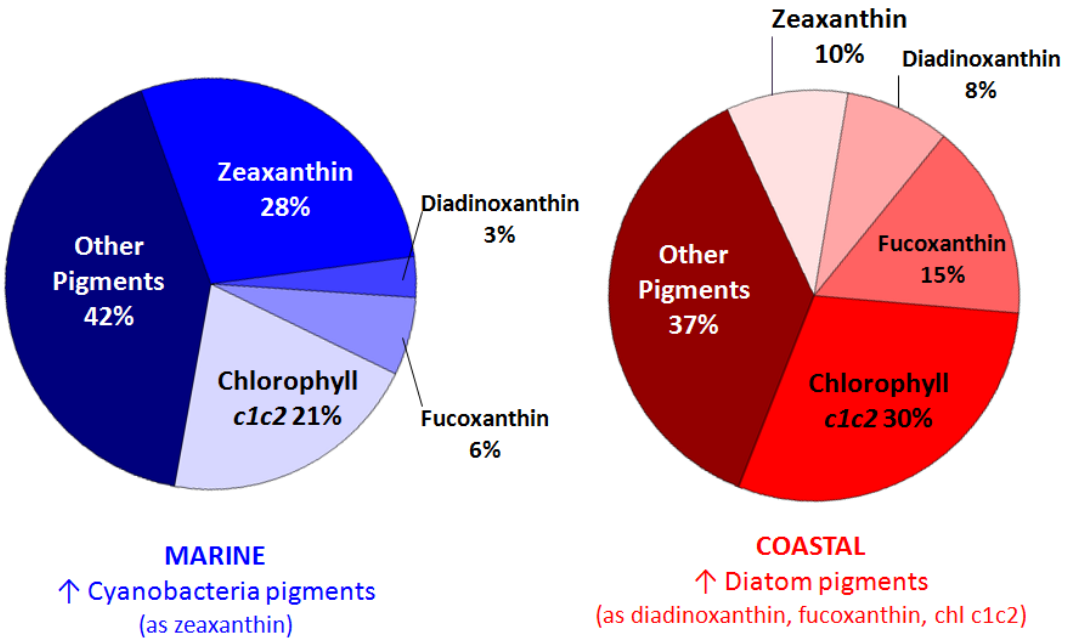

Fig. 9. Accessory pigment distribution. Percent contribution of accessory pigments per chlorophyll *a* molecule for the coastal and marine water masses sampled. Pigments which did not correlate to the nMDS solution were pooled in the "other pigments" fraction.

Molar C:N has been used in conjunction with $\delta^{13}C$ and $\delta^{15}N$ values to evaluate the sources of POM in the Mississippi River (Wissel et al. 2005). We compare POM molar C:N values measured at our sites to those from the Mississippi River further elucidate trends in our data (Fig.10). POM molar C:N at the marine stations was lower than ratios observed at coastal stations and resemble that of marine phytoplankton and bacteria. For the coastal stations, molar C:N increased and approached C:N values characteristic of Mississippi River POM. The shift from marine to coastal environment was also accompanied by an increase in POM $\delta^{15}N$. This may suggest that the POM

measured at coastal stations were composed of terrestrial organic matter or that phytoplankton in the coastal environments are utilizing anthropogenic N originating from the Mississippi River whose $\delta^{15}N$ values are characteristically enriched (Wissel & Fry, 2005). Molar C:N of *Trichodesmium* and zooplankton, however, decreased at marine stations which may reflect differences in their lipid composition (data not shown in Fig, 10).

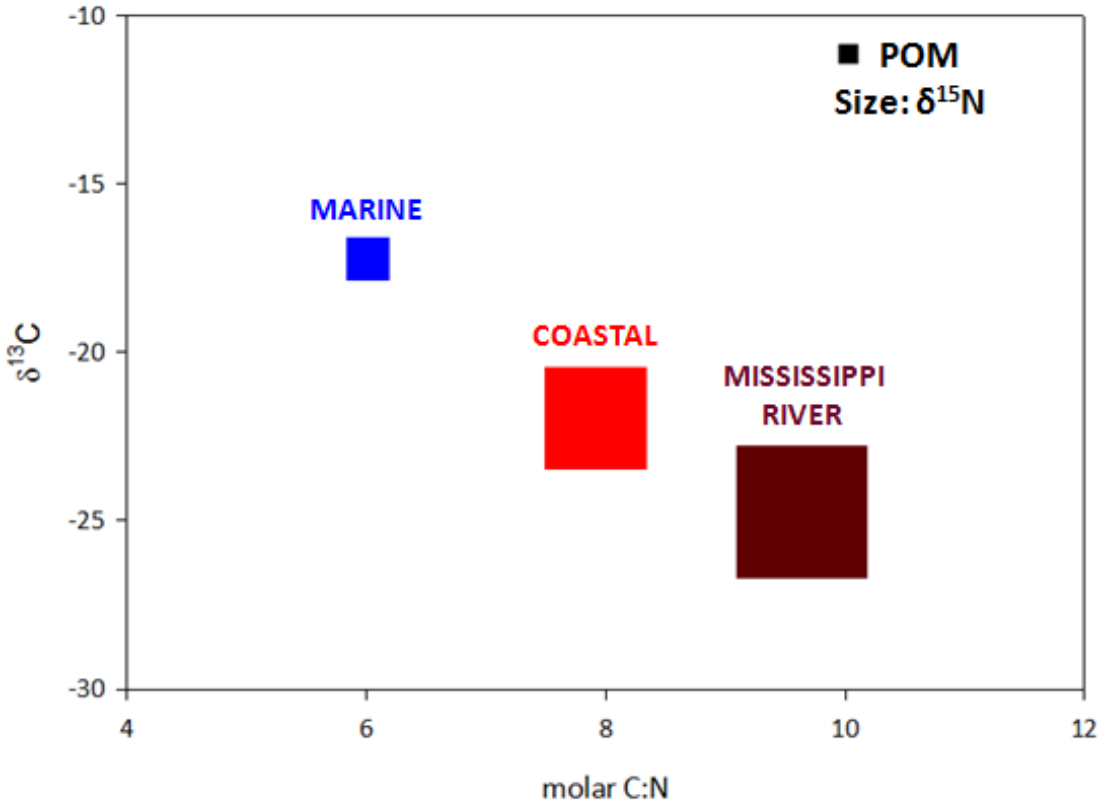

Fig. 10. Bubble cross-plot of $\delta^{13}C$ and molar C:N values. Coastal and marine POM station averages are compared to average POM values from the Mississippi River (Wissel & Fry, 2005). Size indicates relative $\delta^{15}N$ values which are increased in the coastal and Mississippi River POM.

We attribute enriched $\delta^{15}N$ values of the POM and zooplankton collected from coastal stations to the utilization of terrestrial/ anthropogenic derived N sources by chlorophyll *c* eukaryotic phytoplankton (e.g., diatoms), which are then consumed by zooplankton and transferred up the marine food web. In contrast, depleted $\delta^{15}N$ values of the POM and zooplankton at marine stations likely reflect the importance of cyanobacteria in these environments, where N from diazotrophy supports secondary production. The baseline shift caused by diazotrophy has been documented in the GoM (Holl et al. 2007) and between basins (Pajuelo et al. 2010), and results from this study support the premise that biological N_2 fixation provides N compounds to higher trophic levels, potentially relieving N limitation in these oligotrophic open ocean environments.

4.4 Food Web Comparison

Depleted POM and *Sargassum* spp. $\delta^{15}N$ values have been shown to occur in offshore regions of the GoM that serve as spawning habitats of pelagic fishes (Wells and Rooker, 2009). Despite the presence of *Trichodesmium* in surface waters, the impact of N_2 fixation by this genus on pelagic producers and consumers has received little attention. Meta-analysis of $\delta^{13}C$ and $\delta^{15}N$ values of producers and consumers from multiple publications highlight the potential importance of *Trichodesmium* in pelagic food webs (Fig. 11). These results clearly show that *Trichodesmium* is an important producer within these systems and its prevalence will alter stable isotopic baselines and the interpretation of trophic models using these dietary markers.

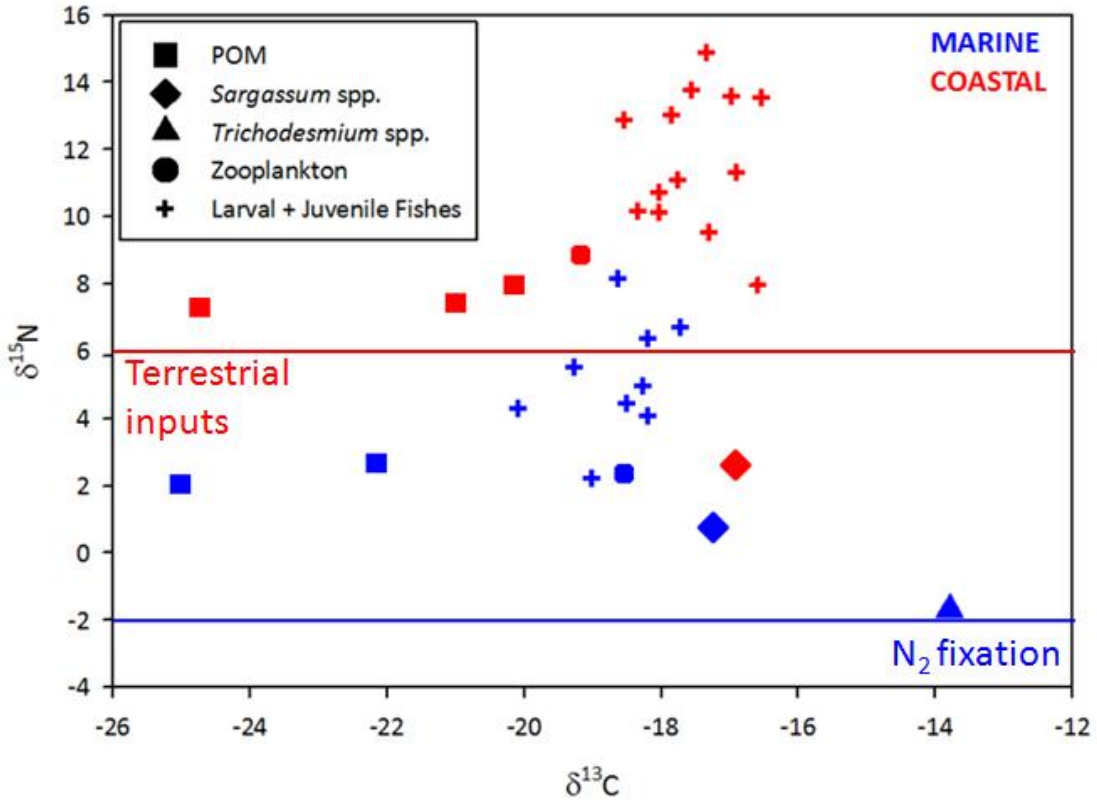

Fig. 11. Published producer and consumer $\delta^{13}C$ and $\delta^{15}N$ values. Proposed isotopic baseline shifts denoted by horizontal lines where N_2 fixation reduces $\delta^{15}N$ values in marine environments and terrestrial inputs increase $\delta^{15}N$ values in coastal habitats.

POM $\delta^{15}N$ values in the published marine food webs are depleted by approximately 4 to 5‰ relative to POM from coastal regions. This is also the case for *Sargassum* where $\delta^{15}N$ values are depleted by approximately 2 ‰ in the marine relative to the coastal data water masses. $\delta^{15}N$ values of *Trichodesmium* originating from marine environments were depleted compared to all other organisms which suggests N compounds from this genus may influences other producers and consumers in the GoM.

This isotopic baseline shift is also apparent in consumer data with $\delta^{15}N$ values of zooplankton being enriched by 5 to 7‰ in the coastal water masses when compared to marine environments. Similarly, the $\delta^{15}N$ values of larval and juvenile fishes are depleted by 7 to 9‰ depletion in the marine water masses when compared to coastal taxa. *Trichodesmium* exhibited the most depleted $\delta^{15}N$ values seen in the producers and we suggest it may shift the isotopic baseline for food webs in marine environments. Furthermore, the enriched producer $\delta^{15}N$ values in the coastal data resemble Mississippi River POM data which suggests that material from the Mississippi River also has the potential to shift isotopic baselines in coastal environments. Our data suggest that *Trichodesmium* and freshwater inputs can be utilized by pelagic producers and consumers and future efforts to construct food webs in the GoM should not overlook salinity or the presence of diazotrophs (Fig. 11).

Differences in the $\delta^{13}C$ values of the producers and consumers seem to vary less when the coastal and marine environments are compared. POM values are concentrated within a relatively narrow range (-25 to -20 ‰), and *Trichodesmium* exhibits a unique carbon signature (~-14‰) that is enriched compared to other phytoplankton measured to date (Tchernov & Lipshultz, 2008). The $\delta^{13}C$ signature of *Sargassum* are enriched compared to the POM and values fall within a narrow range (-18 to -16‰). Results of the meta-analysis show consumer $\delta^{13}C$ values fall within the -16 to -21‰ range, suggesting that *Trichodesmium* is not directly grazed upon. My results are in accord with other literature stating that this phytoplankton indirectly supports production of higher trophic levels through the release of dissolved organic N such as NH_4^+ and amino acids.

5. CONCLUSIONS

We utilized the natural abundance of stable isotopes to evaluate shifts in isotopic baselines between coastal and marine water masses and found that variation relates to changes in salinity, silicate concentrations, DIN:P, phytoplankton community composition, and molar C:N. This study represents a first attempt to use natural abundance of stable carbon and nitrogen isotopes to document how the entrainment of coastal surface waters may impact food-web dynamics in the pelagic GoM. Results highlight the importance of the diazotrophic activity of *Trichodesmium* in sustaining production in marine ecosystems while terrestrial material and anthropogenic sources appear to be important in coastal water masses. My data are in accord with published literature showing that isotopic baselines shift on an ecosystem level. I show that isotopic signatures of producers and consumers are useful for describing differences in the flow of energy between coastal and marine food webs. Further research to measure the isotopic composition of larval fishes will help to validate the importance of *Trichodesmium* and answer questions dealing with the early life ecology of important larval and juvenile fish species.

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