Journal of FISH BIOLOGY
Published for the Fisheries Society of the British Isles

Journal of Fish Biology (2009) **75**, 1719–1732
doi:10.1111/j.1095-8649.2009.02424.x, available online at www.interscience.wiley.com

Feeding ecology of pelagic fish larvae and juveniles in slope waters of the Gulf of Mexico

R. J. D. Wells* and J. R. Rooker

Department of Marine Biology, Texas A&M University, 5007 Avenue U, Galveston, TX 77551, U.S.A.

(Received 5 January 2009, Accepted 3 August 2009)

Stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) were used to investigate feeding patterns of larval and early juvenile pelagic fishes in slope waters of the Gulf of Mexico. Contribution of organic matter supplied to fishes and trophic position within this pelagic food web was estimated in 2007 and 2008 by comparing dietary signatures of the two main producers in this ecosystem: phytoplankton [based on particulate organic matter (POM)] and *Sargassum* spp. Stable isotope ratios of POM and pelagic *Sargassum* spp. were significantly different from one another with δ^{13}C values of POM depleted by 3–6‰ and δ^{15}N values enriched by 2‰ relative to *Sargassum* spp. Stable isotope ratios were significantly different among the five pelagic fishes examined: blue marlin *Makaira nigricans*, dolphinfish *Coryphaena hippurus*, pompano dolphinfish *Coryphaena equiselis*, sailfish *Istiophorus platypterus* and swordfish *Xiphias gladius*. Mean δ^{13}C values ranged almost 2‰ among fishes and were most depleted in *I. platypterus*. In addition, mean δ^{15}N values ranged 4–5‰ with highest mean values found for both *C. hippurus* and *C. equiselis* and the lowest mean value for *M. nigricans* during both years. Increasing δ^{13}C or δ^{15}N with standard length suggested that shifts in trophic position and diet occurred during early life for several species examined. Results of a two-source mixing model suggest approximately an equal contribution of organic matter by both sources (POM = 55%; pelagic *Sargassum* spp. = 45%) to the early life stages of pelagic fishes examined. Contribution of organic matter, however, varied among species, and sensitivity analyses indicated that organic source estimates changed from 2 to 13% for a δ^{13}C fractionation change of ±0·25‰ or a δ^{15}N fractionation change of ±1·0‰ relative to original fractionation values.

Key words: larval fishes; POM; *Sargassum*; stable isotopes; trophic ecology.

INTRODUCTION

Naturally occurring stable isotopes are commonly used to elucidate the origins of organic matter and food web complexity within marine communities (Peterson & Fry, 1987; Kaehler *et al*., 2000; Nagelkerken & van der Velde, 2004). While stable isotopes alone do not elucidate direct prey species in complex and omnivorous food webs like traditional stomach content analysis, these methods are valuable in tracking organic matter through ecosystems (Peterson & Fry, 1987; Post, 2002). Given the

*Author to whom correspondence should be addressed. Tel.: +1 409 740 4784; fax: +1 409 740 4787; email: wellsr@tamug.edu

utility of these natural dietary tracers, few studies have attempted to use stable isotopes to examine the early life feeding ecology of pelagic fishes in offshore marine ecosystems (Rooker *et al*., 2006; Pepin & Dower, 2007).

Slope waters in the northern Gulf of Mexico (Gulf) serve as important habitat to several pelagic fishes (Kraus & Rooker, 2007; Neilson *et al*., 2007; Rooker *et al*., 2007). Shelf regions of the Gulf are generally recognized as productive areas relative to offshore slope waters due to significant nutrient loading from the Mississippi River coupled with unique oceanographic conditions (*i.e.* Loop Current) that influence the distribution and abundance of pelagic fishes (Richards *et al*., 1989, 1993). Studies describing distribution and abundance patterns of the early life stages of pelagic fishes have found high concentrations in slope waters of the northern Gulf (Richards *et al*., 1993; Tidwell, 2008; Simms, 2009), including several migratory species: blue marlin *Makaira nigricans* Lacépède, dolphinfish *Coryphaena hippurus* L., pompano dolphinfish *Coryphaena equiselis* L., sailfish *Istiophorus platypterus* (Shaw) and swordfish *Xiphias gladius* L. (Ditty *et al*., 1994; Govoni *et al*., 2003). Despite the importance of large pelagic fishes on ecosystem productivity, little is known about the feeding ecology of these fishes in slope waters during early life nor the pathways of energy flow through this offshore component of the pelagic food web.

A characteristic feature of slope waters in the Gulf is the limited number of carbon sources compared with benthic and estuarine systems. In addition to phytoplankton, pelagic *Sargassum* spp. is a ubiquitous brown alga present in surface waters of the northern Gulf that provides both food and protection from predators to fishes (Wells & Rooker, 2004; Rooker *et al*., 2006). Early life stages of several pelagic species such as billfishes, tunas, swordfishes and dolphinfishes are found in association with *Sargassum* spp. in the northern Gulf (Hoffmayer *et al*., 2005). Moreover, *Sargassum* spp. has been designated as essential fish habitat (EFH) due to its importance as nursery habitat to several pelagic fish and invertebrate species (NOAA, 1996). To date, assessments of food web structure of pelagic fauna associated with *Sargassum* spp. are limited and centred on late juvenile to adult stages (Rooker *et al*., 2006; Turner & Rooker, 2006), and complementary studies on pelagic fishes during the early life are rare.

Goals of this study were to estimate the contribution of phytoplankton- and *Sargassum* spp.-derived organic matter to the early life stages of pelagic fishes using natural dietary tracers (δ^{13}C and δ^{15}N). Temporal differences in both producers and consumers were evaluated over a 2 year study period, and a range of consumer sizes were collected to investigate ontogenetic shifts in feeding during early life.

MATERIALS AND METHODS

SAMPLING METHODOLOGY

Sampling was conducted in slope waters of the northern Gulf of Mexico during June and July of 2007 and 2008, and both producers and consumers were collected within a corridor ranging from 27·0–27·5° N and 89–92° W off the Louisiana and Texas coasts. Sampling stations were located *c*. 8 nautical miles (14·8 km) apart from one another during the surveys. Two replicate particulate organic matter (POM) and *Sargassum* spp. samples were obtained at six stations within the sample corridor during each year. Surface seawater collections of POM were used as a proxy for phytoplankton, and samples were obtained by

filtering 7 l of sea water over 47 mm GF/F filters (precombusted for 1 h at 450° C) with an effective pore size of 0·8 μm. *Sargassum* spp. and fishes were obtained using two neuston nets (500 and 1200 μm mesh) with opening dimensions of 2 m (width) × 1 m (height). Nets were concurrently towed at 2·5 knots (4·6 km h^{-1}) for 10 min per station. Five pelagic fish species were targeted and analysed in this study: *M. nigricans*, *C. hippurus*, *C. equiselis*, *I. platypterus*, *X. gladius*. Several larger fishes (early juveniles) were collected using dip-nets during the survey. In addition, due to the low sample size of *X. gladius* >10 mm standard length (L_S) collected during 2007 surveys, several samples were obtained within the same sample corridor from previous July surveys in 2005 ($n = 13$) and 2006 ($n = 9$).

STABLE ISOTOPE ANALYSIS

Pelagic fishes were immediately sorted onboard and frozen on dry ice until being moved to a −80° C freezer. Fishes were measured to the nearest mm L_S, and both stomachs and heads were removed to minimize any effect on stable isotope analysis of muscle tissue. Epibiota were removed from *Sargassum* spp. to minimize the influence of other flora and fauna on stable isotope ratios. Plant and fish tissue samples were dried at 60° C for 24 h and stored in individual cryovials. Lipids were not removed from fish tissue; however, C:N ratios were low (<4) across the size spectrum of fishes, indicating a low lipid content and little influence of lipids on fish tissue $\delta^{13}C$ values (Post *et al*., 2007). Isotopic ratios for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in plant and fish tissue were determined with a Finnigan MAT DeltaPlus continuous-flow stable isotope mass spectrometer (www.thermofisher.com). Isotopic ratios are reported relative to Vienna PeeDee belemnite for carbon and atmospheric N_2 for nitrogen.

The potential carbon contribution of POM and *Sargassum* spp. to pelagic fishes was estimated with the two-source mixing model described by Fredriksen (2003) and Rooker *et al*. (2006): $\%C_{Sargassum} = 100\ [(\delta^{13}C_{Consumer} - \delta^{13}C_{POM} - I)\ (\delta^{13}C_{Sargassum} - \delta^{13}C_{POM})^{-1}]$, where I is the average fractionation value of $\delta^{13}C$ per trophic position. A carbon trophic enrichment factor of 1·0‰ was used (DeNiro & Epstein, 1978; Fry & Sherr, 1984); thus I was equal to the estimated trophic position. Trophic position (T_P) was calculated following Hobson & Welch (1992): $T_P = (1 + \delta^{15}N_{Consumer} - \delta^{15}N_{Baseline})I^{-1}$, where $\delta^{15}N$ baseline was the average value of POM and *Sargassum* spp. and I was equal to the average fractionation value of $\delta^{15}N$ per trophic position. $\delta^{15}N$ enrichment values range from 2·5 to 3·5‰ in aquatic systems (Vander Zanden & Rasmussen, 2001; Vanderklift & Ponsard, 2003); therefore, a nitrogen enrichment value of 3·0‰ was used per trophic position (Fry & Sherr, 1984; Rooker *et al*., 2006). Fractionation values can vary in larval fishes (Herzka & Holt, 2000; Bosley *et al*., 2002), thus a sensitivity analysis was performed to investigate the range of possible outcomes to model results using different fractionation factors in the two-source mixing model ($\delta^{15}N$: ±0·5, ±1·0 and ±1·5‰; $\delta^{13}C$: ±0·25 and ±0·5‰).

DATA ANALYSIS

A t-test was used to test for differences in $\delta^{13}C$ and $\delta^{15}N$ between organic sources (POM and *Sargassum* spp.). ANCOVA was used to test for differences in stable isotopes among fish species and between years with L_S as the covariate. Log_{10}-transformed L_S was regressed against $\delta^{13}C$ and $\delta^{15}N$ for each species to test for size-specific trends in consumer values. The equal variance assumption was assessed by examining plots of residuals v. predicted values, and normality was tested with a Shapiro–Wilk test. *A posteriori* differences among means were detected with Tukey's honestly significant difference (HSD) test using SAS (SAS Institute Inc.; www.sas.com). Statistical significance was determined at $P \leq 0{\cdot}05$ for all analyses.

TABLE I. Mean ± S.E. of carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope ratios of primary producers collected in 2007 and 2008

	2007		2008	
	δ^{13}C (‰)	δ^{15}N (‰)	δ^{13}C (‰)	δ^{15}N (‰)
POM	−21·5 ± 0·6	2·8 ± 0·7	−22·7 ± 0·3	2·6 ± 0·5
Sargassum spp.	−18·7 ± 0·3	0·8 ± 0·3	−16·4 ± 0·2	0·8 ± 0·6

POM, particulate organic matter.

RESULTS

Differences in stable isotope ratios of primary producers were significantly different, and both δ^{13}C (2007: *t*-test, $P = 0{\cdot}01$; 2008: *t*-test, $P < 0{\cdot}01$) and δ^{15}N (2007: *t*-test, $P < 0{\cdot}05$; 2008: *t*-test, $P < 0{\cdot}05$) differed between POM and *Sargassum* spp. samples during both years (Fig. 1). POM was depleted in δ^{13}C and enriched in δ^{15}N relative to *Sargassum* spp. during both years investigated (Table I). Furthermore, *Sargassum* spp. δ^{13}C values significantly differed between years (*Sargassum* spp.: *t*-test, $P < 0{\cdot}01$), while δ^{13}C POM values remained similar (POM: *t*-test, $P > 0{\cdot}05$), and δ^{15}N values of primary producers did not significantly differ between years (POM: *t*-test, $P > 0{\cdot}05$; *Sargassum* spp.: *t*-test, $P > 0{\cdot}05$) (Table I). Thus, a δ^{15}N baseline value of 1·7 was used to calculate trophic position of consumers.

Stable isotope ratios of both δ^{13}C and δ^{15}N significantly differed among fishes during both years (Fig. 1). Larval (<20 mm L_S) and juvenile consumers (≥20 mm L_S) are shown separately for visual purposes. Significant differences in δ^{13}C were detected in 2007 and 2008 (ANCOVA, d.f. = 4, 92, $P < 0{\cdot}01$) with mean δ^{13}C values ranging from −20·1‰ (larval *I. platypterus*) to −17·6‰ (juvenile *C. hippurus*) in 2007 and from −20·2‰ (larval *C. equiselis*) to −16·9‰ (juvenile *X. gladius*) in 2008 (Fig. 1). Pair-wise comparisons showed that δ^{13}C values were significantly depleted in *I. platypterus* compared with all other fish species except *M. nigricans* in 2007 ($P < 0{\cdot}01$), and depleted relative to *C. hippurus*, *C. equiselis* and *X. gladius* in 2008 ($P < 0{\cdot}01$). Significant differences in δ^{15}N were also found during both years (2007: ANCOVA, d.f. = 4, 92; $P < 0{\cdot}05$; 2008: ANCOVA; d.f. = 4, 92; $P < 0{\cdot}01$). During both years, mean δ^{15}N values for *C. hippurus* and *C. equiselis* were not significantly different to one another (2007: $P > 0{\cdot}05$, 2008: $P > 0{\cdot}05$), but significantly higher than the other species examined: *M. nigricans*, *I. platypterus* and *X. gladius* ($P < 0{\cdot}05$). Trophic position of the five consumers were consistently higher in 2008 than 2007 and followed a similar pattern as δ^{15}N with highest values for *C. hippurus* and *C. equiselis* (1·7–2·4), followed by *I. platypterus* (1·2–1·4), *X. gladius* (1·1–1·3) and *M. nigricans* (0·4–0·6) (Table II).

Ontogenetic changes in stable isotope composition were found for each of the five species examined (Fig. 2 and Table III). Several species showed significant positive trends of increasing δ^{13}C values with increasing size; these included *M. nigricans*, *C. hippurus* and *I. platypterus* (Table III). Relationships between δ^{15}N and size were more variable, and a significant year effect was found for both *I. platypterus* ($P < 0{\cdot}01$) and *X. gladius* ($P < 0{\cdot}01$). *Istiophorus platypterus* and *X. gladius* had significantly higher δ^{15}N values in 2008 than 2007 (Fig. 2). Significant positive

associations between δ^{15}N and size were detected for *C. hippurus*, *C. equiselis*, *I. platypterus* (2007 and 2008) and *X. gladius* in 2008 (Table III).

Results of the two-source mixing model indicated that POM and *Sargassum* spp. contributed approximately equal amounts of organic matter to consumers (Fig. 3). POM and *Sargassum* spp. contributed 55 and 45%, respectively, of the organic matter to these consumers when averaged over both years. Predicted contribution estimates of organic matter provided from POM were highest for *I. platypterus* (2007 = 76% and 2008 = 77%) and *C. equiselis* (54 and 80%). Conversely, based on the two-source model, *Sargassum* spp. was the primary source of organic matter supplied to

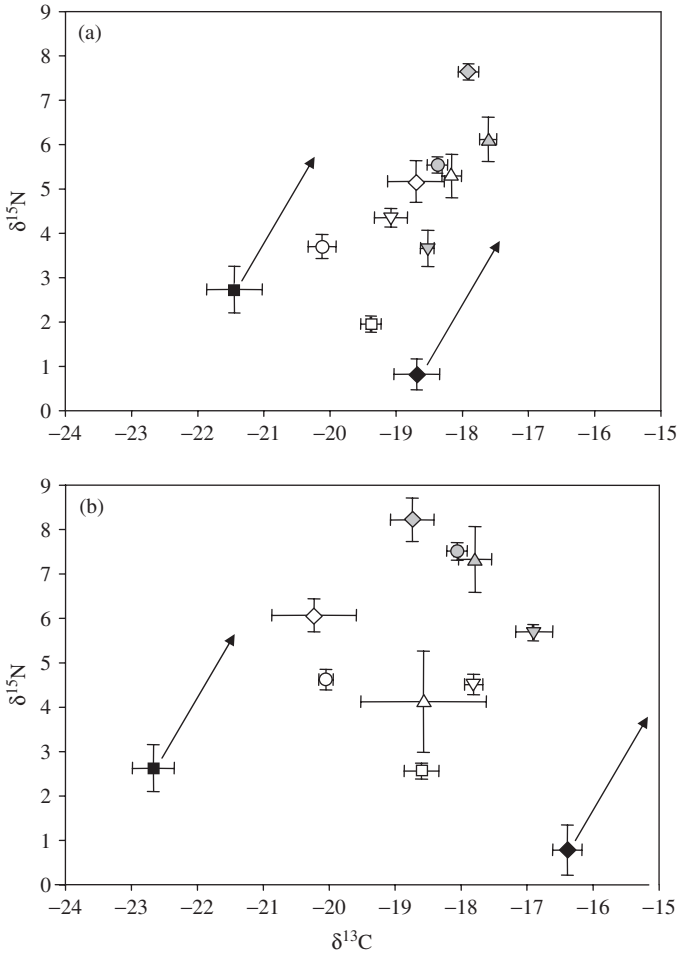

FIG. 1. Biplot of mean ± S.E. carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotopes of primary producers and pelagic fishes during (a) 2007 and (b) 2008. Primary producers include particulate organic matter (■) and *Sargassum* spp. (◆). Pelagic fishes include *Makaira nigricans* (□), *Coryphaena hippurus* (△), *Coryphaena equiselis* (◇), *Istiophorus platypterus* (○) and *Xiphias gladius* (▽). Larval consumers <20 mm standard length (L_S) (open) and juveniles ≥20 mm L_S (grey) are shown separately. Lines with arrows represent expected enrichment with increasing trophic position.

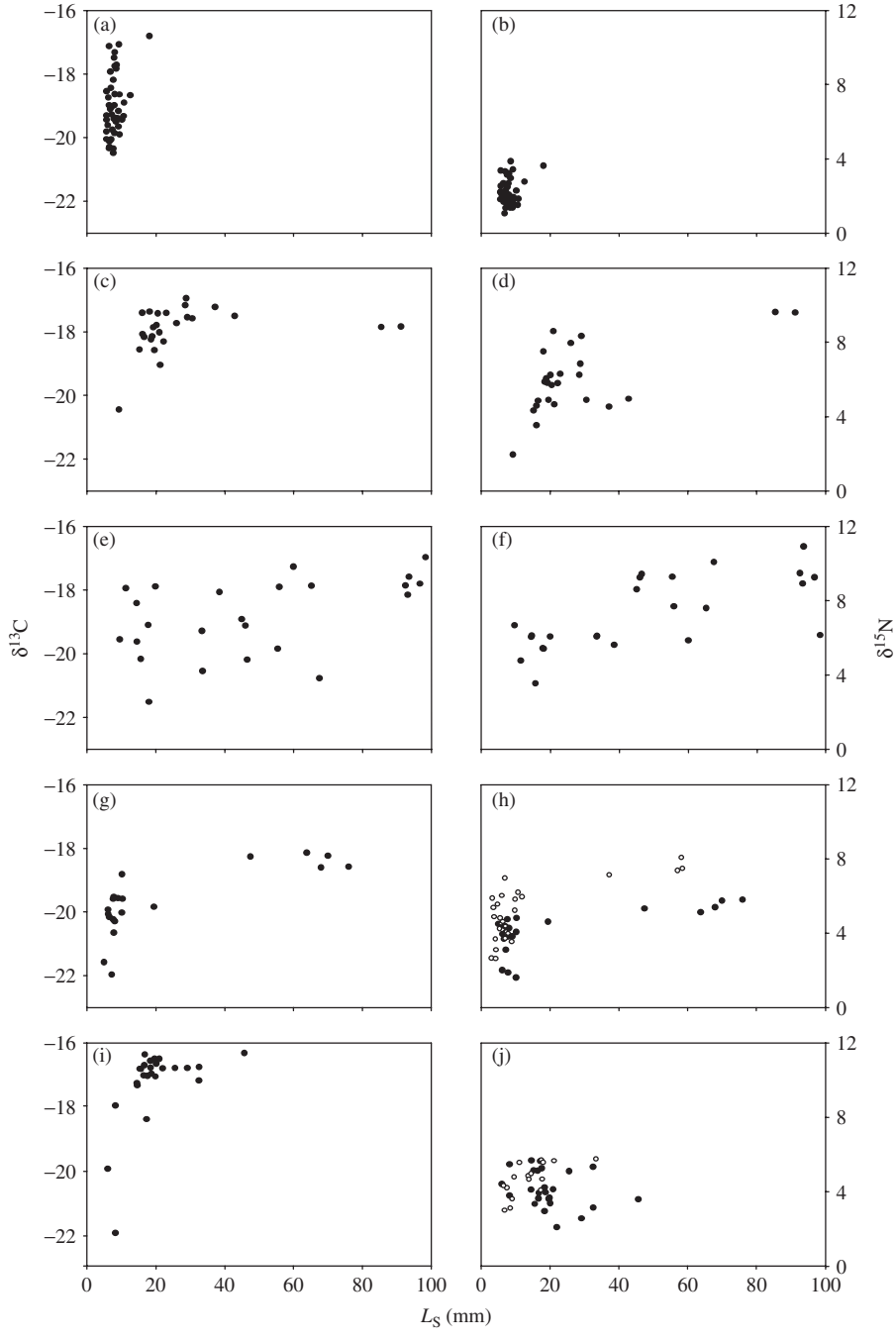

FIG. 2. (a), (c), (e), (g), (i) carbon (δ^{13}C) and (b), (d), (f), (h), (j) nitrogen (δ^{15}N) stable isotopes of (a), (b) *Makaira nigricans*, (c), (d) *Coryphaena hippurus*, (e), (f) *Coryphaena equiselis*, (g), (h) *Istiophorus platypterus* and (i), (j) *Xiphias gladius* relative to standard length (L_S). Years were combined for all species except δ^{15}N for *Istiophorus platypterus* and *Xiphias gladius* due to significant differences between 2007 (●) and 2008 (○).

TABLE II. Standard length (L_S) range, mean L_S, trophic position by year (2007, 2008) and total number of samples for stable isotope analysis of each species

Species	L_S range (mm)	Mean L_S (mm)	Trophic position	*n*
Makaira nigricans	5·6–18·1	8·0	0·4, 0·6	46
Coryphaena hippurus	9·2–91·2	27·8	1·7, 1·9	25
Coryphaena equiselis	9·5–100·0	47·7	1·7, 2·4	24
Istiophorus platypterus	3·2–76·0	18·7	1·2, 1·4	50
Xiphias gladius	6·0–45·7	17·5	1·1, 1·3	41

M. nigricans (62 and 55%) and *X. gladius* (52 and 58%). For *C. hippurus*, *Sargassum* spp. accounted for 70% of organic matter in 2007 and only 44% in 2008 (Fig. 3).

Sensitivity analyses of the two-source mixing model showed that changes in fractionation values affected estimates of POM and *Sargassum* spp.-derived organic

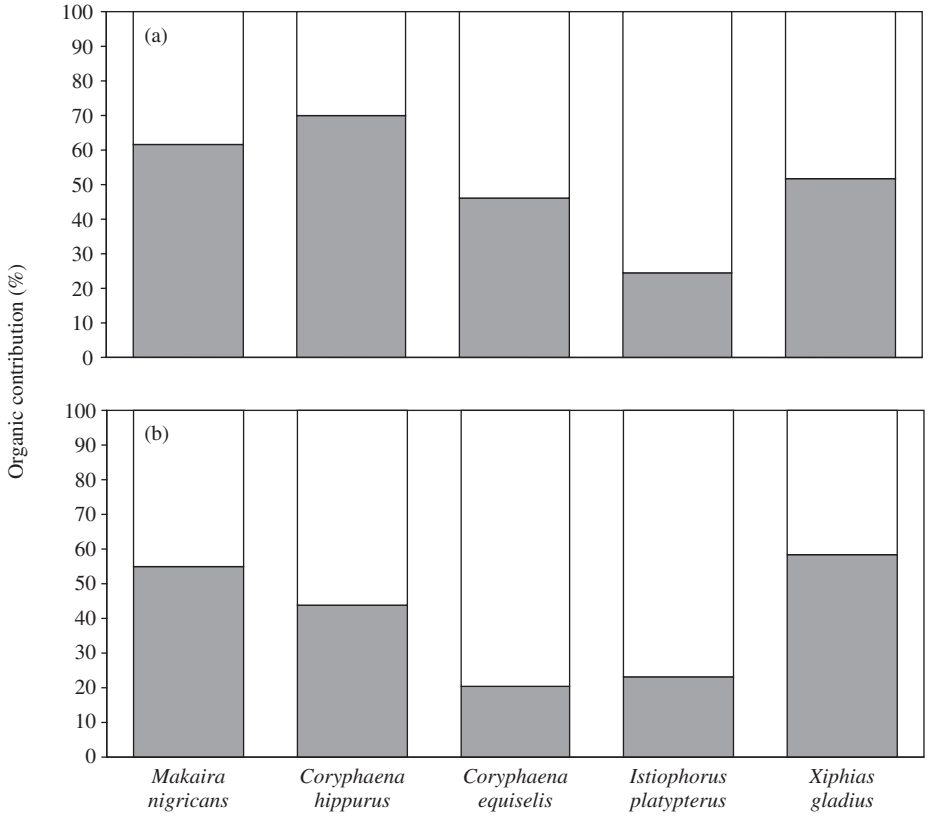

FIG. 3. Organic contribution (%) derived from particulate organic matter (□) and *Sargassum* spp. (■) to five pelagic fishes during (a) 2007 and (b) 2008. Contribution estimates were derived from a two-source mixing model.

TABLE III. Standard length (L_S)-based relationships of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes for five pelagic fish species

Species	$\delta^{13}C$ and L_S	r^2	P value	$\delta^{15}N$ and L_S	r^2	P value
Makaira nigricans	$y = -21{\cdot}85 + 3{\cdot}18 \log_{10} L_S$	0·11	<0·05	$y = 1{\cdot}37 + 0{\cdot}94 \log_{10} L_S$	0·02	>0·05
Coryphaena hippurus	$y = -20{\cdot}05 + 1{\cdot}53 \log_{10} L_S$	0·22	<0·05	$y = -1{\cdot}88 + 5{\cdot}70 \log_{10} L_S$	0·46	<0·001
Coryphaena equiselis	$y = -21{\cdot}19 + 1{\cdot}49 \log_{10} L_S$	0·16	>0·05	$y = 0{\cdot}64 + 4{\cdot}21 \log_{10} L_S$	0·50	<0·001
Istiophorus platypterus	$y = -21{\cdot}56 + 1{\cdot}82 \log_{10} L_S$	0·64	<0·001	2007: $y = 1{\cdot}87 + 2{\cdot}02 \log_{10} L_S$	0·50	<0·001
				2008: $y = 2{\cdot}33 + 2{\cdot}95 \log_{10} L_S$	0·52	<0·001
Xiphias gladius	$y = -19{\cdot}84 + 1{\cdot}18 \log_{10} L_S$	0·06	>0·05	2007: $y = 6{\cdot}02 - 1{\cdot}50 \log_{10} L_S$	0·08	>0·05
				2008: $y = 1{\cdot}09 + 3{\cdot}23 \log_{10} L_S$	0·52	<0·01

matter to consumers. Specifically, changes in the *Sargassum* spp.-derived contribution estimates from original model parameters showed that a δ^{13}C fractionation change of ±0·25‰ was equal to a δ^{15}N fractionation change of ±1·0‰ (Table IV). Increasing δ^{13}C fractionation values by 0·25 and 0·5‰ resulted in increased POM and decreased *Sargassum* spp.-derived organic matter ranging from 2 to 12% and 5 to 21%, respectively. In contrast, increasing δ^{15}N fractionation values increased *Sargassum* spp.-derived (and consequently reduced POM) estimates by 1–8, 2–13 and 3–16% for 0·5, 1·0 and 1·5‰ increases in δ^{15}N, respectively. Largest deviations in contribution estimates were found for both *C. hippurus* and *C. equiselis*, ranging from 4 to 21%, while smallest changes were found for *M. nigricans* (1–7% change).

DISCUSSION

Differences between POM and *Sargassum* spp. in this study were *c*. 3–6‰ for δ^{13}C and 2‰ for δ^{15}N. Depleted δ^{13}C and enriched δ^{15}N values of POM relative to *Sargassum* spp. were found in other studies located in the northern Gulf (Moncrieff & Sullivan, 2001; Rooker *et al*., 2006). Both studies reported that δ^{13}C values of POM were depleted 3–5‰ and δ^{15}N values were enriched 4–5‰ relative to *Sargassum* spp. δ^{13}C values of POM and *Sargassum* spp. were similar to Moncrieff & Sullivan (2001) and Rooker *et al*. (2006) with mean values varying <1‰. Here, δ^{15}N values were depleted relative to these studies with mean POM values 4–7‰ less and *Sargassum* spp. 1–4‰ less. Cross-shelf transport of organic material derived from the Mississippi River drainage basin has been documented in shelf and slope sediments in the northern Gulf (Goni *et al*., 1997). Depleted δ^{15}N values of producers in offshore waters have been attributed to a riverine influence in the Beaufort Sea (Dunton *et al*., 2006) and western North Pacific (Usui *et al*., 2006), and thus it is possible that depleted δ^{15}N values of POM and *Sargassum* spp. observed here may have been due to terrestrially derived nitrogen from the Mississippi River plume. Alternatively, Takai *et al*. (2007) found that δ^{15}N POM values increased from 0·7 to 7·1‰ along an inshore to offshore gradient near south-eastern Japan and attributed differences to ^{15}N-depleted nitrogen from N_2 fixation by blue–green algae of the genus *Trichodesmium*. During both 2007 and 2008 surveys, large amounts of *Trichodesmium* spp. were collected in the 500 μm neuston net at many of the stations, which consequently may have affected δ^{15}N values in this study.

Studies examining feeding dynamics of marine fishes have found rapid isotopic changes occur during early larval stages when a change in diet is accompanied by fast tissue turnover time (Lindsay *et al*., 1998; Herzka & Holt, 2000). Feeding studies analysing stomach contents of pelagic species examined here have observed shifts from planktivorous to piscivorous diets at or *c*. 7–10 mm L_S (Gorbunova, 1969; Gorbunova & Lipskaya, 1975; Lipskaya & Gorbunova, 1977; Govoni *et al*., 2003; Llopiz & Cowen, 2008; R. C. Schekter, unpubl. data). Moreover, Tidwell (2008) recently reported that the stomachs of larval *M. nigricans* and *I. platypterus* collected in the present study area consisted primarily of cyclopoid copepods of the family Corycaeidae (*Corycaeus* and *Farranula* spp.) with piscivory becoming well developed at *c*. 9 mm L_S. Rapid δ^{13}C enrichment and abrupt changes in δ^{15}N values observed at this size interval were an indication of the shift to piscivory for several taxa in the present study.

TABLE IV. Per cent change (expressed as absolute value) in the estimated contribution of *Sargassum* spp.-derived organic matter when fractionation values are varied relative to the original model of 3·0 and 1·0‰ for nitrogen and carbon, respectively. Scenarios represent a ±0·5, ±1·0 and ±1·5‰ change relative to original model for δ^{15}N, and ±0·25 and ±0·5‰ for δ^{13}C in each year

	2007					2008				
	Change in δ^{15}N			Change in δ^{13}C		Change in δ^{15}N			Change in δ^{13}C	
	±0·5‰	±1·0‰	±1·5‰	±0·25‰	±0·5‰	±0·5‰	±1·0‰	±1·5‰	±0·25‰	±0·5‰
Makaira nigricans	2·0	3·5	4·6	3·5	6·6	1·4	2·4	3·1	2·4	4·6
Coryphaena hippurus	7·8	12·6	15·9	12·6	21·4	3·9	6·4	8·2	6·4	11·2
Coryphaena equiselis	7·6	11·8	14·5	11·8	18·8	4·2	6·5	7·8	6·5	9·8
Istiophorus platypterus	4·9	7·5	9·1	7·5	11·5	2·9	4·6	5·8	4·6	7·7
Xiphias gladius	5·4	8·7	11·0	8·7	14·9	2·9	4·8	6·3	4·8	8·9

Even though δ^{15}N enrichment with increasing size was probably linked to a trophic shift (piscivory), results were variable among species examined. δ^{15}N values of both *M. nigricans* and *X. gladius* (2007) initially decreased with increasing size, which deviates from the expected pattern of enrichment. Spatial and temporal variability in stable isotopes of primary producers can confound interpretations (Boon & Bunn, 1994; Jennings *et al*., 1997); however, baseline δ^{15}N values of primary producers were consistent during both years. Alternatively, the decrease in δ^{15}N with increasing size for larval *M. nigricans* and *X. gladius* may have been attributed to a shift from endogenous to exogenous feeding, where a transition from a parental nitrogen source to one dominated by exogenous nitrogen has been shown to influence δ^{15}N of consumers during the larval stage (Vander Zanden *et al*., 1998; Wells *et al*., 2008). Finally, Pepin & Dower (2007) reported highly variable δ^{15}N values with increasing size for six larval fish species and suggested that differences may be related to differential input from herbivorous and microbial food webs. Primary prey organisms consumed by larval fishes examined in this study include species (*i.e.* copepods) that consume a wide range of prey (*e.g.* ciliates and protists) that are associated with the microbial loop (Vincent & Hartmann, 2001; de Figueiredo *et al*., 2005; Olson *et al*., 2006), and therefore differential inputs by taxa associated with the microbial loop may be responsible in part for observed patterns.

POM and *Sargassum* spp. contributed organic matter equally to species examined here from slope waters of the northern Gulf. Rooker *et al*. (2006) found that *Sargassum* spp. contributed 22% of the organic matter to consumers in shelf waters when combined across all taxa; however, results were species-specific and dependent upon trophic position. For example, species with a trophic position $>2{\cdot}0$ obtained an average of only 14% of *Sargassum* spp.-derived organic matter. In contrast, species with a trophic position $<2{\cdot}0$ obtained an average of 47% *Sargassum* spp.-derived organic matter. In the present study, the trophic position of most species (except *C. equiselis* in 2008) was $<2{\cdot}0$, and the estimated contribution from *Sargassum* spp. was remarkably similar to the previous study (45%). *Coryphaena equiselis* in 2008 had the highest trophic position (2·4) and lowest contribution of organic matter from *Sargassum* spp. (20%), which supports the notion that the source of organic matter may be stage-specific. The data clearly show that *Sargassum* spp. serves as a critical source of organic matter to pelagic fishes during the larval period, and survival of several pelagic species may be linked to the distribution and abundance of *Sargassum* spp. in the Gulf.

Results of the sensitivity analyses suggest minor changes (1–16%) in per cent *Sargassum* spp.-derived contribution estimates to consumers, given trophic fractionation of δ^{15}N was in the range of 1·5–4·5‰, while changes between 2 and 21% if δ^{13}C fractionation was between 0·5 and 1·5‰. Herzka & Holt (2000) found fractionation ranges between 1·5 and 4·2‰ for δ^{15}N and 0·2 and 1·9‰ for δ^{13}C in larval red drum *Sciaenops ocellatus* (L.). Trophic fractionation values of 2·5–3·5‰ for δ^{15}N (Vander Zanden & Rasmussen, 2001; Vanderklift & Ponsard, 2003) and 1·0‰ for δ^{13}C (DeNiro & Epstein, 1978; Fry & Sherr, 1984; Rooker *et al*., 2006) are commonly used; however, isotopic fractionation during assimilation, protein synthesis and excretion of nitrogen may cause these conventional fractionation values to change (Ponsard & Averbuch, 1999). The present results are similar to those of Vander Zanden & Rasmussen (2001), who found that changes in mixing model outputs were minor provided primary producers were used as the baseline trophic level,

and that mixing model end members were distinct from one another, as was the case in this study. Nevertheless, given deviations found from sensitivity analyses, values of contribution estimates from primary producers are not absolute and should therefore be viewed as a proxy for source estimates of organic matter provided to consumers. Also, choosing an appropriate baseline can be one of the most difficult methodological issues when using stable isotopes, and therefore it is important to assess any large temporal variation (Post, 2002). δ^{15}N values of primary producers remained similar over the two-year study period, and thus the mean δ^{15}N baseline of 1·7‰ appears suitable for mixing model calculations. POM δ^{13}C values were also similar between years; however, δ^{13}C values of *Sargassum* spp. significantly differed between years and consequently may have affected mixing model results, given a 0·5‰ change in δ^{13}C baseline values resulted in a 5–21% change in contribution estimates of consumers.

We thank the students and staff of the Fisheries Ecology and Ecosystem Research Lab at Texas A & M University at Galveston (J. Simms, R. Schloesser, L. Wetmore and M. Dance) for assistance in the field and laboratory. In addition, a special thanks to the crew of the *Lady Bride*. Support for this work was provided by National Oceanic and Atmospheric Administration and the McDaniel Charitable Foundation.

References

Boon, P. I. & Bunn, S. E. (1994). Variations in the stable isotope composition of aquatic plants and their implications for food web analysis. *Aquatic Botany* **48,** 99–108.

Bosley, K. L., Witting, D. A., Chambers, R. C. & Wainright, S. C. (2002). Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. *Marine Ecology Progress Series* **236,** 233–240.

DeNiro, M. J. & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42,** 495–506.

Ditty, J. G., Shaw, R. F., Grimes, C. B. & Cope, J. S. (1994). Larval development, distribution, and abundance of common dolphin, *Corypheana hippurus*, and pompano dolphin, *C. equiselis* (family: Coryphaenidae), in the northern Gulf of Mexico. *Fishery Bulletin* **92,** 275–291.

Dunton, K. H., Weingartner, T. & Carmack, E. C. (2006). The nearshore western Beaufort Sea ecosystem: circulation and importance of terrestrial carbon in arctic coastal food webs. *Progress in Oceanography* **71,** 362–378.

de Figueiredo, G. M., Nash, R. D. M. & Montagnes, D. J. S. (2005). The role of the generally unrecognised microprey source as food for larval fish in the Irish Sea. *Marine Biology* **148,** 395–404.

Fredriksen, S. (2003). Food web studies in a Norwegian kelp forest based on stable isotope (δ^{13}C and δ^{15}N) analysis. *Marine Ecology Progress Series* **260,** 71–81.

Fry, B. & Sherr, E. B. (1984). δ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science* **27,** 13–47.

Goni, M. A., Ruttenberg, K. C. & Eglinton, T. I. (1997). Sources and contribution of terrigenous organic carbon to surface sediments in the Gulf of Mexico. *Nature* **389,** 275–278.

Gorbunova, N. N. (1969). Breeding grounds and food of the larvae of the swordfish [*Xiphias gladius* Linne (Pisces, Xiphilidae)]. *Problems in Ichthyology* **9,** 375–387.

Gorbunova, N. N. & Lipskaya, N. Y. (1975). Feeding of larvae of the blue marlin, *Makaira nigricans* (Pisces, Istiophoridae). *Journal of Ichthyology* **15,** 95–101.

Govoni, J. J., Laban, E. H. & Hare, J. A. (2003). The early life history of swordfish (*Xiphias gladius*) in the western North Atlantic. *Fishery Bulletin* **101,** 778–789.

Herzka, S. Z. & Holt, G. J. (2000). Changes in isotopic composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: potential applications to settlement studies. *Canadian Journal of Fisheries and Aquatic Sciences* **57,** 137–147.

Hobson, K. A. & Welch, H. E. (1992). Determination of trophic relationships within a high Arctic marine food web using δ^{13}C and δ^{15}N analysis. *Marine Ecology Progress Series* **84,** 9–18.

Hoffmayer, E. R., Franks, J. S., Comyns, B. H., Hendon, J. R. & Waller, R. S. (2005). Larval and juvenile fishes associated with pelagic *Sargassum* in the northcentral Gulf of Mexico. *Proceedings of the Gulf and Caribbean Fisheries Institute* **56,** 259–270.

Jennings, S., Renones, O., Morales-Nin, B., Polunin, N. V. C., Moranta, J. & Coll, J. (1997). Spatial variability in the δ^{15}N and δ^{13}C stable isotope composition of plants, invertebrates, and fishes on Mediterranean reefs: implications for the study of trophic pathways. *Marine Ecology Progress Series* **146,** 109–116.

Kaehler, S., Pakhomov, E. A. & McQuaid, C. D. (2000). Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by δ^{13}C and δ^{15}N analysis. *Marine Ecology Progress Series* **208,** 13–20.

Kraus, R. T. & Rooker, J. R. (2007). Patterns of vertical habitat use by blue marlin (*Makaira nigricans*) in the Gulf of Mexico. *Gulf and Caribbean Research* **19,** 89–97.

Lindsay, D. J., Minagawa, M., Mitani, I. & Kawaguchi, K. (1998). Trophic shift in the Japanese anchovy *Engraulis japonicus* in its early life history stages as detected by stable isotope ratios in Sagami Bay, Central Japan. *Fisheries Science* **64,** 403–410.

Lipskaya, N. Y. & Gorbunova, N. N. (1977). Feeding of sailfish larvae. *Oceanology* **17,** 340–344.

Llopiz, J. K. & Cowen, R. K. (2008). Precocious, selective and successful feeding of larval billfishes in the oceanic Straits of Florida. *Marine Ecology Progress Series* **358,** 231–244.

Moncrieff, C. A. & Sullivan, M. J. (2001). Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. *Marine Ecology Progress Series* **215,** 93–106.

Nagelkerken I. & van der Velde, G. (2004). Are Caribbean mangroves important feeding grounds for juvenile reef fish from adjacent seagrass beds? *Marine Ecology Progress Series* **274,** 143–151.

Neilson, J. D., Paul, S. D. & Smith, S. C. (2007). Stock structure of swordfish (*Xiphias gladius*) in the Atlantic: a review of the non-genetic evidence. *Collective Volume of Scientific Papers ICCAT* **61,** 25–60.

NOAA (1996). Magnuson–Stevens Fishery Conservation and Management Act, as amended through Oct. 11, 1996. *NOAA Technical Memorandum* **NMFS-F/SPO-23**.

Olson, M. B., Lessard, E. J., Wong, C. H. J. & Bernhardt, M. J. (2006). Copepod feeding selectivity on microplankton, including the toxigenic diatoms *Pseudo nitzschia* spp., in the coastal Pacific Northwest. *Marine Ecology Progress Series* **326,** 207–220.

Pepin, P. & Dower, J. F. (2007). Variability in the trophic position of larval fish in a coastal pelagic ecosystem based on stable isotope analysis. *Journal of Plankton Research* **29,** 727–737.

Peterson, B. J. & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18,** 293–320.

Ponsard, S. & Averbuch, P. (1999). Should growing and adult animals fed on the same diet show different δ^{15}N values? *Rapid Communications in Mass Spectrometry* **13,** 1305–1310.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83,** 703–718.

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J. & Montana, C. G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* **152,** 179–189.

Richards, W. J., Leming, T., McGowen, M. F., Lamkin, J. & Kelley-Fraga, S. (1989). Distribution of fish larvae in relation to hydrographic features of the Loop Current boundary in the Gulf of Mexico. *Rapports et Procés-verbaux des Reunions Consiel international pour l'Exploration de la Mer* **191,** 169–176.

Richards, W. J., McGowan, M. F., Leming, T., Lamkin, J. T. & Kelley, S. (1993). Larval fish assemblages at the Loop Current boundary in the Gulf of Mexico. *Bulletin of Marine Science* **53,** 475–537.

Rooker, J. R., Turner, J. P. & Holt, S. A. (2006). Trophic ecology of *Sargassum*-associated fishes in the Gulf of Mexico determined from stable isotopes and fatty acids. *Marine Ecology Progress Series* **313,** 249–259.

Rooker J. R., Alvarado Bremer, J. R., Block, B. A., Dewar, H., De Metrio, G., Corriero, A., Kraus, R. T., Prince, E. D., Rodriguez-Marin, E. & Secor, D. H. (2007). Life history and stock structure of Atlantic bluefin tuna (*Thunnus thynnus*). *Reviews in Fisheries Science* **15,** 265–310.

Simms, J. R. (2009). Early life ecology of sailfish, *Istiophorus platypterus*, in the northern Gulf of Mexico. MSc Thesis, Texas A&M University, TX, U.S.A.

Takai, N., Hirose, N., Osawa, T., Hagiwara, K., Kojima, T., Okazaki, Y., Kuwae, T., Taniuchi, T. & Yoshihara, K. (2007). Carbon source and trophic position of pelagic fish in coastal waters of south-eastern Izu Peninsula, Japan, identified by stable isotope analysis. *Fisheries Science* **73,** 593–608.

Tidwell, M. T. (2008). The feeding ecology of larval billfishes (Family: Istiophoridae) in the Northern Gulf of Mexico. MSc Thesis, University of Texas at Austin, TX, USA.

Turner, J. P. & Rooker, J. R. (2006). Fatty acid composition of flora and fauna associated with Sargassum mats in the Gulf of Mexico. *Marine Biology* **149,** 1025–1036.

Usui, T., Nagao, S., Yamamoto, M., Suzuki, K., Kudo, I., Montani, S., Noda, A. & Minagawa, M. (2006). Distribution and sources of organic matter in surficial sediments on the shelf and slope off Tokachi, western North Pacific, inferred from C and N stable isotopes and C/N ratios. *Marine Chemistry* **98,** 241–259.

Vanderklift, M. A. & Ponsard, S. (2003). Sources of variation in consumer-diet δ^{15}N enrichment: a meta-analysis. *Oecologia* **136,** 169–182.

Vander Zanden, M. J. & Rasmussen, J. B. (2001). Variation in δ^{15}N and δ^{13}C trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanography* **46,** 2061–2066.

Vander Zanden, M. J., Hulshof, M., Ridgway, M. S. & Rasmussen, J. B. (1998). Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. *Transactions of the American Fisheries Society* **127,** 729–739.

Vincent, D. & Hartmann, H. J. (2001). Contribution of ciliated microprotozoans and dinoflagellates to the diet of three copepod species in the Bay of Biscay. *Hydrobiologia* **443,** 193–200.

Wells, R. J. D. & Rooker, J. R. (2004). Spatial and temporal patterns of habitat use by fishes associated with *Sargassum* mats in the northwestern Gulf of Mexico. *Bulletin of Marine Science* **74,** 81–99.

Wells, R. J. D., Cowan, J. H. Jr. & Fry, B. (2008). Feeding ecology of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. *Marine Ecology Progress Series* **361,** 213–225.

