# Determining the Trophic Relationships among Flora and Fauna within *Sargassum* Mat Communities Using Fatty Acids

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ABSTRACT

Trophic relationships among organisms associated with floating *Sargassum* in the northwest Gulf of Mexico was assessed using fatty acids. Nineteen groups were selected as representatives of the *Sargassum* community including 4 autotrophs, 8 invertebrates, 5 juvenile fishes, and 2 adult fishes. Spatial and temporal variability in polyunsaturated fatty acid (PUFA) signatures of selected taxa (*Sargassum fluitans* [autotroph], *Leander tenuicornis* [primary heterotroph], *Balistes capriscus* [secondary heterotroph]) was examined to quantify natural variation within these dietary tracers. Although PUFA signatures varied seasonally for all three taxa, no differences were detected between samples collected in year 2000 and 2001 or from different sample locations in the northwest Gulf. PUFA signatures made up 16.3 - 62.3% of the total fatty acid composition of main autotrophs present in the pelagic environment (particulate organic matter [POM], epiphytic algae, *Sargassum fluitans, S. natans*), and PUFA profiles of selected primary producers were distinct. Specifically, levels of 20:5*n-3* and 22:6*n-3* were higher in POM than *Sargassum spp.* or epiphytic algae (*Cladophora sp*.). Dominant PUFA in the tissue of invertebrate and vertebrate consumers were 18:2*n-6*, 20:4*n-6*, 20:5*n-3*, 22:5*n*-3, 22:6*n*-3 and multivariate analyses indicated that PUFA signatures of all consumers were highly similar to POM. As a result, heterotrophs utilizing the *Sargassum* complex may rely heavily on phytoplankton production rather than production by *Sargassum* or associated epiphytic algae.

KEY WORDS: *Sargassum*, trophic relationships, fatty acids

# Determinación de las Relaciones Tróficas entre Fauna y Flora en las Comunidades de *Sargassum* Usando Ácidos Grasos

Las relaciones tróficas entre organismos asociados al *Sargassum* flotante en el noroeste del Golfo de México fueron evaluadas mediante ácidos grasos. Diecinueve grupos fueron seleccionados como representativos de la comunidad de *Sargassum*. Estos incluyeron 4 autótrofos, 8 invertebrados, 5 peces juveniles y 2 peces adultos. La variación espacial y temporal en el registro del ácido graso polinosaturado (PUFA) de los taxa selecionados (*Sargassum fluitans* [autótrofo], *Leander tenuicornis* [heterótrofo primario], *Balistes capriscus* [heterótrofo secondario]) fue examinada para cuantificar las

variaciones naturales en estos indicadores dietéticos. A pesar de que existieron variaciones estacionales en los 3 taxa, no se detectaron diferencias entre las muestras colectadas en los años 2000 y 2001, ni entre las diferentes localidades muestradeas en el Golfo. Los registros de PUFA contribuyeron del 16.3 al 62.3% del total de la composición de ácidos grasos en los principales autótrofos presentes en ambientes pelágicos (material orgánica particulada [POM], algas epifíticas, *Sargassum fluitans* y *S. natans*) donde los perfiles de PUFA fueron distintos entre los productores primarios. Especialmente, los niveles de 20:5*n-3* y 22:6*n-3* fueron más altos en POM que en *Sargassum spp.* o algas epifiiticas (*Cladophora sp*.). Los PUFA dominantes en los tejidos de invertebrados y vertebrados fueron 18:2*n-6*, 20:4*n-6*, 20:5*n-3*, 22:5*n*-3, 22:6*n*-3, donde un análisis multivariado indicó que los registros PUFA de todos los consumidores fueron muy similares al POM. Como resultado, los heterotrofos que utilizan el conglomerado de *Sargassum* parecen depender fuertemente en la produción fitoplantónica y no en la produción de *Sargassum* o algas epifíticas asociadas.

PALABRAS CLAVES: *Sargassum*, relaciones tróficas, ácidos grasos

## INTRODUCTION

Due to inherent problems associated with conventional measures of diet (*e.g.*, gut content analysis), considerable effort has been afforded to the development of alternative approaches (*e.g.*, stable isotopes, fatty acids) to identify trophic links and determine food web structure within marine systems (Fry and Sherr 1988, Iverson et al. 1997). Although stable carbon and nitrogen isotopes have been used extensively to identify source(s) of primary production has provided important insights on feeding histories of marine fauna, primary producers and secondary consumers often have similar isotopic signatures, thus limiting the usefulness of the approach for examining trophic relationships. In recent years, fatty acid signatures have increasingly been used as natural dietary tracers for a variety of aquatic organisms including invertebrates, fishes, sea turtles, and marine mammals (*e.g.*, Fraser et al. 1989, Graeve et al. 1994, Iverson et al. 1997, Kirsch et al. 1998), and the approach has been shown to overcome deficiencies often associated with stable isotope analysis (Kiyashko et al. 1998, Kharlamenko et al. 2001). Due to biochemical limitations in marine organisms, polyunsaturated fatty acids (PUFAs) are rarely modified and cannot be synthesized *de novo* (Raclot et al. 1998, Hastings et al. 2001, Graeve et al. 2002, Gurr et al. 2002). Therefore, PUFAs in marine consumers are obtained exclusively from dietary sources and useful for reconstructing feeding histories (*e.g.*, Iverson et al. 1997, Graeve et al. 2002).

*Sargassum* is a pelagic, brown algae that dominates a section of the western North Atlantic known as the Sargasso Sea and is present throughout the Caribbean and Gulf of Mexico (Butler et al. 1983). Two species of *Sargassum, S. fluitans and S. natans,* support a large diversity of marine invertebrates and vertebrates, including several commercially, recreationally, and ecologically important fishes (*e.g.*, *Balistes capriscus, Caranx crysos, Seriola dumerili, Coryphaena hippurus, Acanthocybium solandri*) (Dooley

1972, Bortone et al. 1977, Settle 1993). Although *Sargassum* is recognized as essential fish habitat (EFH) by the National Marine Fisheries Service (SAFMC 1998), the role of *Sargassum* has yet to be determined, and data regarding trophic relationships of associated fauna is clearly needed to fully understand its importance within pelagic ecosystems.

In the present study, we examined the feeding ecology of fauna associated with free-floating, pelagic *Sargassum* mats in the northwest Gulf of Mexico using PUFAs. Results of a previous study utilizing stable isotopes (Rooker et al. 2004) indicated that organic matter supplied to heterotrophs inhabiting the mat community might not originate from either *Sargassum* species. However, due to similarities in isotopic signatures of associated autotrophs (phytoplankton and epiphytic algae), this study did not assess the relative importance of producers other than *Sargassum.* Therefore, the aim of the present study was to use fatty acid signature analysis to trace source(s) of primary production to consumers using the *Sargassum* complex and to determine feeding histories of associated fauna. Specific objectives of the present study were to:

i) Characterize PUFAs of autotrophs and consumers, and
ii) Relate PUFAs of community flora and fauna to determine trophic relationships.

## METHODS

### Sample Collection

Samples were collected from three sites within the northwestern Gulf of Mexico, including one inshore and two offshore sites stratified into a northern and southern region (Figure 1). The inshore and offshore sites were < 30 nm and > 30 nm from shore, respectively. Collections were conducted monthly from May through August in 2000 and 2001. *Sargassum* mats were chosen at random within each region during each collection. A 20 m (L) x 3.3 m (H) purse seine with 1,000 mm mesh was deployed around individual mats to collect community flora and fauna. Larger fishes were collected by hook and line opportunistically at each sample site. Samples of POM were collected from seawater pre-filtered through a 125 mm sieve then collected in a 25 mm sieve (to reduce the risk of sample contamination) before being filtered onto 0.7 mm Whatman glass fiber filters for analysis. Samples for zooplankton were collected from seawater in a 125 mm sieve before being filtered onto 0.7-mm Whatman glass fiber filters for analysis. Epibiota (including flora and fauna) were removed from thallus, blades, and pneumatocysts of *Sargassum* using forceps.

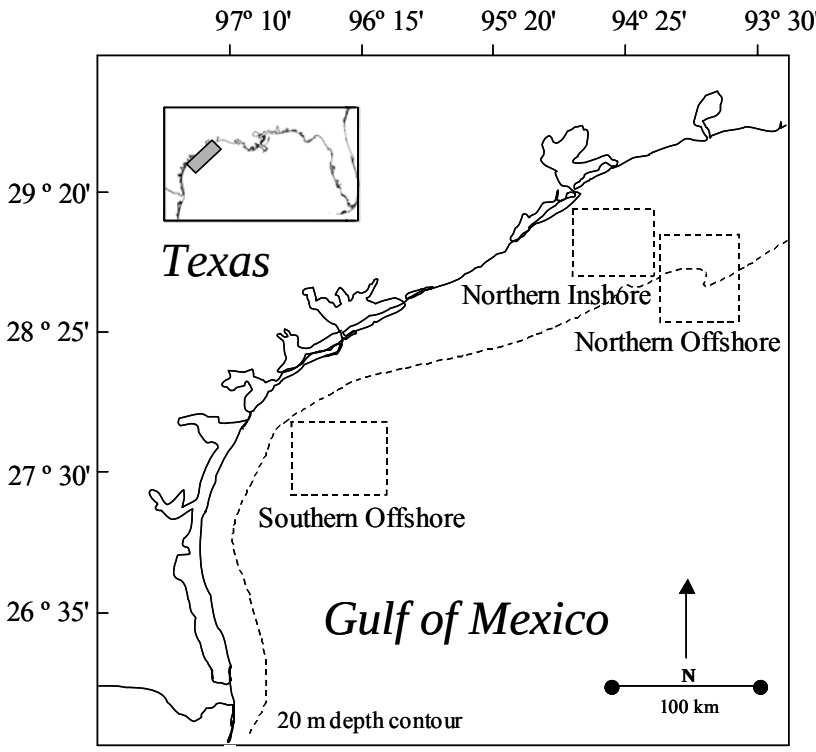


**Figure 1.** Location of sampling sites in the northwestern Gulf of Mexico

**Sample Preparation and Analysis**

Whole samples of autotrophs, invertebrates, and juvenile fishes, and lateral muscle tissue from adult fishes were homogenized thoroughly with blenders and mixing mills. Lipid was then extracted in duplicate aliquots in chloroform:methanol (2:1; v:v) after Folch et al. (1957) as modified by Iverson et al. (2001). Fatty acid methyl esters were prepared by transesterification directly from ≤ 100 mg of pure extracted lipid (filtered and dried over anhydrous sulfate), with 0.5 N sulfuric acid in methanol plus dichloromethane following the Hilditch procedure. Analysis of methyl esters was run using temperature-programmed gas chromatography on a Perkin Elmer Autosystem II Capillary FID Gas Chromatograph fitted with a 30 m x 0.25 mm internal diameter column coated with 50 % cyanopropyl polysilohexane (0.25 mm film thickness, J&W DB-23, Folsom, CA, USA) and linked to a computerized integration system (Turbochrome 4 software, PE Nelson). Identification of fatty acids and isomers was determined by calibrating gas chromatography data with known standards (Nu Check Prep., Elysian, MN, USA). Individual fatty acids were converted to mass percent of total fatty acids using conversion factors from Ackman (1972) after accounting for the contribution of BHT.

**Statistical Analyses**

Multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA) were used to examine differences in composition of PUFA signatures and individual PUFAs, respectively among autotrophs and consumers. Tukey's honestly significant difference (HSD $\alpha = 0.05$) test was used to determine significant differences between groups. Normality and homogeneity of variances were verified using Kolmogorov–Smirnov and Bartlett tests, respectively. Fatty acid data were arcsine-transformed before analyses of variance were run to correct for their binomial distribution (percentages) (Zar 1998). Principal components analysis (PCA) was used to examine distance relationships among autotrophs and subsequent consumers based on PUFA signatures. Factors were extracted using a correlation matrix with minimum eigenvalues of 1.0.

## RESULTS

Sixty-seven individual fatty acids were identified during analysis. The five PUFAs (18:2*n-6* [linoleic acid], 20:4*n-6* [arachidonic acid, AA], 20:5*n-3* [eicosapentaenoic acid, EPA], 22:5*n-3* [docosapentaenoic acid, DPA], and 22:6*n-3* [docosahexaenoic acid, DHA]), were used to assess temporal and spatial variation within the system and determine trophic relationships of the associated community fauna to reduce the number of variables because they were 1) the most abundant and 2) were found to be indicators of diet in previous studies of estuarine and marine consumers (Turner 2004). Further uses of 'PUFA signatures' were based upon these five fatty acids.

Nineteen groups were selected as representatives of the *Sargassum* community including four autotrophs, eight invertebrates, five juvenile fishes, and two adult fishes (Table 1). Although sixty-seven individual fatty acids were identified in the present study, analyses were limited to select PUFAs since marine organisms have extremely limited ability to modify these fatty acids. PUFAs comprised the largest % composition of all fatty acid groups in most of our samples (16.3 – 62.3% of the total fatty acid composition). Furthermore, the five most abundant PUFAs made up 54.1 – 95.9% of the PUFAs and 9.6 – 44.9% of the total fatty acid composition of the samples processed and were used exclusively for further characterization of trophic relationships.

**Spatial and Temporal Variation**

Spatial and temporal variation in PUFA signatures was investigated at three distinct levels in the *Sargassum* mat community: autotroph (*S. fluitans*), primary heterotroph (*Leander tenuicornis*), secondary heterotroph (*Balistes capriscus*) as part of another study (Turner 2004). Although no significant differences were detected between samples collected in 2000 and 2001, and among three sampling regions (northern inshore – NI, northern offshore – NO, and southern offshore - SO), significant seasonal differences in PUFA signatures were identified for *S. fluitans* and *Balistes capriscus*, (but not *L. tenuicornis*) using MANOVA. Therefore, to ensure that seasonal variation in PUFA signatures did not confound our characterization of trophic relation-

ships, only samples from May and June 2000 collected from the northern offshore sampling region (NO) were used for further assessments.

**Table 1.** Representative species of the *Sargassum* community. All specimens collected from Northern Offshore location during May[a] or June[b] 2000. Length represents total length for fishes, carapace length for shrimps, and carapace width for crabs.

| Species | n | Length (mm) |
|---|---|---|
| *Cladophora sp.* (green epiphytic algae)[a] | 10 | n/a |
| *Sargassum fluitans* (brown algae)[a] | 36 | n/a |
| *Sargassum natans* (brown algae)[a] | 24 | n/a |
| POM[ab] | 25 | n/a |
| Zooplankton[a] | 15 | n/a |
| *Membraniporum sp.* (bryozoan)[a] | 6 | n/a |
| *Algaophenia latecarinata* (hydroid cnidarian)[a] | 6 | 8.2-12.5 |
| *Spirorbis sp.* (serpulid polychaete)[a] | 6 | 1.1-2.3 |
| *Latruetes fucorum* (hippolytid shrimp)[a] | 12 | 8.2-15.5 |
| *Leander tenuicornis* (palaemonid shrimp)[a] | 12 | 20.5-36.0 |
| *Portunus sayi* (portunid crab)[a] | 12 | 17.0-38.5 |
| *Scyllaea pelagica* (nudibranch gastrpod)[a] | 12 | 57.3-76.1 |
| *Balistes capriscus* (gray triggerfish)[a] | 27 | 60.5-98.9 |
| *Caranx crysos* (blue runner)[a] | 18 | 46.2-57.7 |
| *Histrio histrio* (sargassum fish)[a] | 19 | 65.5-90.1 |
| *Stephanolepis hispidus* (planehead filefish)[a] | 20 | 65.2-91.4 |
| *Seriola dumerili* (greater amberjack)[a] | 20 | 91.5-145.0 |
| *Coryphaena hippurus* (dolphinfish)[ab] | 9 | 330.0-486.5 |
| *Acanthocybium solandri* (wahoo)[a] | 3 | 1035.0-1115.0 |

## Autotrophs

Significant differences in PUFA signatures of four autotrophs were identified (MANOVA, $p < 0.001$). Levels of 18:2*n-6*, 20:4*n-6*, 20:5*n-3*, and 22:6*n-3* were significantly different (ANOVA, $p < 0.001$) among autotrophs, although levels of 22:5*n-3* were similar in all autotrophs sampled ($p = 0.094$). Tukey's HSD tests indicated that levels of 20:5*n-3*, 22:5*n-3*, and 22:6*n-3* were found in significantly higher concentrations in POM than in *S. fluitans*, *S. natans*, or *Cladophora sp.* (Figure 2).

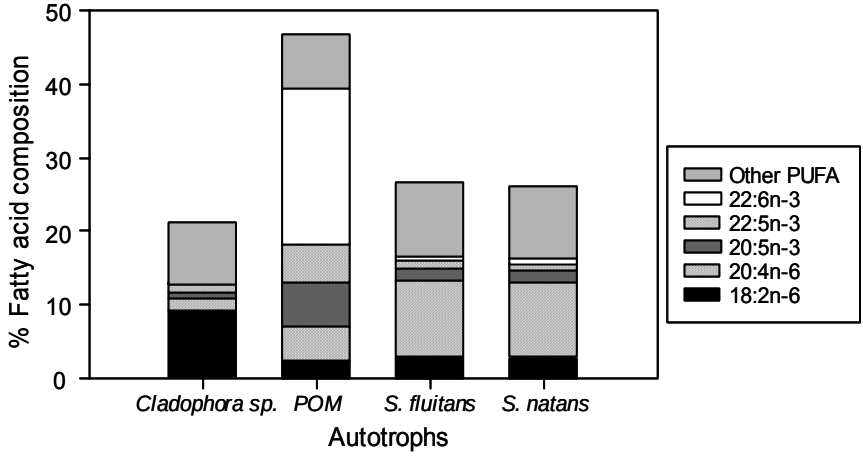


**Figure 2.** Percent composition of five abundant polyunsaturated fatty acids (PUFAs) within autotrophs. Mean values are reported for 18:2*n-6*, 20:4*n-6*, 20:5*n-3*, 22:5*n-3*, 22:6*n-3,*and of all other PUFAs.

**Invertebrates**

PUFA signatures of eight invertebrates included in the present study were significantly different (Figure 3A; MANOVA, $p < 0.001$), and ANOVA results indicate that levels of all PUFAs were significantly different among taxa examined. Levels of 18:2*n-6* were significantly different among invertebrates (ANOVA, $p < 0.001$), but Tukey's HSD test showed that overall significant differences were driven by differences among three groups: crustaceans (*Leander tenuicornis*, *Latruetes fucorum, Portunus sayi*), epibionts-nudibranch (*Membraniporum sp.*, *Spirorbis sp.*, *Algaophenia latecarinata*, and *Scyllaea pelagica*) and zooplankton. Similar trends were observed for thrree of the other four individual PUFAs ( 20:4*n-6*, 20:5*n-3*, and 22:6*n-3* (ANOVA, $p < 0.001$) were significantly different in invertebrates overall. Levels of 22:5*n-3* (ANOVA, $p < 0.001$) were also significantly different among invertebrates, but Tukey's HSD test showed that significant differences described by ANOVA results were driven by differences between two groups: crustaceans-epibionts-nudibranch and zooplankton.

**Fishes**

Significant differences in PUFA signatures of fish taxa were also observed (Figure 3B; MANOVA, $p < 0.001$). Univariate contrasts indicated 18:2*n-6*, 20:4*n-6*, 20:5*n-3*, and 22:6*n-3* were significantly different among fishes (ANOVA, $p < 0.001$), while no effect was observed for 22:5*n-3* (ANOVA, $p = 0.336$).

Tukey's HSD tests indicated that levels of 18:2*n-6*, 20:4*n-6* and 22:6*n-3* were not significantly different among species within two groups: *Caranx crysos*, *Seriola dumerili*, *Coryphaena hippurus*, *Acanthocybium solandri* and *Balistes capriscus*, *Stephanolepis hispidus*, *Histrio histrio*. Tukey's HSD tests for the PUFA 20:5*n-3* revealed significant differences between three groups: *C. crysos* and *S. dumerili*, *C. hippurus* and *A. solandri*, and *B. capriscus*, *S. hispidus*, *H. histrio*.

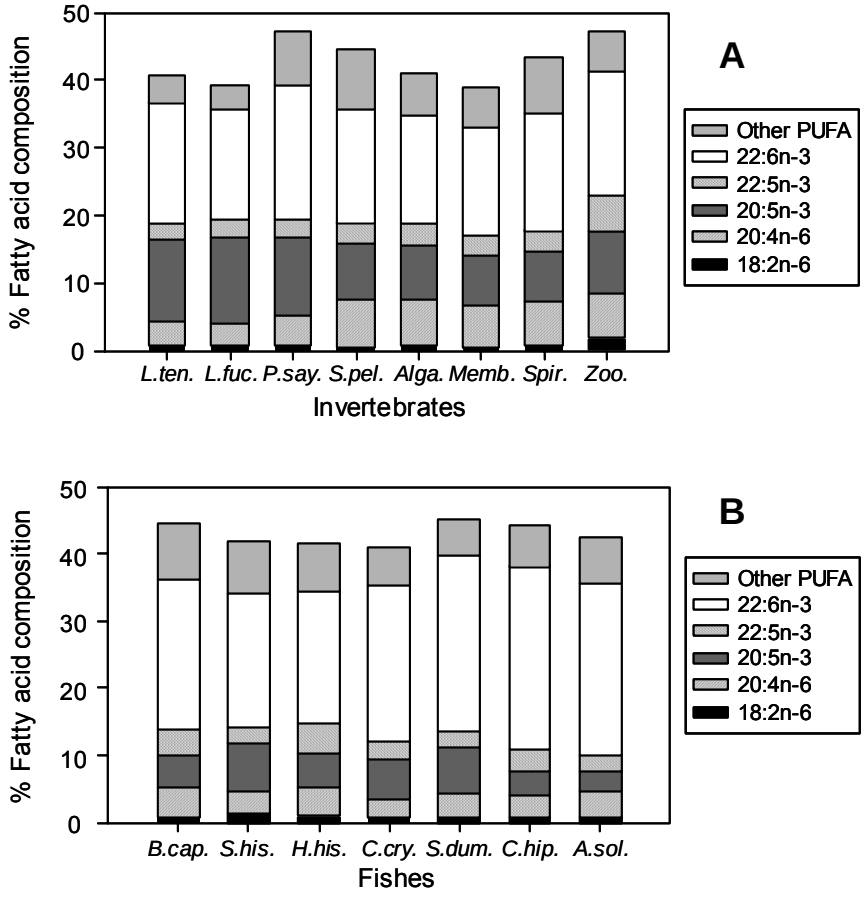


**Figure 3.** Polyunsaturated fatty acid (PUFA) data for invertebrates included in the present study including 18:2*n-6*, 20:4*n-6*, 20:5*n-3*, 22:5*n-3*, 22:6*n-3,*and of all other PUFAs. In Fig. 3A, mean values of percent composition are reported for *L. ten.* = *Leander tenuicornis*, *L. fuc*. = *Latruetes fucorum*, *P. say.* = *Portunus sayi*, *S. pea*. = *Scyllaea pelagica*, *Alga*. = *Algaophenia latecarinata*, *Memb*. = *Membraniporum sp.*, *Spir*. = *Spirorbis sp.*, Zoo. = zooplankton. In Fig. 3B, mean values of percent composition are reported for *B. cap*. = *Balistes capriscus*, *M. his*. = *Stephanolepis hispidus*, *H. his*. = *Histrio histrio*, *C. cry*. = *Caranx crysos*, *S. dum*. = *Seriola dumerili*, *C. hip*. = *Coryphaena hippurus*, *A. sol*. = *Acanthocybium solandri*.

**Trophic Interactions**

Levels of PUFA signatures of consumers were closely related to levels found in POM, while significantly different from quantities found in either *Sargassum* species or *Cladophora sp.* (Figures. 2, 3A, and 3B; MANOVA, $p < 0.001$). PCA was performed using averaged individual PUFA values including all autotrophs, invertebrates, and fishes, and over 68% of the variation in composition of PUFA signatures could be explained by principal components 1 and 2. Scatterplots of components 1 and 2 revealed similarities among individual invertebrates and fishes with POM and separation from other autotrophs (Figure 4).

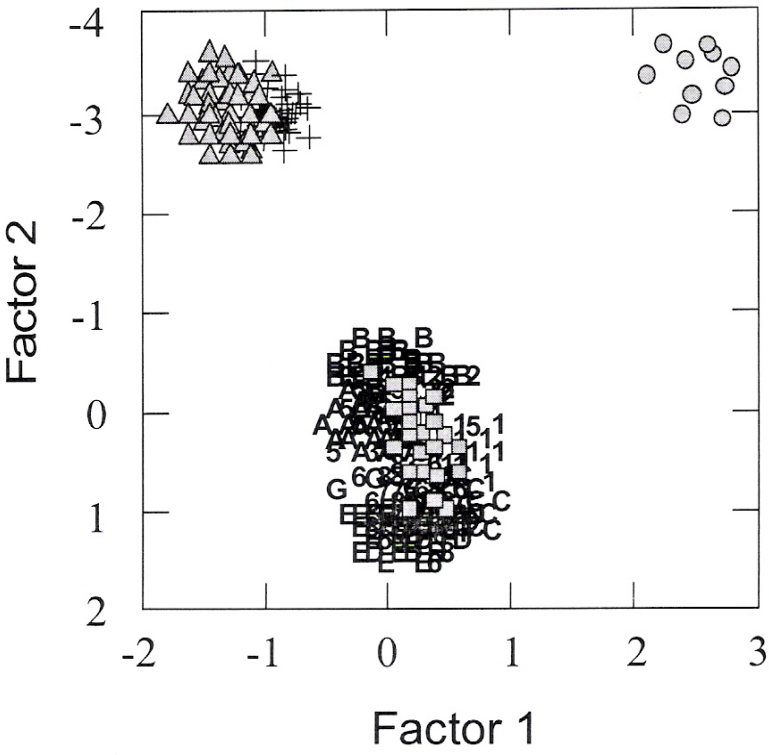


**Figure 4.** Factor scores plot of principal components analysis based upon polyunsaturated fatty acid (PUFA) signatures of autotrophs (■-POM, ▲-*Sargassum fluitans,* **+** - *Sargassum natans,* ●-*Cladophora sp.)*, invertebrates (1-zooplankton, 2- *Membraniporum sp.*, 3- *Spirorbis sp.*, 4- *Algaophenia latecarinata*, 5- *Scyllaea pelagica*, 6- *Leander tenuicornis*, 7- *Latruetes fucorum*, 8-*Portunus sayi*), and fishes (A- *Stephanolepis hispidus*, B- *Balistes capriscus*, C-*Histrio histrio*, D- *Caranx crysos*, E- *Seriola dumerili*, F- *Coryphaena hippurus*, G- *Acanthocybium solandri*) in the *Sargassum* community.

## DISCUSSION

Results suggest that PUFA signatures of POM were significantly different than signatures of *Sargassum spp.* or *Cladophora sp*., and contained high levels of PUFAs including 20:5*n-3*, 22:5*n-3*, and 22:6*n-3*, while levels of 18:2*n-6* and 20:4*n-6* were more abundant in macroalgae. These results were not unexpected since concentrations of long-chain PUFAs (20:5*n-3*, 22:5*n-3*, 22:6*n-3*) are found in many phytoplankton species, although levels of these fatty acids are often minimal or absent in macroalgae (Herbretau et al. 1997, Graeve et al. 2002). Phytoplankton is typically the largest component of POM, though smaller amounts of bacteria and non-living particles are present (Hama 1999), and it often contains substantial amounts of long-chain PUFAs like 20:5*n-3* and 22:6*n-3* (Pedersen et al. 1999, Graeve et al. 2002). As previously reported, large concentrations of long-chain PUFAs including 20:5*n-3*, 22:5*n-3*, and 22:6*n-3* are often found in diatoms, dinoflagellates, and haptophytes (Harrington et al. 1970, Henderson et al. 1988, Pedersen et al. 1999). Conversely, macroalgae typically contain very low quantities of long-chain PUFAs, especially 22:6*n-3* (Herbretau et al. 1997, Graeve et al. 2002). Therefore, PUFA signatures of phytoplankton in POM are most likely contributing to signatures found in the *Sargassum* food web indicating that phytoplankton is the likely source of organic matter in this complex.

Organic matter incorporated into invertebrates and fishes appears to have originated from phytoplankton in POM rather than *Sargassum spp*. or *Cladophora sp*. based upon PUFA signatures. High levels of long-chain PUFA signatures, apparently derived from phytoplankton, were identified in all zooplankton, microinvertebrate, macroinvertebrates, and fishes included in the present study. For example, copepods, the most abundant marine zooplankton in pelagic waters, are highly associated with *Sargassum* and typically feed upon diatom and dinoflagellate species (Yeatman 1962, Cowles et al. 1988, Xu and Wang 2001). As a result, food chains utilizing organic matter from marine phytoplankton tend to be exceptionally enriched in levels of 20:5*n-3* and 22:6*n-3* (Sargent 1978, Pedersen et al. 1999, Domiazon et al. 2000).

Although *Sargassum* does not appear to directly contribute nutrients to the food web, it may play important roles in nutrient recycling, aggregation mechanisms, as substrate, and increasing habitat complexity in pelagic environments. This is seemingly facilitated by similar oceanographic factors involved with "clumping" *Sargassum* and may, in addition, accumulate large concentrations of phytoplankton in these characteristically oligotrophic areas, ultimately shaping the composition of organic matter in this system (Carpenter 1970, Dooley 1972, Stoner and Greening 1984, Woodcock 1993). Additionally, by contributing byproducts of photosynthetic processes, *Sargassum* appears to be an important component in nutrient cycling of the pelagic environment (Shoener and Rowe 1970). *Sargassum* also serves as a substrate for other macro algae, including large amounts of *Cladophora sp*., which often covers portions of whole *Sargassum* plants. Furthermore, drifting macrophytes add to habitat complexity in many surface waters, facilitate redistribution of organisms in pelagic environments, and could affect survival of species that depend upon them for food and refuge (Kingsford 1995). Similarly, by

increasing the complexity of the pelagic environment *Sargassum* may bring about redistribution of organisms and augment survivorship by providing protection from predators and improving food availability during early life stages in several fishes.

In summary, high concentrations of PUFAs found in POM more closely match levels in higher trophic groups of the *Sargassum* community than signatures of *Sargassum fluitans, S. natans,* or *Cladophora sp*., suggesting that phytoplankton is the major source of organic matter entering this food web. Organic matter incorporated into invertebrates including crustaceans, nudibranch, epibionts, and zooplankton, appears to have originated from phytoplankton in POM based upon PUFA signatures. PUFA signatures of juvenile and adult fishes in the complex are similar to prey taxa and thus utilization of *Sargassum* mats is in part may be linked to their value as feeding grounds.

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