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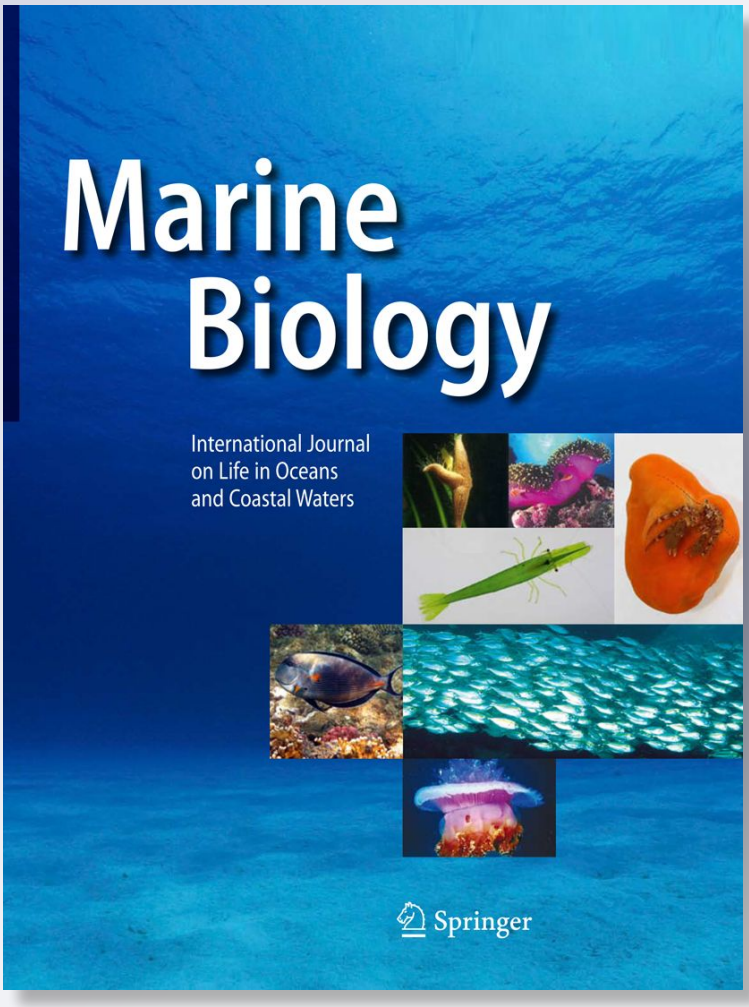

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ORIGINAL PAPER

Flux by fin: fish-mediated carbon and nutrient flux in the northeastern Gulf of Mexico

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Abstract Seagrass meadows are among the most productive ecosystems in the marine environment. It has been speculated that much of this production is exported to adjacent ecosystems via the movements of organisms. Our study utilized stable isotopes to track seagrass-derived production into offshore food webs in the northeastern Gulf of Mexico. We found that gag grouper (*Mycteroperca microlepis*) on reefs as far as 90 km from the seagrass beds incorporate a significant portion of seagrass-derived biomass. The muscle tissue of gag grouper, a major fisheries species, was composed on average of 18.5–25% seagrass habitat-derived biomass. The timing of this annual seagrass subsidy appears to be important in fueling gag grouper egg production. The $\delta^{34}S$ values of gag grouper gonad tissues varied seasonally and were $\delta^{34}S$ depleted during the spawning season indicating that gag utilize the seagrass-derived biomass to support reproduction. If such large scale trophic subsidies are typical of temperate seagrass systems, then loss of seagrass production or habitat would result in a direct loss of offshore fisheries productivity.

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J. Nelson (✉) · R. Wilson · J. Chanton
Department of Oceanography, Florida State University, Tallahassee, FL 32306, USA
e-mail: jnelson2@fsu.edu

R. Wilson
e-mail: younge@ocean.fsu.edu

J. Chanton
e-mail: chanton@ocean.fsu.edu

F. Coleman · C. Koenig
FSU Coastal and Marine Laboratory, 3618 Coastal Highway 98, St. Teresa, FL 32358-2702, USA
e-mail: coleman@bio.fsu.edu

C. Koenig
e-mail: koenig@bio.fsu.edu

D. DeVries · C. Gardner
National Marine Fisheries Service, 3500 Delwood Beach Road, Panama City, FL 32408, USA
e-mail: doug.devries@noaa.gov

C. Gardner
e-mail: chris.gardner@noaa.gov

Introduction

Temperate seagrass meadows are highly productive ecosystems (Valentine and Heck 1993). In the northeastern Gulf of Mexico, the Big Bend of Florida contains some of the most extensive ($\sim$3,000 km^2) seagrass meadows in the world (Iverson and Bittaker 1986; Zieman and Zieman 1989). This habitat, composed primarily of Turtle Grass (*Thalassia testudinum*) and Manatee Grass (*Syringodium filiforme*), hosts thousands of species of fish and invertebrates and is considered critical nursery habitat (Zieman and Zieman 1989; Beck et al. 2001). Most of the species that inhabit these seagrass meadows enter the system in the early spring (April–May), accumulate biomass during the summer (June–September), and then egress to offshore habitats in the fall (October–December) (Muncy 1984). It has been suggested that this pattern of biomass accumulation and subsequent emigration transports a significant amount of seagrass-derived biomass to offshore environments (Odum 1980; Deegan 1993; Heck et al. 2008). However, despite the great number of studies that speculate about the importance of this egress or "outwelling" to adjacent ecosystems via the migration of secondary production, very few attempts have been made to directly

demonstrate or quantify it. One of the few that have (Deegan 1993) found that a single species, gulf menhaden (*Brevoortia patronus*), transported 5–10% of the total estuarine production offshore each year. It is unlikely that the amount of nutrients transported offshore via this annual migration is equivalent to the amount of nutrients transported offshore passively by water movement. However, fish-mediated carbon and nutrient flux is of higher quality because it can be incorporated directly into the food web via predation and is not lost to bacterial respiration or sedimentation (Deegan 1993). In warm temperate areas, such as the southeastern Atlantic and Gulf coasts, where numerous small species egress from inshore to offshore in the late fall, this subsidy could be a significant factor driving offshore secondary production.

In the northeastern Gulf of Mexico, approximately 1.5×10^9 pinfish (*Lagodon rhomboides*) egress in the fall from the Florida Big Bend and spawn on reefs far offshore (at depths of 20–100 m) (Stallings unpublished data, Muncy 1984). This flux of prey could represent a major organic matter source for reef fish that inhabit the offshore environment. Furthermore, the timing of the egress occurs several months prior to the spawning season of gag grouper (*Mycteroperca microlepis*), a known piscivore and valuable fishery species (Bullock and Smith 1991; Coleman et al. 1996). It is during this period that the grouper increase their feeding rate and store large amounts of adipose (fatty) tissue in preparation for spawning (Jorgensen et al. 1997; Marshall et al. 1999). Although comprehensive information on gag grouper diet is lacking, one study (Naughton and Saloman 1985) documented the stomach contents of 1,975 gag caught off northwest Florida. In that study, fishes accounted for 95.1% of the food volume. During the period of pinfish egress, the stomach contents of gag contained 47.4% pinfish (by volume), as compared to 25.1% in the summer.

We hypothesized that the late fall egress of small, seagrass-dwelling species to offshore reefs constitutes a major food source for gag grouper. Furthermore, we hypothesized that this material contributes directly to the reproductive productivity of winter/spring spawning groupers. To address these hypotheses, we employed carbon and sulfur stable isotopes to trace seagrass-derived production transfer to the offshore food web.

We chose carbon and sulfur isotopes because they offer the greatest contrast in observed values between seagrass and marine habitats and fractionate very little between trophic levels (Post 2002; Herzka 2005; Fry 2006). Seagrass-derived production is enriched in ^{13}C and depleted in ^{34}S relative to offshore pelagic-derived production (Chanton and Lewis 2002b; Chasar et al. 2005; Herzka 2005; Wilson 2010). A study, conducted during the same time period as this one, of primary producers and secondary consumers in the Florida Big Bend reports seagrass and associated epiphytes are the dominant primary producers supporting the food web. Epiphytes and seagrass have mean values of $\delta^{13}C = -18.3‰ \pm 0.7$, $\delta^{34} = 14.7‰ \pm 2.0$ and $\delta^{13}C = -8.8‰ \pm 1.6$, $\delta^{34}S = 8.7‰ \pm 3.1$, respectively (Wilson 2010). Feeding on a combination of these sources, secondary consumers, such as pinfish, has isotope values that are -16.4 ‰ $\pm$ 1.6 $\delta^{13}C$ and 9.8 ‰ $\pm$ 3.1 $\delta^{34}S$ (Wilson 2010).

In the offshore environment, phytoplankton is responsible for the majority of primary production. In the northeastern Gulf of Mexico, phytoplankton fixes organic matter with characteristic values of $-20.0‰ \pm 1.0$ $\delta^{13}C$ and 18.0‰ $\pm$ 0.5 $\delta^{34}S$ (Chanton and Lewis 1999). Because phytoplankton accounts for nearly all of the primary production in the pelagic environment of the Gulf of Mexico (Lohrenz et al. 1999), the $\delta^{13}C$ and $\delta^{34}S$ values of secondary consumers closely resemble phytoplankton.

Based on our review of the relevant literature, we would expect to see differences of approximately 2‰ $\delta^{13}C$ and 10‰ $\delta^{34}S$ between seagrass habitat-derived and pelagic-derived secondary production (Allendorf et al. 1979; Fry and Parker 1979; Haines and Montague 1979; Chanton and Lewis 2002a; Fry et al. 2003; Chasar et al. 2005; Herzka 2005; Fry 2006). If secondary consumers from seagrass habitats are an important prey item for gag grouper while in offshore habitats, then we would expect gag grouper tissues to be enriched in ^{13}C and depleted in ^{34}S relative to secondary consumers that feed only on offshore-derived production. Our approach was to construct a two end member mixing model using measured isotope values of secondary consumers from seagrass habitat and deep reef piscivores as contrasting end members. We then used the observed $\delta^{34}S$ of gag grouper muscle tissue to estimate the relative amount of seagrass-derived production transported to offshore food webs.

Our specific objectives for this study were to (1) establish that seagrass-derived secondary production and offshore secondary production have unique isotopic signals, (2) determine the extent to which seagrass-derived organic matter is utilized by gag grouper, (3) determine whether gag grouper utilize energy attained from seagrass-derived production for egg production.

Materials and methods

Study location and sample collection

Samples used in this study were collected in the N.E. Gulf of Mexico from 2007 until 2009 in areas between 86.50°W and 28.00°N (Fig. 1). To establish the seagrass isotopic end member, we sampled fish species (Table 1) from seagrass beds in the Florida Big Bend. Seagrass

species were collected by otter trawl in summer from the seagrass beds along the northern coast of Florida from St. Joseph Bay to Cedar Key, Fl. To establish the offshore habitat end member, we sampled fish species from shallow and deep hard bottom reefs in the northeastern Gulf of Mexico. Hard bottom habitat sampling locations were divided into sites shallower than 30 m "shallow" and deeper than 30 m "deep". Collection of offshore reef species was done at fixed locations from 20 to 50 m water depth using hook and line, spear, and fish trap. Deep and shallow reefs samples were collected seasonally (March, December, and June weather permitting) from 2007 to 2009. After submersion in an ice bath, individuals were filleted and the skeletal muscle frozen until they could be processed. Five individuals from each species at each depth range were collected for analysis. The species were selected because they occur on both deep and shallow reef habitat, represent a broad range of trophic levels, feeding styles, are potential prey items for gag grouper, and represent the deep water end member. The gag grouper were collected by hook and line aboard the F/V Lady J II in >30 m of water on monthly sampling trips, weather permitting, from November 2007 until March 2009. This area is the heart of the gag grouper fishery and accounts for >90% of the total catch in the Gulf of Mexico (SEFSC 2004). All 286 gag grouper samples collected for this study were from adult females >75 cm in total length. After submersion in an ice bath, gonads and a sample of skeletal muscle tissue from each gag grouper were removed and placed on ice, returned to laboratory and frozen until they could be processed. Although we did observe seagrass species in the stomach contents of a few gag grouper (e.g., pinfish and pigfish), the vast majority of gag grouper collected suffered barotraumas that evacuated their stomachs prior to capture, thus quantitative gut content analysis was not possible in this study.

Lipids

In animals, lipids are depleted by approximately 7‰ relative to the carbon source (DeNiro and Epstein 1977). Therefore, tissues that contain significant quantities of lipids require lipid extraction to obtain values that are representative of the carbon source. Studies have shown that in aquatic animals, lipid content of <5% does not significantly alter the δ^{13}C values of the tissue (Sweeting et al. 2006; Post et al. 2007). Because lipid does not contain nitrogen, the C/N ratio of the tissue can be used as a proxy for lipid content. In aquatic animals, a C/N ratio of 3.5 indicates a lipid content of less than 5% and thus does not require lipid extraction (Post et al. 2007). Gag gonad tissue C/N ratios varied with season with the highest values occurring in the winter during gag spawning. For values greater than 3.5, the δ^{13}C values were corrected using the lipid correction method from Nelson et al. (2011)

$$\Delta\delta^{13}\mathrm{C\ value} = 0.86 * \mathrm{C/N} - 2.66. \quad (1)$$

Stable isotope analysis

In the laboratory, specimens were thawed and 5 g subsamples of skeletal muscle tissue or gonad tissue were excised using a MeOH-cleaned scalpel. The tissue was placed in a 50°C drying oven for 48 h. Once dry, the samples were ground to a fine powder using an electric mill. Approximately, 500 µg of tissue for carbon analysis and 3 mg of tissue for sulfur analysis were wrapped in tin capsules for analysis. Carbon stable isotope analysis was performed at the National High Magnetic Field laboratory in Tallahassee, Fl. Sulfur stable isotope analysis was done at the Stable Isotope Core Facility at Washington State University in Pullman, WA. All analyses were done using a continuous flow ThermoFinnigan DeltaPlus mass spectrometer coupled to a CHNS analyzer that provides % carbon and sulfur. PeeDee Belemnite (PDB) and Canyon Diablo Troilite (CDT) were used as the reference standards for C and S, respectively.

All isotope values are reported in the following notation:

$$\delta X = [(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] * 1,000 \quad (2)$$

where R is the ratio of the heavy to light isotope of the element X. We estimated analytical variability using internal standards of fish muscle tissue. Repeated analysis of these standards yields standard deviations of 0.2‰ δ^{13}C and 0.4‰ δ^{34}S. We were unable to collect primary producers from each of the habitats in order to determine the species-specific trophic fractionations for C and S. For this paper, we assume a trophic fractionation of 1‰ for δ^{13}C and 0‰ for δ^{34}S values (DeNiro and Epstein 1976; Post 2002; McCutchan et al. 2003).

Statistical analysis

Carbon and sulfur biplot data was analyzed with the hypothesis testing framework proposed by Turner et al. (2010). This method provides a powerful way to analyze how a food web component responds to gradients in isotope values. The procedure uses nested linear models and residual permutation procedure to evaluate the difference in centroid location of the isotope values for the seagrass habitat fishes, shallow reef fishes, deep reef fishes, and gag grouper muscle tissue. The Euclidean distance between centroids was used to determine whether the centroid location of each habitat type differed from zero. A t test was used to determine whether there was a significant relationship between gag grouper gonad δ^{34}S value with the time of year. Gonad δ^{34}S values were normalized using

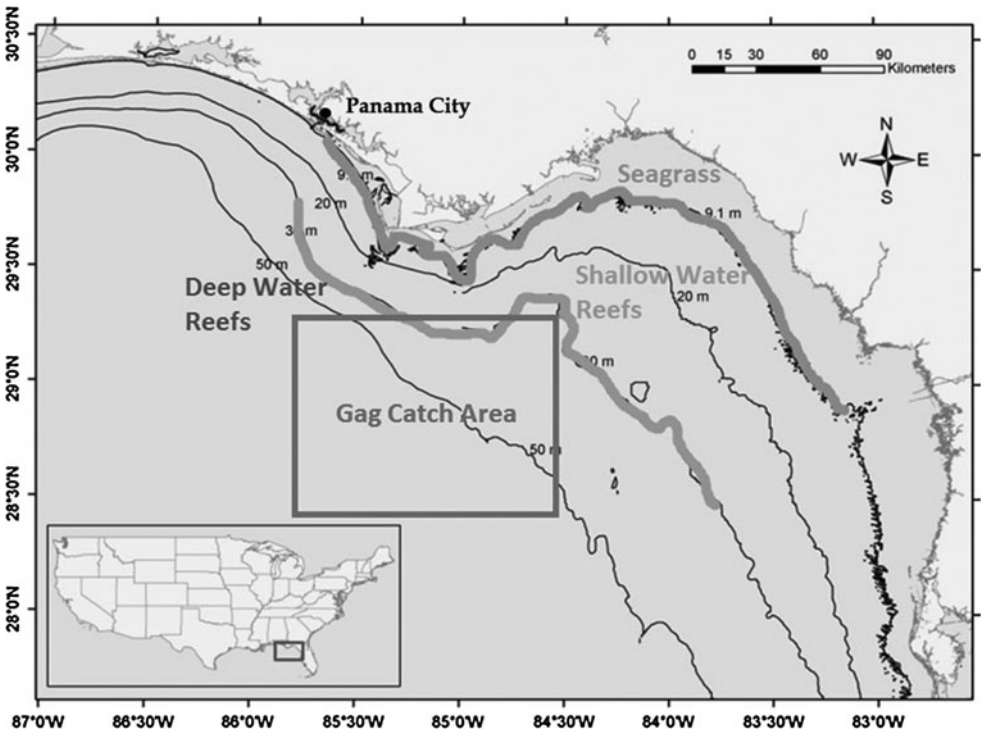

Fig. 1 Map of the N.E. Gulf of Mexico showing the sampling locations. The *black line* on the 30 m isobaths denotes the separation between the shallow and offshore reefs. The *black box* outlines the area where all gag grouper samples were taken

a square root transformation. An ANOVA was used to determine any seasonal and annual changes in isotope values on offshore reefs. All statistics were done using R v2.13 (www.r-project.org).

Mixing model

We use the δ^{34}S and δ^{13}C values of secondary consumers to establish the contrast in isotope values between offshore and seagrass-derived production. To determine the percent contribution of seagrass-derived fish tissue in gag grouper, we measured the mean δ^{34}S and δ^{13}C values of croaker (*M. undulates*), gulf flounder (*P. albigutta*), mojarra (*E. argenteus*), pigfish (*O. chrysoptera*), pinfish (*L. rhomboids*), seatrout (*C. nebulosus*), silver perch (*B. chrysoura*), and spot (*L. xanthurus*) and assumed that the values represent the seagrass habitat end member and the measured values for δ^{34}S and δ^{13}C values of sand perch (*D. formosum*), inshore lizard fish (*S. foetens*), tomtate (*H. aurolineatum*), and scamp (*M. phenax*) as the deep reef habitat end member. We then used IsoError (Phillips and Gregg 2001) to determine two estimates of seagrass habitat-derived biomass to gag grouper diet using carbon and sulfur separately. The same procedure was used to determine the contribution of seagrass-derived organic matter to gonad tissue during peak spawning (February) with the δ^{13}C values corrected using the lipid correction method from Nelson et al. (2011).

Results

The mean and standard deviation of the δ^{13}C values, δ^{34}S values, and length of all species collected are presented in Table 1. The centroid location of the δ^{13}C and δ^{34}S values of the fishes collected in the seagrass beds differed significantly from all other groups (Fig. 2). The nearest group was gag grouper muscle tissue (distance = 7.89, $p = 0.0001$). The δ^{13}C and δ^{34}S values of the shallow reef species were most similar to those of the deep reefs but were significantly enriched in ^{13}C and depleted in ^{34}S relative to these species (Figs. 2, 3). Fish captured on shallow reefs differed significantly in centroid location to all groups except gag grouper muscle tissue. Deep reef fishes were the next nearest group (distance = 1.41, $p = 0.002$). Isotope values for shallow reef fishes did not vary significantly with season (ANOVA $p = 0.227$, $F = 1.539$). Deep reef species differed significantly in centroid location to all groups (Fig. 2). Shallow reef species were the nearest group (distance = 1.41, $p = 0.002$). Like shallow reef species, we did not observe a significant seasonal shift in stable isotope values for deep reef species (ANOVA, $p = 0.115$, $F = 2.224$).

We collected gonad and muscle tissue from 286 adult female gag grouper during the study, all caught at depths greater than 30 m. Gag grouper muscle tissue differed significantly in centroid location from all groups except shallow reef species (distance = 0.59, $p = 0.26$, Fig. 2). Both gag grouper and deep reef species were collected

Table 1 Mean and standard deviation of isotope values for all fish sampled

Depth	Common name	Species name	Mean $\delta^{13}C$	Mean $\delta^{34}S$	Mean length (cm)
Seagrass	Croaker	*Micropogonias undulatus*	−17.3 ± 1.3	10.1 ± 2.6	15.2 ± 1.0
	Gulf flounder	*Paralichthys albigutta*	−16.1 ± 1.5	8.4 ± 2.6	16.1 ± 7.6
	Mojarra	*Eucinostomus argenteus*	−16.9 ± 0.5	7.2 ± 1.2	7.7 ± 0.6
	Pigfish	*Orthopristis chrysoptera*	−16.9 ± 0.8	6.8 ± 2.7	12.4 ± 2.1
	Pinfish	*Lagodon rhomboides*	−17.0 ± 1.4	8.1 ± 2.8	11.5 ± 1.3
	Seatrout	*Cynoscion nebulosus*	−16.6 ± 0.7	7.5 ± 2.0	14.7 ± 6.2
	Shrimp	*Penaeidae*	−16.8 ± 0.8	3.7 ± 2.4	NA
	Silver perch	*Bairdiella chrysoura*	−17.5 ± 1.3	11.6 ± 2.2	8.3 ± 1.5
	Spot	*Leiostomus xanthurus*	−15.9 ± 0.9	12.0 ± 0.8	11.5 ± 1.0
	Mean		−16.9 ± 0.6	8.6 ± 2.5	
<30 m	Bank sea bass	*Centropristis ocyurus*	−17.4 ± 0.3	17.3 ± 0.5	15.5 ± 2.1
	Blue angelfish	*Holacanthus bermudensis*	−19.7 ± 0.4	18.2 ± 0.8	27.9 ± 6.5
	Gray snapper	*Lutjanus griseus*	−16.6 ± 1.0	15.0 ± 2.7	37.1 ± 11.1
	Inshore lizard fish	*Synodus foetens*	−17.6 ± 0.3	17.2 ± 1.2	25.0 ± 9.2
	Red grouper	*Epinephelus morio*	−17.3 ± 0.2	18.0 ± 0.6	34.6 ± 5.8
	Red snapper	*Lutjanus campeachinus*	−17.6 ± 0.5	18.3 ± 1.0	36.9 ± 3.4
	Sand perch	*Diplectrum formosum*	−17.0 ± 0.2	16.6 ± 0.7	14.7 ± 5.4
	Scamp	*Mycteroperca phenax*	−17.5 ± 0.1	17.0 ± 0.7	29.2 ± 10.0
	Tomtate	*Haemulon aurolineatum*	−17.8 ± 0.3	17.5 ± 0.7	23.2 ± 0.9
	Mean		−17.4 ± 1.0	17.2 ± 1.0	
>30 m	Bank sea bass	*Centropristis ocyurus*	−17.8 ± 0.3	17.1 ± 0.7	19.4 ± 5.5
	Blue angelfish	*Holacanthus berudensis*	−19.2 ± 0.5	18.4 ± 0.7	34.2 ± 4.1
	Gray snapper	*Lutjanus griseus*	−17.5 ± 0.3	18.5 ± 0.6	51.7 ± 4.8
	Inshore lizard fish	*Synodus foetens*	−17.6 ± 0.6	18.5 ± 0.8	18.5 ± 0.8
	Red grouper	*Epinephelus morio*	−17.3 ± 0.4	17.7 ± 0.8	58.1 ± 11.3
	Red snapper	*Lutjanus campeachinus*	−17.7 ± 0.4	18.5 ± 0.6	48.4 ± 6.7
	Sand perch	*Diplectrum formosum*	−18.4 ± 0.2	18.5 ± 0.4	23.6 ± 1.9
	Scamp	*Mycteroperca phenax*	−17.7 ± 0.5	19.0 ± 0.5	48.0 ± 5.0
	Tomtate	*Haemulon aurolineatum*	−18.2 ± 0.2	18.4 ± 0.4	19.4 ± 1.9
	Gag	*Mycteroperca microlepis*	−16.8 ± 0.5	16.7 ± 1.6	79.1 ± 8.9
	Mean		−18.1 ± 0.6	18.1 ± 0.6	

For prey items $n = 5$, For gag $n = 286$

from the same sites and the same depths but differed significantly in centroid location (distance = 1.41, $p = 0.002$). Gag grouper muscle tissue had a mean C/N ratio of 3.38 ± 0.20 and did not require lipid correction of $\delta^{13}C$ values. We observed mean C/N ratios of 3.3 ± 0.24 and 3.5 ± 0.31 for shallow and deep reef fishes, respectively, indicating the $\delta^{13}C$ values did not need to be lipid corrected.

The IsoError single isotope, two-source model for carbon indicates that gag grouper muscle tissue contained 25% (S.E. 7%) seagrass habitat-derived carbon. This result assumes a 1 ‰ enrichment of $\delta^{13}C$ for the trophic step between gag and the prey (Post 2002). IsoError gives a contribution of 18.5% (S.E. 1%) seagrass-derived biomass when run using the $\delta^{34}S$ values. We assumed that sulfur does not fractionate with trophic level.

We used IsoError to calculate the maximum contribution of seagrass-derived biomass to gag grouper gonad tissue by using the same end members for muscle tissue and the mean $\delta^{13}C$ and $\delta^{34}S$ values for the peak spawning month (February). The mean $\delta^{13}C$ value of lipid corrected gonad tissue in February was −16.8 (±0.52 S.D.). Based on the corrected carbon isotope values for the month of February, IsoError gives a seagrass-derived biomass contribution of 25% (S.E. 6%). This value assumes a 1‰ enrichment for the first trophic step. The contribution of seagrass biomass to gag grouper gonad tissue based on the $\delta^{34}S$ values was 13.6% (S.E. 1%). The mean pre-egress gonad tissue $\delta^{34}S$ values was 19.3 (±0.91 S.D.) and post-egress was 17.4 (±1.5 S.D.). The pre- and post-egress gonad tissue was significantly different (*t* test $t = 79.27$, p value < 0.0001, Fig. 4).

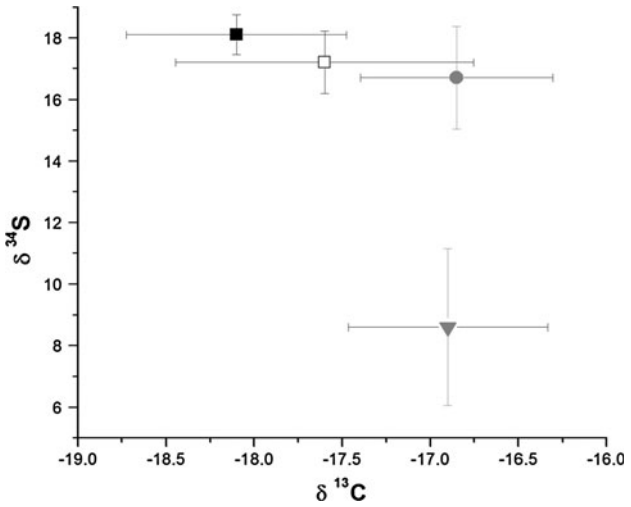

Fig. 2 Biplot of mean stable carbon and sulfur isotope values of the species in Table 1, seagrass species (*gray triangle*), gag grouper muscle tissue (>30 m, *gray circle*), shallow reef species (<30 m, *open square*), and deep reef species (>30 m, *black square*).The *error bars* indicate the standard deviation of the mean of the individuals

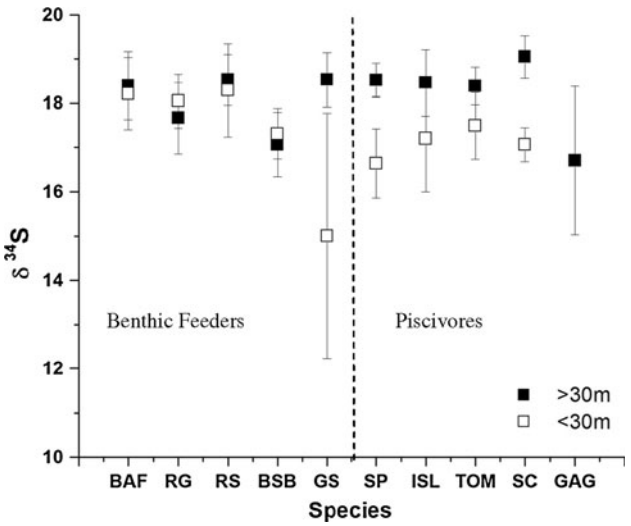

Fig. 3 Bioplot of stable sulfur isotope values of shallow reef species (*open squares*) and offshore reef species (*black squares*). The *error bars* indicate the standard deviation of the mean of the individual samples. The *dotted line* separates benthic feeders from piscivorous species. The species abbreviations are as follows: *BAF* blue angelfish, *BSB* bank sea bass, *GS* gray snapper, *ISL* inshore lizardfish, *RG* red grouper, *RS* red snapper, *SP* sand perch, *SC* scamp, *TOM* tomtate, *GAG* gag grouper

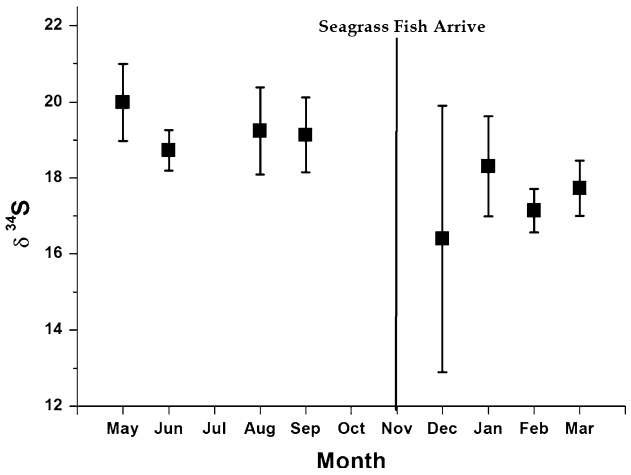

Fig. 4 Change in sulfur isotope value of gag grouper gonad tissue by month. Each *point* represents the mean monthly gonad values of 10 individuals from the 2 years of the study. The months of Feb–Mar are the peak of gag grouper spawning. The *error bars* indicate the standard deviation of the mean of the individual samples. The *black line* indicates the approximate time of the seagrass fish egress

Discussion

Detecting seagrass production in offshore food webs

Our results indicate that seagrass-derived production enters offshore food webs via the annual migration of seagrass-dwelling fishes based on the δ^{13}C and δ^{34}S values observed in the tissues of the shallow reef fishes and gag grouper (Fig. 2). Several lines of evidence based on the isotope data lead to this conclusion.

In a review of the use of stable isotope to assess habitat connectivity, Herzka (2005) notes that the large differences in the δ^{34}S isotopic composition of sediment and seawater sulfate makes this isotope particularly useful for discriminating benthic and pelagic sources. We are able to eliminate the possibility of an alternative source of deepwater ^{34}S depleted sulfur to the shallow reef food webs because marine planktonic production and marine particulate organic matter have a range of 17–21‰ (Chanton and Lewis 2002b; Chasar et al. 2005). In coastal marine environments, the two significant sources of depleted sulfur are runoff of terrestrial organic matter or sulfate reduction in anoxic sediments (Peterson et al. 1986; Chanton and Lewis 2002b; Herzka 2005). The outer shelf of the northeastern Gulf of Mexico is characterized by sandy sediments that contain little organic matter and therefore do not produce depleted sulfur through sulfate reduction (Chipman et al. 2010). The typical range of coastal marine secondary consumers δ^{34}S values are similar to those observed here for seagrass-dwelling species (Chanton and Lewis 1999, 2002b; Chasar et al. 2005).

Gag grouper

The IsoError two-source single isotope mixing model provides similar estimates for the contribution of seagrass-derived biomass to gag muscle tissue for carbon and sulfur isotope values of 25 and 18.5%, respectively. Due to the greater variability, overlap of source values, and need to correct for trophic fractionation, the δ^{13}C values do not

provide as accurate an estimate as $\delta^{34}S$ values but both isotopes indicate that seagrass habitat-derived biomass is a significant food source for gag captured in deep water habitat (Fig. 2). When we examine the isotope values of individual species in the offshore environment, gag are the only species in deep water habitat to feed significantly on seagrass habitat-derived prey and isotopically appear similar to piscivorous species on shallow reefs (Fig. 3). It is unlikely that gag would be the only species to prey upon these small fishes if a significant number of them migrated to water deeper than 30 m. Therefore, we hypothesize that gag undergo a pre-spawning migration to shallow water that is timed specifically to intercept the prey migrating from the seagrass habitat. Once in shallow water, gag feed intensively to store energy in preparation for spawning later in the winter. The hypothesis is supported by preliminary evidence that gag appear on shallow reefs in greater numbers in November–January (Nelson unpublished data). Much more work is needed to test this hypothesis, but the isotope data provides some of the first evidence of this unknown aspect of gag grouper ecology.

Our tertiary hypothesis was that the timing of the egress contributed directly to gag grouper reproductive productivity. We observed a significant contribution of seagrass-derived biomass to gag gonads (Fig. 4). Gonad tissue $\delta^{34}S$ values decrease from more deep reef-derived values in May, the end of the gag spawning cycle, to more seagrass-derived values during peak spawning in February (Coleman et al. 1996, Fig. 4). The difference between the pre- and post-egress $\delta^{34}S$ values was 1.7‰. Using this value, we compute a gonad tissue turnover rate of 0.014‰/day for the 4 months prior to peak spawning. This is in agreement with the mean value for adult female gag grouper turnover calculated in Nelson et al. (2011) of 0.012‰. The rapid incorporation of seagrass organic matter into gag gonad tissue suggests that the gag grouper population's spawning potential in the northeastern Gulf of Mexico may be linked to the strength of the seagrass subsidy in a given year. Therefore, the continued health of coastal habitats such as seagrass meadows would be vital in maintaining gag populations by providing needed energy for spawning as well as critical nursery habitat.

Conclusions

Our results demonstrate that seagrass-derived production contributes significantly to gag grouper in adjacent offshore habitats. While this concept is not novel, the use of stable isotope analysis to calculate the contribution of such fluxes makes quantifying ecosystem subsidies much more feasible (Polis et al. 2004). In temperate marine coastal systems such as ours, where organisms move from coastal habitats to marine habitats, or vice versa, $\delta^{34}S$ analysis may be particularly useful because coastal marine habitats tend to have greater amounts of sulfate reduction in the sediments and thus lower $\delta^{34}S$ values than those found offshore.

Our observations also have major implications from a fisheries management perspective. The shelf edge waters of the N.E. Gulf of Mexico are characterized by generally low primary productivity (Lohrenz et al. 1999). Therefore, failure of this annual coastal subsidy from loss of seagrass habitat or large scale fish kills inshore would directly reduce the spawning potential of the gag grouper population offshore, reducing fishery yields, and sustainability.

Globally, seagrass habitat is being lost at a rate of $\sim$7% year^{-1} (Waycott et al. 2009). If such large scale trophic subsidies are typical of temperate seagrass systems, then loss of seagrass production or habitat could result in a direct loss of offshore fisheries productivity.

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