Retrospective Determination of Trophic Relationships among Pelagic Fishes Associated with *Sargassum* Mats in the Gulf of Mexico

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ABSTRACT

Stable isotopic composition of flora and fauna associated with floating *Sargassum* mats in the NW Gulf of Mexico was measured to identify the origin(s) of organic matter used by pelagic fishes and determine trophic relationships of associated taxa. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes were quantified from tissue samples of the primary autotrophs and several other members of the *Sargassum* complex (herbivores through apex predators). Distinct $\delta^{13}C$ values were observed among autotrophs: *Sargassum natans* (-17.1 ± 0.4‰), *Sargassum fluitans* (-16.3 ± 0.2‰), epiflora (-18.3 ± 0.9‰), and phytoplankton (particulate organic matter) (-21.0 ± 0.5‰), suggesting the approach has promise for identifying source(s) of organic matter. In addition, nitrogen stable isotopes indicated that four distinct trophic levels were present in the *Sargassum* complex. Spatial and temporal patterns in $\delta^{13}C$ and $\delta^{15}N$ values were observed for selected autotrophs and heterotrophs; however, signatures were relatively stable over scales investigated. Based upon expected modification due to fractionation, a simple mixing model predicted that the majority of organic matter reaching top-level consumers originates from phytoplankton production. Findings from this study challenge the theory that *Sargassum* is a direct source of energy to pelagic fishes, and therefore the value of this unique complex may be limited to its role as refuge for pelagic fauna.

KEY WORDS: Diet, pelagic fishes, stable isotopes, Gulf of Mexico

Determinación Retrospectiva de las Relaciones Tróficas entre Peces Pelágicos Asociados a las Matas de *Sargassum* en el Golfo de México

La composición isotópica de la flora y fauna asociada con el sargazo flotante en el noroeste del Golfo de México fue medida para identificar el origen ó origines de la materia orgánica utilizada por los peces pelágicos y así determinar la relación

trófica asociadas a estos grupos taxonómicos. Isótopos estables de carbono ($\delta^{13}C$) y nitrógeno ($\delta^{15}N$) fueron cuantificados en muestras de tejidos provenientes de tres autotrofos primarios y de varios otros miembros del complejo de *Sargassum* (herbívoros hasta depredadores superiores). Valores muy distintivos de ($\delta^{13}C$) fueron observados entre los autótrofos: *Sargassum natans* (-17.1 ± 0.4‰), *Sargassum fluitans* (-16.3 ± 0.2‰), algas epifitas (-18.3 ± 0.9‰), and fitoplancton (partículas de materia orgánica) (-21.0 ± 0.5‰), lo que sugiere que este acercamiento es prometedor para la identificación la(s) fuente(s) energéticas. Además, los isótopos estables de nitrógeno indican la presencia de cuatro niveles tróficos dentro del complejo de *Sargassum*. Patrones de variación temporal y espacial en las medidas de los $\delta^{13}C$ y $\delta^{15}N$ fueron observadas en un grupo selecto de autótrofos y heterótrofos; sin embargo, las señales fueron relativamente estables dentro de las escalas estudiadas. Partiendo de modificaciones esperadas por fragmentación, un modelo de mezcla simple predijo que la mayoría de la materia orgánica que llega a los consumidores superiores se origina en la producción del fitoplancton. Este estudio es contrario a la teoría de que el sargazo (*Sargassum*) es la fuente directa de energía para los peces pelágicos, por lo que el valor de este complejo esta limitado al papel de proveedor de refugio para la fauna pelágica.

PALABRAS CLAVES: *Sargassum*, peces pelágicos, Golfo de México, composición isotópica

INTRODUCTION

Sargassum is a free-floating brown alga that is of particular interest to scientists and fishery managers because of its putative value as essential fish habitat. Survey work indicates that several highly valued species utilize *Sargassum* mats (e.g. amberjack, cobia, dolphin, triggerfish, tuna), particularly during early life (e.g. Coston-Clements et al. 1991, Wells and Rooker In press). Due to the complexity this habitat affords, *Sargassum* may enhance early life survival by reducing predation-mediated mortality and enhancing prey availability. If this assumption is valid, survival and recruitment success of certain pelagic fishes will be linked to the distribution and abundance of *Sargassum*, and these mats will play a critical role sustaining marine fisheries throughout the Gulf. In response, the National Oceanic and Atmospheric Administration (NOAA) recently classified *Sargassum* as essential marine fish habitat and recommended that research on ecosystem structure and function of pelagic *Sargassum* was critical for maintaining and enhancing living resources in the Gulf (SAFMC 1998).

In addition to its presumed importance as habitat of pelagic fishes, *Sargassum* is one of the few sources of energy for pelagic food webs, and scientists have speculated that this unique complex (defined here as *Sargassum* + epiphytic algae + heterotrophic bacteria) contributes to primary and secondary production in nearshore and offshore waters (Peres 1982). Preliminary findings from stable isotope and fatty acid analyses support the premise that a portion of organic material

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supplied to pelagic fishes originates in the *Sargassum* complex, indicating these mats afford more than "refuge" to associated fauna (Rae 2002). Despite its potential value as both habitat and source of organic matter to consumers, we know very little about the role or significance of this complex. As a result, integrated studies establishing relationships of habitat use, primary productivity, trophodynamics, and biogeochemical cycling are critically needed.

In this study we examine the structure and dynamics of pelagic *Sargassum* in the Gulf of Mexico, and evaluate its ecological value using stable carbon and nitrogen isotopes. Stable isotopes serve as useful tracers of nutritional history since consumer tissues reflect the isotopic composition of prey in a predictable manner (Peterson and Fry 1987). The stable isotope approach is based is based on premise that primary producers take up naturally occurring isotopes of carbon and nitrogen in different ratios, and the isotopic ratio is conserved to some degree by consumers. Stable isotope ratios of carbon ($\delta^{13}C$) provide information on the source(s) of organic matter to heterotrophs while isotope ratios of nitrogen ($\delta^{15}N$) are often used to delineate trophic positions of consumers (Michener and Schell 1994, Hobson and Wassenaar 1999). The aim of the current study is to determine the source(s) of organic matter supporting pelagic fishes and delineate pathways of energy flow through the *Sargassum* complex (from juvenile fishes to apex predators) using stable carbon and nitrogen isotopes. Our working hypothesis is that the *Sargassum* complex plays a significant role in primary and secondary production, and is a primary source of organic matter to pelagic fishes.

METHODS

Natural variation in stable isotope signatures of primary and secondary producers was examined for taxa associated with *Sargassum* in the northwest Gulf of Mexico in 2000. Flora and fauna associated with *Sargassum* were collected in northwest regions of the Gulf (20 - 70 km offshore), and collections were taken over a 4-month period (May - August) to assess temporal variation in stable isotope signatures. Several different sampling gears were used to collect primary producers [(*Sargassum*, epiflora (*Cladophora sp.*), phytoplankton (based on < 40 µm size fractions of particulate organic matter-POM)] and consumers associated with the *Sargassum* mats, including plankton nets and purse seines (Wells and Rooker In press). In addition, large apex predators feeding within or near the *Sargassum* complex (wahoo, dolphin, yellowfin and blackfin tuna) were collected using hook and line. Plants and animals were placed on dry ice in the field and later moved to freezers in the laboratory.

Plant and animal tissues were ground and approximately 0.6 mg of material were used for isotopic determination. Isotopic ratios and percent carbon and nitrogen were determined using a Finnigan MAT DeltaPlus continuous-flow stable isotope mass spectrometer attached to a Carlo Erba elemental analyzer at the University of Texas at Austin Marine Science Institute.

Stable carbon and nitrogen ratios are expressed as $\delta^{13}C$ or $\delta^{15}N$ according to the following equation: $\delta^{13}C$ or $\delta^{15}N$ (‰) = $[R_{sample}/R_{standard}]$-1 x 1000 where R is $^{13}C:^{12}C$ or $^{15}N:^{14}N$. Isotopic values of carbon and nitrogen are reported relative to Peedee belemnite and atmospheric nitrogen standards, respectively. Similar to Herzka and Holt (2000), accuracy of isotopic measurements was verified using a secondary standard reference material (chitin of marine origin, Sigma Aldrich Co., USA, No. C-8908).

RESULTS

Primary Producers

Stable carbon and nitrogen isotope ratios were estimated for *S. fluitans*, *S. natans*, epiflora (*Cladophora* sp.), and POM (Table 1). Distinct $\delta^{13}C$ values (mean ± 1 SE) were observed for each autotroph, and *S. fluitans* (-16.3 ± 0.2‰) *Sargassum natans* (-17.1 ± 0.4‰) were enriched (1-4‰) relative to epiflora (-18.3 ± 0.9‰), and POM (-21.0 ± 0.5‰). Stable nitrogen isotope ratios of primary producers varied between 2.3 and 9.1‰, and $\delta^{15}N$ of both *S. fluitans* (2.8 ± 0.5‰) and *S. natans* (2.3 ± 1.0‰) were depleted by 6 –7‰ compared to POM (8.5 ± 0.8‰) and epiflora (9.1± 0.5‰).

Consumers

Consumers exhibited patterns of enrichment in $\delta^{13}C$ and $\delta^{15}N$ with increasing trophic position (Table 1). $\delta^{13}C$ values were most depleted for juvenile shrimps and crabs (-17.4 to -18.2‰). A shift in $\delta^{13}C$ values was observed for juvenile fishes (blackfin tuna *Thunnus atlanticus*, yellowfin tuna *Thunnus albacares*, gray triggerfish *Balistes capriscus*), and values increased by approximately 1.0 to 1.5‰; however, certain taxa maintained relatively depleted $\delta^{13}C$ values: planehead filefishes *Monacanthus hispidus* (-18.4 ± 0.7‰), greater amberjack *Seriola dumerili* (-18.3± 0.1‰), and sargassumfish *Histrio histrio* (-17.8± 0.8‰). Stable carbon isotope ratios of adult fishes varied (-15.9 to -17.5‰), and the heaviest $\delta^{13}C$ values were observed for wahoo *Acanthocybium solanderia* (-15.9± 0.4‰), blue marlin (-16.8± 0.3‰), and king mackerel (-16.9± 0.4‰). Enrichment of $\delta^{15}N$ also occurred with increasing trophic position and patterns were more consistent than $\delta^{13}C$. Again, the lowest values were observed for juvenile crustaceans: *Leander* sp. (6.5± 0.4‰), *Latreutes* sp. (6.0± 0.7‰), *Portunus* sp. (8.7± 0.7‰). Juvenile fishes ranged from 8.0 to 10.2‰ with $\delta^{15}N$ values over 10.0‰ for blackfin tuna, planehead filefish, and greater amberjack. $\delta^{15}N$ values of tertiary consumers ranged from 11.8 to 15.6‰, and estimated trophic position was highest for king mackerel *Scomberomorus cavalla*, blackfin tuna, and yellowfin tuna ($\delta^{15}N$ ranged: 13.0 to 15.6‰).

Table 1. $d^{13}C$ and $d^{15}N$ values (mean ± 1 SE) of producers and consumers associated with *Sargassum* in the NW Gulf of Mexico. Sample size (N) and stage (P = primary producer, I = intermediate, J = juvenile, A = Adult) are given. Predicted trophic position based on POM $d^{15}N$ value and fractionation of 3‰ per trophic level.

Common Name	Scientific Name	Stage	N	$d^{13}C$	$d^{15}N$	Trophic Position
Macroalgae	**Sargassum natans**	P	7	-17.08±0.41	2.32±1.03	
Macroalgae	*Sargassum fluitans*	P	10	-16.27±0.14	2.82±0.40	
POM		P	10	-21.00±0.49	8.59±0.82	
Epiflora	*Cladophora sp.*	P	6	-18.83±0.85	9.12±0.47	
Shrimp	*Latreutes fucorum*	I	6	-18.01±0.33	5.95±0.74	0.65
Shrimp	*Leander tenuicornis*	I	16	-17.40±0.23	6.50±0.35	0.83
Yellowfin Tuna	*Thunnus albacares*	J	2	-16.60±0.35	7.97±1.19	1.32
Gray Triggerfish	**Balistes capriscus**	J	39	-16.61±0.90	8.41±0.23	1.47
Sargassum Crab	*Portunus sayi*	J	6	-18.17±0.20	8.67±0.66	1.56
Sargassumfish	*Histrio histrio*	J	10	-17.94±0.26	9.00±0.34	1.67
Blue Runner	*Caranx crysos*	J	23	-17.29±0.07	9.53±0.19	1.84
Planehead Filefish	*Monocanthus hispidus*	J	11	-18.36±0.20	10.11±0.37	2.04
Blackfin Tuna	*Thunnus atlanticus*	J	7	-16.42±0.10	10.14±0.58	2.05
Greater Amberjack	*Seriola dumerili*	J	6	-18.34±0.13	10.22±0.44	2.07
Wahoo	*Acanthocybium solandri*	A	5	-15.86±0.38	11.82±0.70	2.61
Dolphinfish	*Coryphaena hippurus*	A	9	-17.07±0.11	12.24±0.27	2.75
Blue Marlin	*Makaira nigricans*	A	3	-16.79±0.27	12.37±1.19	2.79
Lesser Blue Crab	*Callinectes sapidus*	A	7	-17.16±0.64	12.98±0.23	2.99
Greater Amberjack	*Seriola dumerili*	A	6	-17.54±0.11	12.99±0.37	3.00
Yellowfin Tuna	*Thunnus albacares*	A	5	-17.40±0.43	13.08±0.46	3.03
Blackfin Tuna	*Thunnus atlanticus*	A	5	-17.41±0.67	13.90±0.19	3.30
King Mackerel	*Scomberomorus cavalla*	A	6	-16.85±0.35	15.57±0.51	3.86

Temporal Variability

Seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ values was evaluated for *S. fluitans* and two consumers (gray triggerfish, *Balistes capriscus* and blue runner *Caranx crysos*)(Table 2). $\delta^{13}C$ values of *S. fluitans* were similar among months examined; values ranged from -16.2 to -17.8‰ (ANOVA, $p > 0.05$). Monthly differences in $\delta^{13}C$ values of blue runner were negligible, with mean monthly values all within 0.1‰ (ANOVA, $p > 0.05$). Conversely, monthly shifts in $\delta^{13}C$ of gray triggerfish were more apparent (range: -14.5 to –18.8‰) and a significant difference in monthly signatures was detected (ANOVA, $p > 0.05$). Seasonal shifts in $\delta^{15}N$ values of *S. fluitans* ranged from 2.0 - 4.0‰ with values lower during June and July; nevertheless, no seasonal difference in $\delta^{15}N$ was observed. $\delta^{15}N$ values of both consumers were similar across months investigated (ANOVA, $p > 0.05$).

DISCUSSION

Three main autotrophs were present in offshore waters of the Gulf of Mexico (*Sargassum spp.*, epiflora, and phytoplankton), and stable isotopic ratios of these producers were distinct. Based on $\delta^{13}C$ values, *Sargassum* was the most enriched producer while epiflora (*Cladophora* sp.) is more depleted (1 - 2‰). Observed values for both taxa are within the common range of values observed for marine marcoalgae (Michener and Schell 1994, Kaehler et al. 2000), and both are markedly different from our observed value for phytoplankton (-21‰), which is consistent with the estimate provided by other researchers in the Gulf of Mexico (Herzka and Holt 2000). $\delta^{13}C$ values between -18 to -24‰ are typically observed for marine phytoplankton while nearshore and estuarine phytoplankton are often more depleted (-22 to 30‰) (Fry and Sherr 1984,Fogel et al. 1992, Deegan and Garritt 1997). $\delta^{15}N$ of primary producers was also distinct with both species of *Sargassum* being depleted (6–7‰) relative to phytoplankton or epiflora, and the large disparity likely indicates differences in the ability of these plants to fix N_2 from the atmosphere (Peterson and Fry 1987). Marked differences in $\delta^{13}C$ and $\delta^{15}N$ values of producers in this study indicate that the approach has promise for delineating trophic relationships of consumers in the *Sargassum* complex.

Table 2. Intrannual variation in d^{13}C and d^{15}N values (mean $\pm$ 1 SE) of selected roducer and consumers associated with *Sargassum* in the NW Gulf of Mexico.

Common Name	Scientific Name	May	June	July	August
Macroalgae	*Sargassum fluitans*	d^{13}C −16.20±0.03 d^{15}N 4.03±0.54	d^{13}C −17.48±0.49 d^{15}N 1.98±0.24	d^{13}C −16.61±0.21 d^{15}N 1.97±0.24	d^{13}C −17.75±0.18 d^{15}N 3.72±0.44
Blue Runner	*Caranx crysos*	N/A N/A	d^{13}C −17.36±0.30 d^{15}N 9.75±0.65	d^{13}C −17.37±0.04 d^{15}N 9.76±0.28	d^{13}C −17.27±0.06 d^{15}N 9.43±0.22
Gray Triggerfish	*Balistes capriscus*	d^{13}C −18.80±0.17 d^{16}N 9.34±0.49	d^{13}C −17.84±0.20 d^{16}N 8.17±0.72	d^{13}C −17.54±0.09 d^{16}N 8.89±0.16	d^{13}C −14.50±2.27 d^{16}N 7.75±0.35

Enrichment of $\delta^{13}C$ and $\delta^{15}N$ was observed with increasing trophic position in the *Sargassum* community and comparable results have been reported in other studies examining multiple trophic levels (e.g. Jennings et al. 1997, Kaekler et al. 2000). Based on $\delta^{13}C$ and $\delta^{15}N$ enrichment of approximately 1.5 ‰ and 3.0 per trophic level, respectively, signatures of consumers exhibited $\delta^{13}C$ and $\delta^{15}N$ values similar to phytoplankton (based on POM signature) and epiflora production. As a consequence, the majority of organic matter reaching top-level consumers appears to originate from phytoplankton and epiflora production. Unfortunately, $\delta^{13}C$ and $\delta^{15}N$ values of both producers overlap, thus limiting our ability distinguish the relative contribution of organic matter provided by each producer to consumers. $\delta^{13}C$ and $\delta^{15}N$ values exhibited by consumers were not consistent with the enrichment of organic matter derived from either *S. natans* or *S. fluitans*. As a consequence, findings from this study challenge the theory that *Sargassum* is a direct source of energy to pelagic fishes, and therefore the value of this unique complex may be limited to its role as refuge of pelagic fauna.

Temporal variation in $\delta^{13}C$ is commonly reported for primary producers and consumers (Wiencke and Fisher 1990, Bouillon et al. 2000, Rolff 2000). Observed trends appear related to changes in environmental conditions and several factors (e.g. CO2 supply, growth rate, temperature) have been shown to modify $\delta^{13}C$ of phytoplankton and macroalgae (Fry 1996). No conspicuous seasonal patterns were observed in this study; however, our seasonal assessment was based on a few taxa (*S. fluitans* and two consumers), and the duration of the study was limited to four months. In addition, recent data suggests that isotopic signatures of phytoplankton in the Gulf of Mexico vary over spatial and temporal scales (J. Rooker, unpublished data). Therefore, precaution must be exercised when interpreting stable isotope data, and detailed information on spatial and temporal variation is needed to fully understand energy flow and trophic relationships within the *Sargassum* complex.

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