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Trophic ecology of *Sargassum*-associated fishes in the Gulf of Mexico determined from stable isotopes and fatty acids

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ABSTRACT: Natural dietary markers (stable isotopes and fatty acids) were used to determine the trophic structure and characterize carbon source(s) of juvenile and adult fishes associated with floating *Sargassum* in mid-shelf waters of the Gulf of Mexico. Stable carbon isotope ratios ($\delta^{13}C$) of 4 autotrophs (*Cladophora* sp., phytoplankton [based on particulate organic matter, POM], *S. fluitans*, *S. natans*) were distinct (range –16.3 to –21.0‰), with *S. fluitans* and *S. natans* enriched by 2 to 5‰ relative to *Cladophora* sp. and POM. Stable nitrogen isotope ratios ($\delta^{15}N$) of both *S. fluitans* and *S. natans* were depleted by 5 to 7‰ compared to *Cladophora* sp. and POM. The majority of $\delta^{13}C$ values of consumers were between –16 and –18‰, and $\delta^{13}C$ values were most depleted for juvenile shrimps, juvenile crabs and certain juvenile fishes (e.g. *Aluterus heudeloti*, *Monacanthus hispidus*, *Abudefduf saxatilis*, *Histrio histrio*, *Seriola dumerili*). Stable carbon isotope ratios of adult fishes varied from –16.1 to –17.5‰. Enrichment of $\delta^{15}N$ occurred with increasing trophic position, and the lowest values were observed for juvenile crustaceans, which ranged from 6.0 to 8.7‰. The majority of juvenile fishes were secondary heterotrophs ($\delta^{15}N$ values ca. 8.0 to 11.0‰), while most adult fishes were tertiary consumers with $\delta^{15}N$ values ranging from 11.9 to 14.3‰. Carbon source estimates from a 2-source mixing model indicated that the 78% of organic matter supplied to consumers (pooled across taxa) in the *Sargassum* complex was derived from POM. Fatty acid signatures of the primary producers were significantly different, and were used to further evaluate organic matter contribution to *Sargassum*-associated consumers. C_{22} polyunsaturated fatty acids (PUFAs) (22:6n-3, 22:5n-3) were most abundant in POM, while high levels of C_{18} and C_{20} PUFAs were observed for *Cladophora* sp. and *Sargassum* spp. (18:2n-6 and 20:4n-6, respectively). Consumer signatures were dominated by 22:6n-3, and principal component analysis indicated that fatty acid signatures of each of the 6 juvenile and 6 adult fish species were highly similar to POM and distinct from the other producers within the S*argassum* complex.

KEY WORDS: Food web · Diet · Pelagic ecosystem · Trophic position · Large pelagic fishes

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INTRODUCTION

A broad goal of oceanographers and marine ecologists has been to understand trophic relationships in shelf and open ocean communities (Botsford et al. 1997, Estes & Peterson 2000). To date, considerable work has focused upon characterizing benthic ecosystems, and these efforts have led to valuable information on energy flow and food web structure (Lindeman et al. 2000). Unfortunately, information on the structure and dynamics of pelagic ecosystems has not received the same attention, despite the fact that these

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systems contribute substantially to total primary production, biogeochemical cycling and global fishery yields (Pauly & Christensen 1995). The lack of information on pelagic food webs is of particular concern since large predatory fishes within these ecosystems have experienced substantial declines over the past few decades, thus altering food web structure and the relative impact of top–down controls (Jackson et al. 2001, Watson & Pauly 2001, Meyers & Worm 2003). As a result, there is a clear need to investigate pelagic food webs and this information is prerequisite for maintaining biodiversity and fishery yields.

Apart from floating *Sargassum* spp. (hereafter '*Sargassum*'), the pelagic zone of the Atlantic Ocean and the Gulf of Mexico is characterized by lack of structure and low levels of primary production. *Sargassum*, a brown macroalgae (Phaeophyceae) comprised of 2 species (*S. natans* and *S. fluitans*), is a ubiquitous surface feature in this region. *Sargassum* often accumulate in large mats or windrows, thereby forming a structured habitat for pelagic fauna, and survey work indicates that these floating mats represent a critical habitat for several members of the pelagic community, including a variety of invertebrates, fishes and sea turtles (Kingsford & Choat 1985, Coston-Clements et al. 1991). Moreover, several recreationally and commercially important finfish use *Sargassum* mats as refuge during early life (e.g. Coston-Clements et al. 1991, Wells & Rooker 2004a,b), and it is likely that the structural complexity afforded by floating *Sargassum* reduces predation-mediated mortality. If this assumption is valid, survival and recruitment success of certain pelagic fishes will be linked to the distribution and abundance of *Sargassum* (SAFMC 2002).

In addition to its presumed importance as a habitat for pelagic taxa, *Sargassum* is one of the few potential sources of organic matter available to pelagic communities. Scientists have speculated that the *Sargassum* complex contributes to primary and secondary production, and represents hot spots of production in otherwise oligotrophic waters (Pérès 1982). *Sargassum* productivity rates range from 0.3 to 2.4 mg C g^{-1} dry wt) and these rates are typically lower than observed for epiphytes that colonize its surface (Carpenter & Cox 1974, Lapointe 1995). Although the contribution of *Sargassum* to total production in neritic and oceanic waters is believed to be low, its production can account for up to 60% of the total primary production in the upper 1 m of the water (Carpenter & Cox 1974, Pérès 1982). Moreover, nitrogen fixation by epiphytic algae attached to *Sargassum* (e.g. cyanobacteria) may provide a substantial source of new nitrogen, representing over 40% of nitrogen for the total community (Carpenter & Cox 1974, Phlips & Zeman 1990). While recent findings in other communities dominated by large stands of macroalgae (e.g. kelps) indicate that these producers are major contributors of organic matter to higher trophic levels (Duggins 1989, Kaehler et al. 2000, Fredriksen 2003), comparable studies assessing the role or significance of *Sargassum* do not exist.

Understanding the trophic structure of *Sargassum* communities requires a detailed understanding of the feeding histories of associated fauna. In recent years, stable isotopes and fatty acids have been used extensively to investigate marine food web structure, since consumer tissues reflect the isotopic and fatty acid composition of prey in a predictable manner (e.g. Yoshii et al. 1999, Kaehler et al. 2000, Gurney et al. 2001, Fredriksen 2003). These natural biomarkers provide time integrated or long term measures of diet, and both approaches afford information on source(s) of organic matter supporting local food webs as well as trophic relationships of associated consumers (Peterson & Fry 1987, Fry 1988, Iverson et al. 1997). To date, stable isotopes have been used extensively to identify source(s) of primary production within estuarine and marine food webs (e.g. Hobson & Wassenaar 1999, Martineau et al. 2004). Although the approach provides important insights into feeding histories of marine fauna, primary producers and secondary consumers often have similar isotopic signatures, limiting the usefulness of the approach for delineating trophic relationships. Moreover, obtaining baseline stable isotope ratios is difficult due to temporal and spatial discontinuities in source signatures, and this problem becomes more complicated when multiple sources contribute to a food web (Post 2002). In turn, fatty acid signatures have been used increasingly as natural dietary tracers since they are incorporated into consumer tissue in largely unmodified form (Sargent et al. 1981). The approach provides additional discriminatory power of source material and finer resolution of prey types than bulk isotopic analysis, and has been used recently in conjunction with carbon and nitrogen isotopes to investigate food web structure (Kiyashko et al. 1998, Kharlamenko et al. 2001, Graeve et al. 2002).

In this study, we used both stable isotope and fatty acid signatures to identify the source(s) of organic matter supporting pelagic fishes in mid-shelf waters of the Gulf of Mexico. In addition, these natural dietary markers were used to delineate pathways of energy flow through the *Sargassum* complex from autotrophs to apex predators, with a species emphasis on fishes and their presumed prey (e.g. crabs, shrimps). The goals of this work were to enhance our understanding of food web dynamics within this prominent yet poorly understood component of the mid-shelf shelf ecosystem, and to determine whether *Sargassum* is an important source of energy for pelagic fishes.

MATERIALS AND METHODS

Sample collection. Flora and fauna associated with *Sargassum* were collected in 2000 and 2001 from the NW Gulf of Mexico (27° 30′ to 29° 20′ N, 96° 30′ to 93° 30′ W). Collections were taken from May to August when *Sargassum* is commonly observed in this region. Several different sampling gears were used to collect primary producers and consumers. *S. natans*, *S. fluitans*, and epiflora (*Cladophora* sp.) were picked from plankton nets and purse-seine collections, while surface waters in the general vicinity of *Sargassum* mats were sampled for particulate organic matter (POM). Samples of POM were obtained by filtering water samples through Nitex sieves, the <40 µm size fraction being used for stable isotope and fatty acid analyses. Since phytoplankton is typically the largest component of POM, we used POM as a proxy for phytoplankton even though smaller amounts of bacteria and nonliving particles are often present (Hama 1999). Epibiota (including flora and fauna) were removed from thallus, blades and pneumatocysts of *Sargassum* using forceps to minimize contamination of stable isotope and fatty acid signatures from epiphytes. Small consumers (invertebrates, juvenile fishes) were collected with a larval purse seine (1000 µm mesh) and plankton net (500 µm mesh). In addition, larger predators present within or near *Sargassum* (*Acanthocybium solandri*, *Coryphaena hippurus*, *Scomberomorus cavalla*, *Seriola dumerili*, *Thunnus albacares*, *T. atlanticus*) were collected using hook and line. Flora and fauna from several different trips and locations were used for developing stable isotope and fatty acid signatures.

Stable isotope analysis. Plants and animals were placed on dry ice in the field and later moved to freezers in the laboratory. In the laboratory, plant and animal tissues were ground for isotopic determination. Isotopic ratios were determined using a Finnigan MAT Delta-Plus 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 here as $\delta^{13}C$ or $\delta^{15}N$ according to the following equation:

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

where R is $^{13}C:^{12}C$ or $^{15}N:^{14}N$. Isotopic values of carbon and nitrogen are reported relative to Pee Dee Belemnite and atmospheric nitrogen standards, respectively. The accuracy of isotopic measurements was verified using a secondary standard reference material (chitin of marine origin, Sigma Aldrich No. C-8908).

The trophic level of heterotrophs relative to primary producers (baseline) was calculated using the equation

$$TL_{consumer} = 1 + (\delta^{15}N_{consumer} - 6.2)/3 \quad (2)$$

where TL is the trophic level, 6.2 is the baseline $\delta^{15}N$ value of primary producers (based upon estimated contribution rates by POM and *Sargassum* of 80 and 20%, respectively), and 3 is the $\delta^{15}N$ enrichment value per trophic level. Recent meta-analyses revealed that $\delta^{15}N$ enrichment values in aquatic ecosystems typically range from 2.5 to 3.5 (Vander Zanden et al. 2001, Vanderklift & Ponsard 2003), and our value of 3 represents an intermediate point of $\delta^{15}N$ enrichment.

Contribution of organic matter derived from *Sargassum* and phytoplankton production was estimated using a 2-source mixing model modified from Fredriksen (2003):

$$\text{Carbon } (\%)_{\text{Sargassum-derived}} = \frac{(\delta^{13}C_{\text{consumer}} - \delta^{13}C_{\text{POM}} - I) \times 100}{\delta^{13}C_{\text{Sargassum}} - \delta^{13}C_{\text{POM}}} \quad (3)$$

Where I represents the average fractionation of $\delta^{13}C$ per trophic level. We used a $\delta^{13}C$ enrichment value of 1.0‰ per tropic level (DeNiro & Epstein 1978), and thus I was equal to the estimated trophic level (from Eq. 2). Although the epiphyte *Cladophora* sp. is another producer associated with the *Sargassum* complex and included in this study, the biomass of this autotroph was very low (<1% of *Sargassum* biomass), and it was only observed in a small fraction of our collections. Thus *Cladophora* sp. was not included in mixing equations.

Fatty acid analysis. To broaden the scope of our fatty acid assessment, collections of pelagic fishes were extended into 2002. Additional collections were needed to comprehensively examine the fatty acid signatures of several species at each trophic level. Similar to stable isotope analysis, plants and animals remained in freezers until analytical runs. Plant tissue, whole samples of invertebrates and juvenile fishes, and lateral muscle tissue from adult fishes were homogenized for fatty acid analysis. Lipids were first extracted in duplicate aliquots in chloroform:methanol (2:1 by volume) similar to the method of Iverson et al. (2001), and fatty acid methyl esters were prepared following Iverson et al. (1992). Analysis of methyl esters was conducted using a temperature-programed Perkin Elmer Autosystem II capillary FID gas chromatograph fitted with a 30 m × 0.25 mm internal diameter column coated with 50% cyanopropyl polysilohexane (0.25 mm film thickness, J&W DB-23), and linked to a computerized integration system (Turbochrome 4 software). Identification of fatty acids and isomers was determined from known standards (Nu-Check Prep), and mass percentages were estimated from conversion factors (Ackman 1972, Ackman et al. 1991). Since polyunsaturated fatty acids (PUFAs) cannot be synthesized by consumers and are rarely modified, they are obtained exclusively from dietary sources and often used for

reconstructing dietary histories (Iverson et al. 1997, Raclot et al. 1998, Hastings et al. 2001, Graeve et al. 2002, Gurr et al. 2002, Turner & Rooker, 2005a,b). Our assessment focused primarily on the 5 most abundant PUFAs (18:2n-6 [linoleic acid], 20:4n-6 [arachidonic acid, AA], 20:5n-3 [eicosapentaenoic acid, EPA], 22:5n-3 [docosapentaenoic acid, DPA] and 22:6n-3 [docosahexaenoic acid, DHA]) observed in the tissue of primary producers and consumers, and the relative PUFA abundances (percent of total) were calculated. In addition, the relative abundance of all saturated (e.g. 14:0, 16:0, 18:0) and monounsaturated (e.g. 16:1n-7, 18:1n-9, 18:1n-7) fatty acids were considered as separate categories and their relative abundance calculated.

Data analysis. Multivariate analysis of variance (MANOVA) was used to examine differences in stable isotope and fatty acid signatures of producers and consumers. Normality and homogeneity of variance assumptions were verified using Kolmogorov–Smirnov and Bartlett tests, respectively. Principal components analysis (PCA) was used to identify interrelationships of producers and consumers based on their fatty acid composition. Since fatty acids were expressed as percentages, all data were arcsine transformed prior to testing (Zar 1996).

Collections from several summer cruises were used to generate stable isotope and fatty acid signatures of *Sargassum*-associated flora and fauna. Temporal variation in stable isotope ratios of *S. natans* and *S. fluitans* and selected consumers (*Caranx crysos*, *Balistes capriscus*) was previously investigated by Rooker et al. (2004). No seasonal differences in $\delta^{13}C$ values of *S. natans* and *S. fluitans* were detected (CV = 1.0 and 4.8%, respectively). Although more variable (2 to 4‰ shifts), $\delta^{15}N$ values did not vary significantly among collection periods. Seasonal variation in $\delta^{13}C$ values of consumers was also low but a significant seasonal effect was observed for *B. capriscus* (range –14.5 to –18.8‰). $\delta^{15}N$ values of both *C. crysos* and *B. capriscus* were similar across all months investigated. Temporal variation in the PUFA signatures at 3 distinct levels in the *Sargassum* mat community (autotroph *S. fluitans*; primary heterotroph *Leander tenuicornis*; secondary heterotroph-*B. capriscus*) was also investigated previously (Turner 2004). Although PUFA signatures varied seasonally for certain taxa, no significant differences were detected between samples collected in different years or from different locations within the NW Gulf of Mexico. These results indicated that temporal trends in stable isotope and fatty acid signatures occur. Nevertheless, differences among groups (e.g. producers) are often greater than the temporal variability within groups, suggesting that stable isotope and fatty acid signatures of *Sargassum* associated flora and fauna are relatively robust indicators of feeding history. Therefore, we pooled collections from different sampling periods to obtain sufficient sample sizes for statistical testing.

RESULTS

Stable isotopes

Stable carbon and nitrogen isotope ratios of 4 autotrophs examined (*Cladophora* sp., *Sargassum fluitans*, *S. natans* and phytoplankton [based on POM]) were distinct (MANOVA, $p < 0.001$). $\delta^{13}C$ values of *S. fluitans* and *S. natans* were enriched compared to those

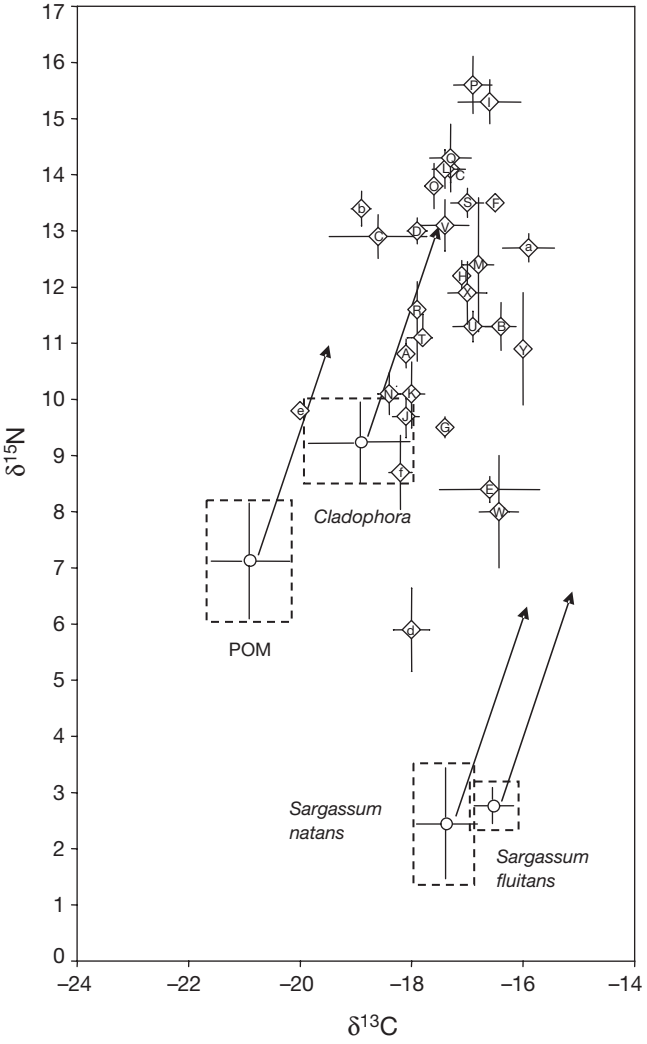

Fig. 1. Stable carbon and nitrogen isotope ratios (‰, mean ±1 SE) of producers and consumers associated with the *Sargassum* complex in the NW Gulf of Mexico in 2000 and 2001. Dashed-line boxes represent stable isotope ratios of producers; arrows denote expected trajectory of enrichment with increasing trophic position. Lower- and upper-case letters denote invertebrates and fishes, respectively (codes in Table 1)

for *Cladophora* sp. and POM (Fig. 1). Stable nitrogen isotope ratios of primary producers varied between 2.3 and 9.1‰, and $\delta^{15}N$ of both *S. fluitans* and *S. natans* were depleted by approximately 4 to 6‰ compared to *Cladophora* sp. and POM.

Stable carbon isotope ratios of fishes and potential prey (crabs, shrimps) associated with *Sargassum* ranged from −15.9 to −18.9‰ (Fig. 1). The most depleted $\delta^{13}C$ values were observed for juvenile shrimps (*Leander tenuicornis*, *Latreutes fucorum*) and juvenile crabs (*Callinectes sapidus*, *Portunus sayi*). In addition, $\delta^{13}C$ values of certain juvenile fishes *Aluterus heudeloti*, *Monacanthus hispidus Abudefduf saxatilis Histrio histrio* were depleted relative to those for other juvenile fishes e.g. *Thunnus albacares*, *T. atlanticus*, *Balistes capriscus*. Stable carbon isotope ratios of adult fishes varied (−16.4 to −17.5‰), and adults with the heaviest $\delta^{13}C$ values were *Acanthocybium solandri*, *Euthynnus alletteratus*, *Makaira nigricans* and *Scomberomorus cavalla*.

Enrichment of $\delta^{15}N$ also occurred with increasing trophic position. Values of $\delta^{15}N$ in consumers were low for juvenile crustaceans (*Leander tenuicornis*, *Latreutes fucorum*, *Portunus sayi*), ranging from 6.0 to 8.7‰; however, $\delta^{15}N$ values of certain crabs (*Callinectes sapidus*, *C. similis*) were enriched by 4 to 5‰ relative to other crustaceans (Fig. 1). The majority of juvenile fishes were secondary heterotrophs (trophic level 2.0 to 3.0, Table 1), with $\delta^{15}N$ values ranging from 8.0 to 11.0‰. $\delta^{15}N$ values of tertiary consumers (trophic level 3.0 to 4.0) ranged from 11.9 to 14.3‰, and this group was comprised primarily of juvenile and adult fishes. Estimates of trophic positions of 4.0 were only observed for 2 species (*Euthynnus alletteratus*, *Scomberomorus cavalla*, with $\delta^{15}N$ values of 15.3 ± 0.9‰ and 15.6 ± 1.2‰, respectively).

Ontogenetic shifts in $\delta^{13}C$ values and $\delta^{15}N$ values were examined for 4 species of teleosts *Seriola dumerili*, *Thunnus atlanticus*, *T. albacares*, *Histrio histrio*. Mean differences in $\delta^{13}C$ values between juveniles and adults were relatively minor and ranged from 0.1 to 1.2‰, with the largest difference observed for *S. dumerili*. Mean $\delta^{15}N$ values were enriched in adults relative to juveniles, indicating a shift to a higher trophic position with increasing size or age. Mean differences in $\delta^{15}N$ values ranged from 0.1 to 5.1‰, and $\delta^{15}N$ values of both *S. dumerili* and *T. albacares* were greater than 3.0‰, suggesting that juvenile life stages of these taxa were feeding at one trophic level below subadults or adults.

Relationships between primary producer and consumer signatures were further examined to elucidate the source(s) of organic matter supplied to consumers associated with the *Sargassum* complex. Based upon expected trophic enrichment of carbon and nitrogen (1.0 and 3.0‰ per tropic level, respectively), stable isotope signatures in the tissues of many consumers correlated well with expected fractionation patterns of POM as well as those of *Cladophora* sp. (Fig. 1); however, as noted in 'Materials and methods', the biomass of *Cladophora* sp. was relatively low and not included in the mixing equations. Carbon source estimates from the 2-source mixing model (sources POM and *Sargassum*) indicated that the 78% of organic matter supplied to consumers (pooled across taxa) in the *Sargassum* complex was derived from POM (Table 1). The contribution of *Sargassum*-derived organic matter was greater for invertebrates (34.9%) than for fishes (20.5%), and comprised the primary source for 1 invertebrate species (*Leander tenuicornis*: 58.1%). Contribution of carbon from *Sargassum* was typically less than 20% for the majority of juvenile and adult fishes. Nonetheless, for 3 juvenile fishes *Balistes capriscus*, *Thunnus atlanticus*, *T. albacares*, the predicted contribution of *Sargassum*-derived organic matter was greater than 50%.

Fatty acids

We quantified 67 individual fatty acids in producers, and profiles of *Cladophora* sp., POM, *Sargassum fluitans* and *S. natans* were dominated by 5 PUFAs (18:2n-6, 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3) as well as monounsaturated and saturated fatty acids. Fatty acid signatures of the 3 primary producer categories (*Cladophora* sp., POM, *Sargassum*) were significantly different (MANOVA, $p < 0.001$). In POM, a single C_{22} PUFA (22:6n-3) accounted for 21.5% of the fatty acids quantified (46.3% of total PUFAs), while the other 4 selected PUFAs comprised 2.3 to 6.1% of the fatty acid signature (4.8 to 13.1% of total PUFAs) (Fig. 2). POM also contained low levels of saturated fatty acids (23.1%) relative to both *Sargassum* and *Cladophora* sp. (59.8 and 65.4%, respectively). For both species of *Sargassum*, 20:4n-6 was the dominant PUFA measured, accounting for 10.6% of the fatty acid signature (39.7% of total PUFAs). While C_{22} and C_{20} PUFAs were the main PUFAs in POM and *Sargassum*, a C_{18} fatty acid, 18:2n-6, was the primary PUFA in *Cladophora* sp., comprising 9.4% of the fatty acid signature (44.3% of total PUFAs).

Multivariate testing was also used to contrast fatty acid signatures of consumers from 4 different trophic levels (determined from stable isotope analysis). Unlike the marked differences observed among producers, profiles of consumers were similar, and 22:6n-3 was the dominant PUFA at each of the 4 trophic levels examined, with values ranging from 20.3 to 27.8% (Fig. 2). Nevertheless, modest changes in fatty acid contributions were sufficient to separate the different trophic

Table 1. Predicted trophic levels of invertebrate and finfish taxa associated with *Sargassum* spp. mats in the NW Gulf of Mexico and contribution of *Sargassum* (% organic matter derived from *Sargassum*) to their diet, based on stable carbon and nitrogen isotopes; for calculation of parameters see 'Materials and methods'. Sample sizes and stage (J = juvenile; A = adult) of specimens used for stable isotope analysis are provided. Code letters are used in Fig. 1

Scientific name	Common name	Stage	N	Trophic level	% contribution	Code
Invertebrates						
Callinectes similis	Lesser blue crab	J	4	3.2	41.9	a
Callinectes sapidus	Blue crab	J	3	3.4	0.0	b
Latreutes ensiferus	Shrimp	J	6	0.9	44.2	c
Leander tenuicornis	Shrimp	J	16	1.1	53.5	d
Portunus sayi	Sargassum crab	J	6	1.8	18.6	e
Fishes						
Abudefduf saxatilis	Sergeant major	J	5	2.5	7.0	A
Acanthocybium solandri	Wahoo	A	10	2.7	39.5	B
Aluterus heudeloti	Dotterel filefish	J	3	3.2	0.0	C
Aluterus scriptus	Scrawled filefish	J	4	3.3	0.0	D
Balistes capriscus	Gray triggerfish	J	39	1.7	58.1	E
Caranx bartholomaei	Yellow jack	J	4	3.4	20.9	F
Caranx crysos	Blue runner	J	23	2.1	32.6	G
Coryphaena hippurus	Dolphin	A	9	3.0	16.3	H
Euthynnus alletteratus	Atlantic bonito	A	5	4.0	4.7	I
Histrio histrio	Sargassum fish	A	4	2.3	9.3	J
		J	11	2.2	14.0	K
Kyphosus sectatrix	Bermuda chub	A	3	3.6	0.0	L
Makaira nigricans	Blue marlin	A	3	3.1	20.9	M
Monocanthus hispidus	Planehead filefish	J	11	2.3	2.3	N
Psenes cyanophrys	Freckled driftfish	A/J	5	3.5	0.0	O
Scomberomorus cavalla	King mackerel	A	6	4.1	0.0	P
Seriola dumerili	Greater amberjack	A	3	3.3	4.7	Q
		J	12	2.3	14.0	R
Seriola rivoliana	Almaco jack	J	4	3.7	7.0	S
Syngnathus louisianae	Chain pipefish	A/J	5	2.6	9.3	T
Syngnathus pelagicus	Sargassum pipefish	A/J	5	2.7	27.9	U
Thunnus albacares	Yellowfin tuna	A	5	3.3	2.3	V
		J	2	1.6	60.5	W
Thunnus atlanticus	Blackfin tuna	A	10	2.9	20.9	X
		J	2	2.6	51.2	Y

levels (MANOVA, $p < 0.001$). Similar to our trophic level assessment, 22:6n-3 was the most abundant PUFA observed for all juvenile and adult fishes examined. Levels of this PUFA and others quantified in these 12 taxa were similar to levels found in POM but distinct from those in *Sargassum* (Fig. 3). PCA analysis of fatty acid signatures of autotrophs, juvenile fishes and adult fishes indicated that fatty acid signatures of fishes were highly similar to POM and distinct from the other producers associated with S*argassum* (Fig. 4). Component loadings indicated that 20:5n-3, 22:5n-3, and 22:6n-3 were important variables for Axis 1, while 18:2n-6 and other PUFAs contributed to Axis 2; 78% of the total variation in composition of fatty acid signatures was explained by Principal Components 1 and 2.

DISCUSSION

The stable isotope ratios of producers examined in this study were distinct, and observed values were within the range commonly reported for marine macroalgae and POM. Mean $\delta^{13}C$ values of both species of *Sargassum* ranged from approximately –16 to –17‰ and were enriched 4 to 5‰ relative to POM. Moncreiff & Sullivan (2001) also quantified $\delta^{13}C$ values of both *Sargassum* and POM in the Gulf, and reported *Sargassum* being enriched by 5‰ relative to phytoplankton. Similarly, Ishihi et al. (2001) measured carbon isotope ratios of 4 species of *Sargassum* in the western Pacific Ocean and reported that $\delta^{13}C$ values of *Sargassum* were 4 to 5‰ heavier than those of phytoplankton, suggesting that the observed differences in isotopic signatures were taxonspecific characters that may be based on phylogenetic relationships. $\delta^{15}N$ values of primary producers were also unique among producers, with both species of *Sargassum* being depleted relative to *Cladophora* sp. or POM. The lower $\delta^{15}N$ values observed for *Sargassum* may be a function of nitrogen fixation, which is often accomplished by epiphytic cyanobacteria associated with pelagic *Sargassum* (Carpenter & Cox 1974, Phlips & Zeman 1990). In the present study, cleaned blades were not enriched relative to blades with epiphytes, indicating that contami-

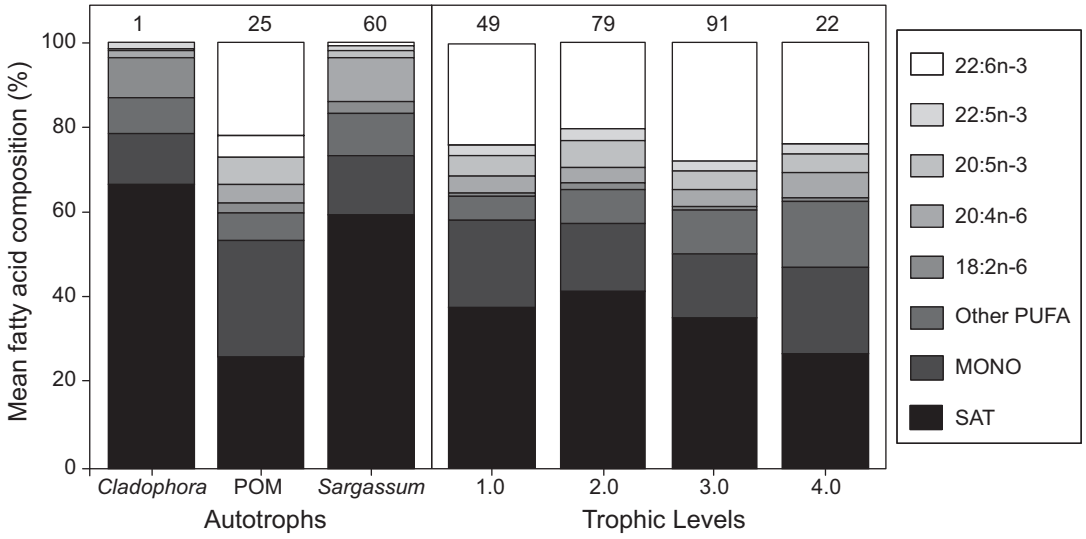

Fig. 2. Percent composition of fatty acids within autotrophs, and trophic levels (TL) 1.0 to 4.0. Suite of invertebrates and fishes was included in each trophic level category: TL 1.0 = *Balistes capriscus*, *Latreutes fucorum*, *Leander tenuicornis*; TL 2.0 = *Acanthocybium solandri*, *Caranx crysos*, *Histrio histrio*, *Monocanthus hispidus*, *Portunus sayi*; TL 3.0 = *Coryphaena hippurus*, *Kyphosus saxatilis*, *Makaira nigricans*, *Seriola dumerili*, *Thunnus atlanticus*, *T. albacares*; TL 4.0 = *Euthynnus alletteratus*, *Scomberomorus cavalla*. Mean values for grouped saturated, monounsaturated, 5 abundant polyunsaturated fatty acids (PUFAs) 18:2n-6, 20:4n-6, 20:5n-3, 22:5n-3, 22:6n-3, and all other PUFAs. Sample size for each category given above bars

nation from epiphytic form did not affect the $\delta^{13}C$ or $\delta^{15}N$ values observed for *Sargassum*. Our $\delta^{15}N$ values for *Sargassum* (2.5 to 2.8‰) compared well with measurements reported by Moncrieff & Sullivan (2001), and our mean $\delta^{15}N$ value of 7.1‰ for phytoplankton was similar to values (7 to 9‰) reported in other studies conducted in the Gulf of Mexico (Sullivan & Moncreiff 1990, Herzka & Holt 2000). Since observed signatures of producers present in and around the *Sargassum* complex were distinct, our results suggest these markers are useful for determining source(s) of organic matter supplied to consumers in the *Sargassum* complex.

Stable carbon and nitrogen isotope ratios of consumers were heavier relative to producers and patterns of enrichment indicated that 4 trophic levels of consumers were present in the *Sargassum* community. Marine food webs with 4 or more trophic levels have been reported for kelp (Kaehler et al. 2000), rocky intertidal (Menge et al. 1986) and coastal, phytoplankton-based (Bouillon et al. 2000) communities. Although stable isotope signatures of consumers associated with *Sargassum* showed some signs of vertical separation, there was a fair degree of overlap among trophic levels. The lack of trophic discreteness may be a function of omnivorous or opportunistic feeding strategies, which can obscure trophic level separation (Persson et al. 1996). Also, disturbance has been shown to constrain foraging opportunities, leading to significant shifts in trophic structure and food web length (e.g. Menge et al. 1986, Pimm & Kitching 1987). Because *Sargassum* is an ephemeral phenomenon and its physical state (e.g. large mats, windrows, scattered clumps) varies almost daily in response to changes in sea conditions. Consumers are constantly exploiting new habitats that may differ with respect to refuge, prey resources and predator fields. Under these conditions, consumers must be capable of utilizing a variety of prey resources and trophic positions.

Plots of producer and consumer stable isotope ratios, along with mixing model results, indicated that consumers appear dependent on phytoplankton (based on POM) and possibly *Cladophora* sp. production. Although POM and *Cladophora* sp. had significantly different isotopic values, the absolute differences in average $\delta^{13}C$ and $\delta^{15}N$ values were not sufficiently large to claim that these sources were isotopically distinct in terms of their relative important to the local *Sargassum* food web. Thus, our ability to distinguish the relative contribution of organic matter derived from each source to consumers could not be determined using only stable isotope ratios. Still, *Cladophora* sp. biomass was very low in the *Sargassum* complex, and therefore we did not attempt to include this producer in mixing equations. Based on our 2-source model, the largest fraction of the organic matter supplied to juvenile and adult fishes was derived from POM or phytoplankton production. Since the biomass of producers other than phytoplankton is typically low in oceanic waters, phytoplankton often constitute the primary source of

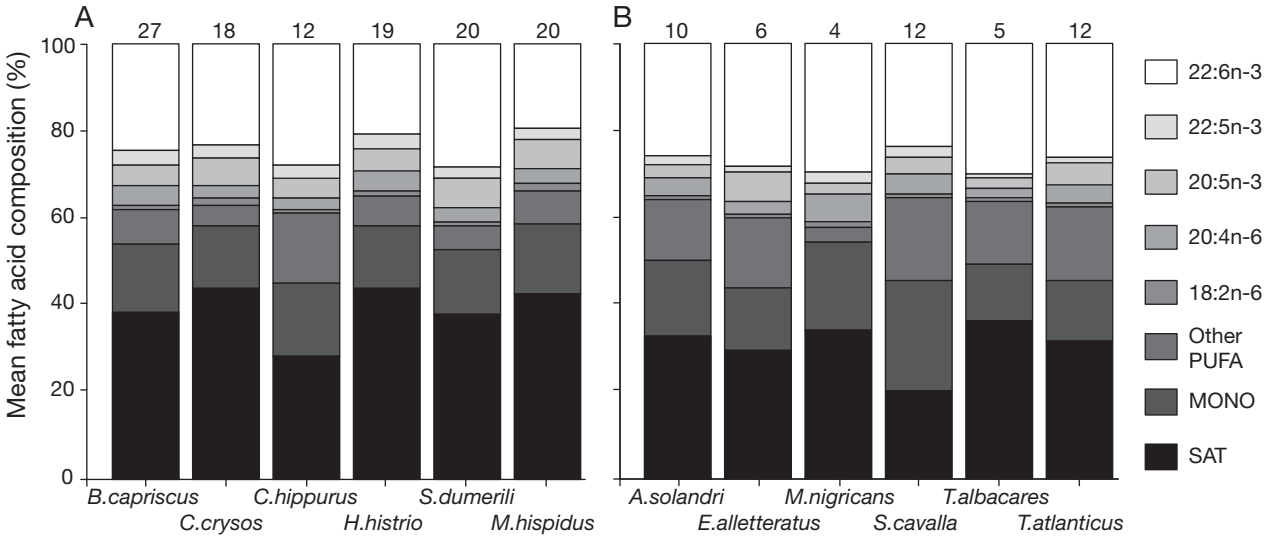

Fig. 3. Percent composition of fatty acids within (A) juvenile and (B) adult fishes. Mean values for grouped saturated, monounsaturated, 5 abundant polyunsaturated fatty acids (PUFAs) 18:2n-6, 20:4n-6, 20:5n-3, 22:5n-3, 22:6n-3, and all other PUFAs. Sample size for each category given above bars

organic matter to these food webs (e.g. Davenport & Bax 2002). However, food webs located in areas with substantial macroalgae or seagrass biomass often incorporate substantial amounts of organic matter from these producers (kelp: Kaehler et al. 2000, Fredriksen 2003; seagrass: Kharlamenko et al. 2001; epiflora: Moncreiff & Sullivan 2001). In the present study, the areal coverage and biomass of *Sargassum* was substantial, but unlike the kelp or seagrass-based food webs described above, this producer does not appear to be the main source of organic matter for the majority of higher-order consumers. Similar to other brown algae, *Sargassum* has high levels of polyphenols, which serve as a chemical defense against grazers (Pereira & Yoneshigue-Valentin 1999, Taylor et al. 2003), and the presence of polyphenols is likely to be one reason that a limited amount of organic matter is incorporated to higher trophic levels.

Fatty acid signatures of producers were distinct, and allowed us to further investigate links between producers and consumers as well as clarify the importance of *Cladophora* sp. as an autotrophic source. In the present study, PUFA signatures of POM were significantly different from those of *Sargassum* or *Cladophora* sp., and contained substantial amounts of a single long-chain C_{22} PUFA (22:6n-3). Our findings are in accord with earlier work characterizing PUFA composition of phytoplankton in coastal and offshore environments (Henderson et al. 1988, Pedersen et al. 1999, Kharlamenko et al. 2001). In contrast, the dominant PUFA present in *Sargassum* included 20:4n-6, and the high relative abundance of C_{20} PUFAs in brown algae (Phaeophyta) has been documented previously (Graeve et al. 2002). Our assessment of epiflora was limited to the green algae *Cladophora* sp., and the PUFA composition of this taxon was also unique compared to *Sargassum* and POM, with higher amounts of 18:2n-6. Graeve et al. (2002) noted that the formation of C_{20} PUFAs from C_{22} PUFAs does not readily occur in the Chlorophyta and thus high levels of C_{18} PUFAs in *Cladophora* sp. suggest that it shares this phylogenetic character with other green algae.

Trophic relationships were further examined by comparing fatty acid signatures of producers to consumers. In support of stable isotope analysis, fatty acid profiles of consumers indicated that the mid-shelf *Sargassum* food web in the Gulf was more directly linked to phytoplankton production than *Sargassum* or *Cladophora* sp. production. PUFA signatures of all groups examined (trophic levels, juvenile fishes, adult fishes) were similar to each other, and signatures matched the POM signature to a high degree. The dominant PUFA for every consumer category or species was 22:6n-3, the primary long-chain PUFA found in POM. Moreover, the relative proportion of other PUFAs (e.g. 22:5n-3, 20:5n-3, 20:4n-6) as well as saturated and monounsaturated fatty acids, was similar to the POM signature but different from *Sargassum* or *Cladophora* sp. In fact, the primary PUFA present in *Cladophora* sp. (18:2n-6) was present in low percentages in all consumer profiles. Consequently, fatty acid analysis allowed us to differentiate the contribution of producers with similar isotopic signatures, and supported the decision to omit *Cladophora* sp. from mixing equations. The high degree of similarity among consumer profiles indicated that the dominant juvenile and adult fishes inhabiting the complex were heavily dependent on phytoplankton production.

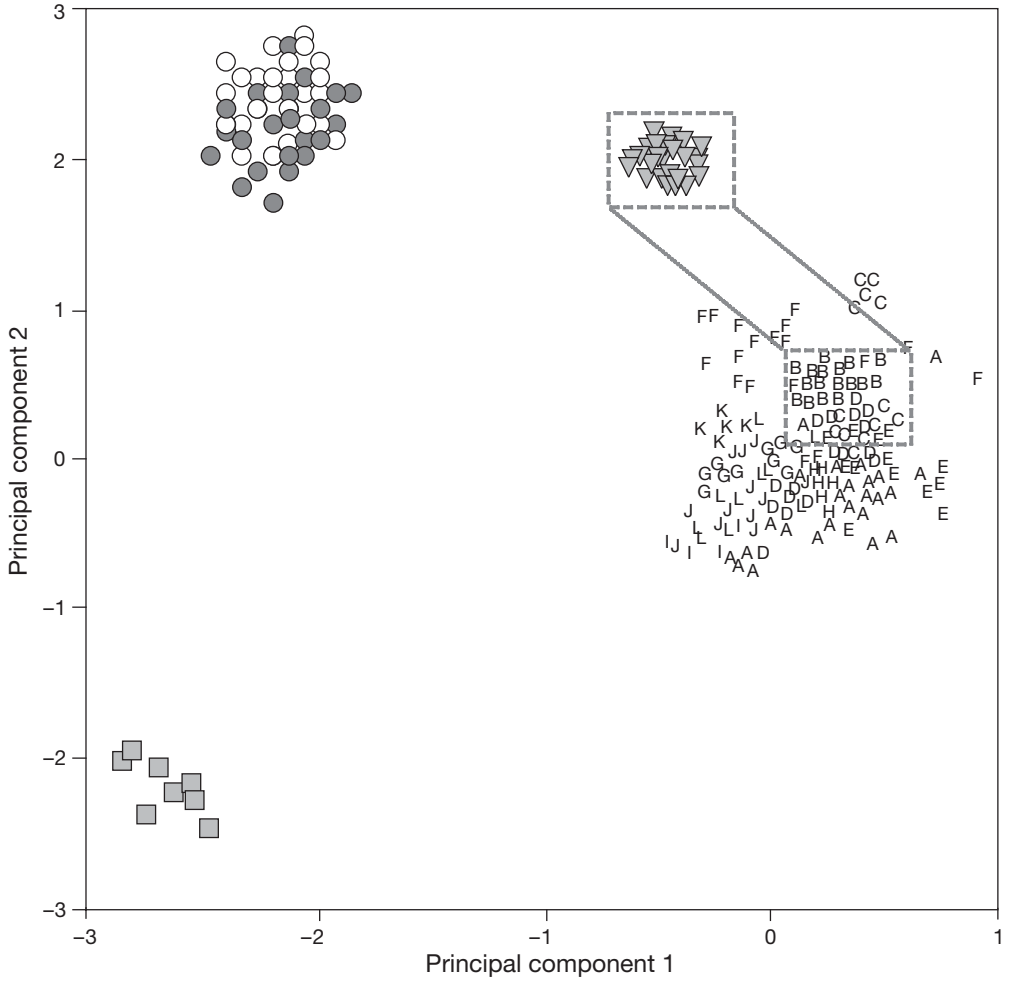

Fig. 4. Principal Components 1 and 2 for grouped saturated, monounsaturated, 5 abundant polyunsaturated fatty acids (PUFAs) 18:2n-6, 20:4n-6, 20:5n-3, 22:5n-3, 22:6n-3, and all other PUFAs of autotrophs and fishes in *Saragassum* community. (□) *Cladophora* sp., (▽) POM; (○) *Sargassum fluitans;* (●) *S. natans.* (A)–(F) juvenile fishes: (A) *Balistes capriscus*; (B) *Caranx crysos*; (C) *Coryphaena hippurus*; (D) *Histrio histrio*; (E) *Seriola dumerili*; (F) *Monocanthus hispidus.* (G)–(L) adult fishes: (G) *Acanthocybium solandri*; (H) *Euthynnus alletteratus*; (I) *Makaira nigricans*; (J) *Scomberomorus cavalla*; (K) *Thunnus albacares*; (L) *T. atlanticus*

This study effectively demonstrated that the majority of organic matter supplied to pelagic consumers in the study area did not originate from *Sargassum* production. Stable isotope and fatty acid results showed that the largest fraction of organic matter used by *Sargassum*-associated fauna was derived from POM. Still, the contribution of organic matter from *Sargassum* was important for certain taxa, particularly certain invertebrates and juvenile fishes. Moreover, *Sargassum* may enhance overall food web productivity by serving as a substrate for epiphytic algae, which may provide a substantial source of new nitrogen to the pelagic community (Dauby & Poulicek 1995). Although the present study focused on the epipelagic zone, sinking *Sargassum* may also serve as an important carbon source for benthic communities, and therefore the structure and dynamics of communities far removed from surface waters may be linked to *Sargassum* production (Schoener & Rowe 1970, Snelgrove et al. 1996). Clearly, pelagic *Sargassum* is an integral component of pelagic food webs in the Gulf, and the complex has many functional roles (e.g. energy source, physical habitat, substrate for epiflora). While this study has shed light on the functional role of *Sargassum* in mid-shelf pelagic food webs, more research is needed to fully understand its ecological value with respect to primary and secondary productivity, especially in oligotrophic waters off the continental shelf.

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