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NOTE

Food Web Structure in a Chesapeake Bay Eelgrass Bed as Determined through Gut Contents and ^{13}C and ^{15}N Isotope Analysis

James G. Douglass · J. Emmett Duffy · Elizabeth A. Canuel

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Abstract Changes in seagrass food-web structure can shift the competitive balance between seagrass and algae, and may alter the flow of energy from lower trophic levels to commercially important fish and crustaceans. Yet, trophic relationships in many seagrass systems remain poorly resolved. We estimated the food web linkages among small predators, invertebrate mesograzers, and primary producers in a Chesapeake Bay eelgrass (*Zostera marina*) bed by analyzing gut contents and stable C and N isotope ratios. Though trophic levels were relatively distinct, predators varied in the proportion of mesograzers consumed relative to alternative prey, and some mesograzers consumed macrophytes or exhibited intra-guild predation in addition to feeding on periphyton and detritus. These findings corroborate conclusions from lab and mesocosm studies that the ecological impacts of mesograzers vary widely among species, and they emphasize the need for taxonomic resolution and ecological information within seagrass epifaunal communities.

Keywords Mesograzer · Diet · Seagrass · Stable isotope · Omnivory · Food web

J. G. Douglass (✉)
Northeastern University Marine Science Center,
430 Nahant Road,
Nahant 01908, MA, USA
e-mail: j.douglass@neu.edu

J. Emmett Duffy · E. A. Canuel
Virginia Institute of Marine Science,
1208 Greate Road,
Gloucester Point, VA 23062, USA

Introduction

Theory and experiments suggest that the diversity and feeding behaviors of consumers at intermediate trophic levels are important in determining how top-down and bottom-up trophic effects are compounded or attenuated within a community (Duffy 2002; Cardinale et al. 2006; Douglass et al. 2008). Unfortunately, the species-specific feeding ecology of middle trophic levels remains poorly resolved in some seagrass ecosystems. Consequently, the intermediate consumers in seagrass food webs have often been lumped into broad functional categories (e.g., Wetzel and Neckles 1986; Cerco and Moore 2001), which helps to create tractable models but may obscure important differences among species. For example, the small epifaunal invertebrates known as "mesograzers" are generally believed to benefit seagrass by consuming algal epiphytes (Jernakoff et al. 1996; Hughes et al. 2004; Valentine and Duffy 2006), but species-specific studies suggest a less benign role for some mesograzer taxa. While many mesograzers do consume mostly epiphytic microalgae, others graze directly on seagrass and macroalgae and have caused destruction of macrophytes in cultures (Kirkman 1978; Short et al. 1995; Duffy and Harvilicz 2001) and in the field (Kangas et al. 1982; Haahtela 1984). Conversely, certain mesograzer species, such as *Gammarus mucronatus*, have been shown to facilitate growth of macroalgae, and others, i.e., *Idotea balthica*, have displayed intra-guild predation (Duffy and Hay 2000; Duffy et al. 2005; Jaschinski et al. 2009). Most of these findings come from mesocosm and laboratory experiments, however, making it difficult to assess the prevalence and importance of these mesograzer feeding habits in the wild, where a wider range of available

resources, greater threats from predators, and other factors may alter mesograzer behavior (Douglass et al. 2010).

In Chesapeake Bay, as in many temperate estuaries, mesograzers in eelgrass (*Zostera marina*) beds are consumed by a suite of small predators including demersal fishes and decapod crustaceans (Orth et al. 1984; Teixeira and Musick 1994). These predators vary in the strength and selectivity of their mesograzer consumption, and most appear to be generalists that also feed on other organisms. For example, blue crabs, *Callinectes sapidus*, have been variously documented to feed upon infaunal bivalves (Virnstein 1978; Mansour 1992), mesograzers (Tagatz 1968; Stoner and Buchanan 1990; Hines et al. 1990), and seagrass and epiphytes (Perkins-Visser et al. 1996), while pipefish, *Syngnathus* spp., have a stronger predilection towards mesograzers (Teixeira and Musick 1994).

In this study, we used gut contents and stable C and N isotope ratios to assess the trophic links among mesograzers, small predators, and primary producers and basal resource pools in a Chesapeake Bay eelgrass bed. Based on previous studies of mesograzer feeding (Kitting 1984; van Montfrans et al. 1984; Jernakoff et al. 1996) and the results of $\delta^{13}C$ isotope analyses in a North Carolina, USA eelgrass bed (Thayer et al. 1978), we hypothesized that periphyton, detritus, and macroalgae would be the primary food sources for most species of mesograzers, with eelgrass making a lesser contribution. However, based on the results of mesocosm experiments (Duffy and Hay 2000; Duffy and Harvilicz 2001; Duffy et al. 2005; Jaschinski et al. 2009), we also expected to find significant diet differences among mesograzer species, with some directly grazing on eelgrass or omnivorously consuming other mesograzers.

At the predator trophic level, we expected *Syngnathus* spp. pipefish to have a diet of primarily mesograzers, as shown by previous gut contents surveys (Teixeira and Musick 1994), while we expected other fishes and predatory crustaceans to show a more varied diet. In particular, we thought that the reportedly omnivorous grass shrimp *Palaemonetes* sp. would show evidence of being at a lower trophic level than the other predators (Nelson 1979). We also hypothesized that blue crabs (*C. sapidus*), omnivores that have been demonstrated to consume mesograzers readily in mesocosm experiments (Duffy et al. 2005), might consume more detritus and infauna when in the field.

Materials and Methods

Study Location Samples were collected in an area of perennially dense eelgrass at the Goodwin Islands National Estuarine Research Reserve, located near the mouth of the York River estuary in Chesapeake Bay (Virginia, USA, 37° 13′ N; 76° 23′ W). Detailed information on the physico-chemical and biological characteristics of the site can be found in Douglass et al. (2010).

Sample Collections Collections for gut contents and stable isotope analyses were made on 21 April 2005, 19 May 2006, and 21 August 2006. Primary producers and hypothesized basal resource pools sampled for stable isotope analysis included surficial sediment (the surface layer down to approximately 1 cm depth), live eelgrass shoots, all abundant macroalgae species, and periphyton (the matrix of microalgal epiphytes, sediment, and other organic material removed from eelgrass shoots by light scraping). For April 2005, periphyton on shoots was too scarce for analysis, so periphyton and eelgrass were obtained from frozen samples taken for a related study in March 2005. Isotopic ratios for seston were indirectly estimated by back-calculating from the signatures of filter feeders like sponges, tunicates, and hydroids (see Canuel et al. 1995 for a similar approach using other biomarkers). The back-calculated values were within the typical range of $^{13}C/^{12}C$ ratios reported for estuarine phytoplankton (Chanton and Lewis 1999), and hereafter we refer to seston and phytoplankton interchangeably. Mobile epifauna were collected with a dip net and quickly placed on ice or in 10% formalin. (Only April 2005 gut contents samples were preserved in formalin because preliminary analyses indicated that the quality of gut contents in frozen samples was superior.) The number of species and individuals sampled at each date was dictated by their abundance at the time of sampling. Some species were not present in all months or were represented only by one or a few individuals (Tables 1 and 2).

Gut Contents Analysis Fish stomachs were dissected according to the procedures of Teixeira and Musick (1994) and blotted on a Petri dish under a dissecting microscope at 16× magnification. Identifiable prey individuals were counted and the percent cover of all items in the blot, including mineral grains and organic "unidentified material", was estimated visually by a single observer. Crab stomach contents were treated similarly. Shrimp stomachs were too small for analysis under the dissecting microscope and were therefore blotted on a glass slide and examined under a compound microscope at 100× according to the same procedure that was used for mesograzer guts (Kitting 1984).

Mean ± SEM percent composition for each food item was calculated for each consumer species for each sample date (Tables 1 and 2). Blue crabs, *C. sapidus*, were divided into three size classes based on carapace width, which were treated as separate consumer types. Crabs <20 mm, between 20 and 40 mm, and >40 mm carapace width formed size classes one, two, and three, respectively.

Table 1 Gut contents of mesograzers sampled at Goodwin Islands on 21 April 2005, 19 May 2006, and 21 August 2006

Sample Date	Species	*n*	Unidentified	Sediment	*Zostera*	Macrophyte	Macroalgae	Diatoms	Dinoflagellates	Unidentified protists	Crustacean parts
21-Apr-05	*Ampithoe longimana*	3	58 (3.3)	32 (1.7)			3 (2.7)	3 (1.7)	4 (3.1)		
19-May-06	*Ampithoe longimana*	9	57 (2.6)	34 (4.4)		8 (3.3)	1 (0.6)	0 (0.2)			
21-Aug-06	*Ampithoe longimana*	3	56 (4.5)	25 (5.8)		18 (8.3)		0 (0.3)			
21-Apr-05	*Ampithoe valida*	1	60	40							
19-May-06	*Ampithoe valida*	8	14 (3.2)	8 (2.5)		78 (4.4)					
21-Apr-05	*Caprella penantis*	9	79 (3.5)	13 (2.5)				1 (0.6)	1 (0.7)	6(2.4)	
19-May-06	*Caprella penantis*	11	69 (1.6)	31 (1.6)					0 (0.1)		
21-Apr-05	*Cymadusa compta*	6	58 (4.2)	30 (4.7)	1 (0.8)		3 (1.9)	1 (1.2)	6 (2.2)		
19-May-06	*Cymadusa compta*	1	35	5			60				
21-Aug-06	*Cymadusa compta*	9	63 (5.2)	36 (5)		2 (1.7)					
21-Aug-06	*Elasmopus levis*	3	53 (8.8)	23 (6.7)		18 (10.1)					5 (5.0)
21-Apr-05	*Erichsonella attenuata*	10	58 (2.5)	31 (2.3)	1 (0.5)		1 (0.5)	2 (2.0)	7 (2.0)		
21-Aug-06	*Erichsonella attenuata*	12	87 (2.3)	9 (2.1)			4 (1.5)				
21-Apr-05	*Gammarus mucronatus*	10	72 (3.8)	27 (4)	1 (0.5)				2 (0.5)		
19-May-06	*Gammarus mucronatus*	11	67 (2.6)	32 (2.7)		0 (0.5)			1 (0.5)		
19-May-06	*Hippolyte pleuracantha*	1	55	45							
21-Aug-06	*Hippolyte pleuracantha*	10	69 (3.5)	29 (3.7)							2 (2.0)
21-Apr-05	*Idotea balthica*	10	43 (9.1)	5 (1.1)	27 (13)		1 (1.0)		1 (0.5)		23 (7.4)
19-May-06	*Idotea balthica*	1	90	10							

Values in cells are mean and SEM (in parentheses) percent cover of each type of item or material as observed in a blot of total gut contents on a microscope slide at 100× magnification. The "macrophytes" category includes multicellular plant or algal material that could not be positively identified as *Zostera marina* or macroalgae

Table 2 Gut contents of small predators sampled at Goodwin Islands on 21 April 2005, 19 May 2006, and 21 August 2006

Gut contents of blue crabs

Sample date	Species/size class	*n*	Unidentifiable	Sediment	*Zostera*	Macroalgae	*Cladophora* sp.	Ostracoda	Gam. amp.	Isopods	Crustacean p.	Polychaete	Barnacles	Bivalves	Fish
19-May-06	*C. sapidus* 1	1	75	20									15		
21-Aug-06	*C. sapidus* 1	12	56 (5)	38 (5)	1 (1)		0 (0.4)		2 (1.7)		1 (1)	1 (1)			
21-Apr-05	*C. sapidus* 2	1		10								90			
19-May-06	*C. sapidus* 2	8	17 (2)	8 (3)	2 (1)	3 (3)					5 (3)		65 (3)	2 (1)	
21-Aug-06	*C. sapidus* 2	25	47 (4)	29 (3)	7 (2)		6 (2)	1 (0.3)	2 (1.6)	1 (1)	1 (0.7)	0 (0.4)	1 (0.8)	0 (0.4)	1 (1)
19-May-06	*C. sapidus* 3	2	20	13 (3)	3 (3)								60 (10)	5 (5)	
21-Aug-06	*C. sapidus* 3	3	37 (10)	18 (3)	23 (13)						11 (3)			3 (3)	3 (3)

Gut contents of other predators

Sample date	Species	*n*	Unidentifiable	Sediment	Macroalgae	*Cladophora* sp.	Diatoms	Copepoda	Ostracoda	Gam. amp.	Capr. amp.	Iso pods	*Pala.* spp.	Crustacean p.	Polychaete
21-Apr-05	*Crangon sept.*	5	32 (10)	24 (15)	8 (8)										36 (19)
19-May-06	*Crangon sept.*	1	60	40											
21-Aug-06	*Gobiesm. bosci*	10	52 (6)	11 (3)						3 (3)	3 (2)	1 (1)			29 (5)
21-Aug-06	*Gobiesox strum.*	2	45 (5)					10 (10)	18 (3)	28 (8)					
21-Apr-05	*Palae. vulgaris*	1	10						90						
19-May-06	*Palae. vulgaris*	5	73 (17)	3 (2)						16 (16)				8 (8)	
21-Aug-06	*Palae. vulgaris*	17	62 (3)	26 (3)			4 (1)		2 (2)			0 (0.4)		5 (3)	
21-Aug-06	*Penae. aztecus*	1	15	15											70
19-May-06	*Syng. floridae*	1	20							20			60		
21-Aug-06	*Syng. floridae*	4	53 (5)							36 (6)	5 (3)	4 (4)			
21-Apr-05	*Syng. fuscus*	13	30 (4)					26 (6)	0 (0.1)	38 (6)	0 (0.4)			2 (2)	
19-May-06	*Syng. fuscus*	8	38 (4)					0 (0.3)		60 (4)	1 (0.6)		1 (0.6)		
21-Aug-06	*Syng. fuscus*	6	58 (8)			0 (0.2)		9 (7)	0 (0.2)	26 (5)	1 (0.8)	4 (3)			

Values in cells are mean and SEM (in parentheses) percent cover of each type of item or material as observed in a blot of total gut contents on a glass dish at 16× magnification

Gam. amp. gammaridean amphipods, *Pala. sp. Palaemonetes* spp. shrimp, *Crustacean p.* unidentifiable crustacean parts, *C. Callinectes*, *sept. septemspinosa*, *Gobiesm. Gobiesoma*, *strum. strumosus*, *Palae. Palaemonetes*, *Penae. Penaeus*, *Syng. Syngnathus*, *Callinectes sapidus 1, 2, and 3* crabs of carapace widths <20, 20–40, and >40 mm, respectively

Isotope Sample Preparation and Analysis Organisms and materials for isotope analysis were cleaned with deionized water and dried for 5 days at 60°C, homogenized, acidified with hydrochloric acid (0.1 N), and dried for several more days at 60°C. For samples of mesograzers, and the shrimps *Palaemonetes vulgaris* and *Crangon septemspinosa*, multiple individuals were pooled and homogenized. Larger predators were analyzed as separate individuals, except for the April 2005 sample in which three *Syngnathus fuscus* between 100 and 120 mm in length were pooled. One to three procedural replicates from each homogenized sample were weighed, packed into tin capsules, and shipped to the University of California Davis Stable Isotope Facility for dual $^{13}C/^{13}C$ and $^{15}N/^{14}N$ analyses on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). We report isotopic composition as δ values (units per mil) based on the conventional formula and standards (Fry 2006).

Mean and standard error of $\delta^{13}C$ and $\delta^{15}N$ within procedural replicates were calculated for each item analyzed, and were graphed on dual isotope plots (Figs. 1, 2, and 3). Between one and five possible food items were inferred for each consumer based on gut contents, the ecological literature, and the items' position relative to the consumer on the dual isotope plot (filled cells in Table 3). The percent composition of each candidate food item was estimated based on fractionation factors of +1.0‰ $\delta^{13}C$ and ±3‰ $\delta^{15}N$ with each trophic step (Peterson and Fry 1987). We are aware that trophic enrichment levels can deviate from the values we used (Fry 2006), but it was beyond the scope of this study to experimentally determine the precise fractionation factors for every taxon surveyed.

We used a variety of mixing equations to estimate relative contributions of multiple food sources to a consumer. If there were only two candidate food items, we used a simple, weighted-distance estimate of diet composition based on $\delta^{13}C$ values (Table 3, estimation method 1). Diet mixing between three candidate food sources was calculated in one of two ways. When a single solution was possible, a linear mixing model based on both $\delta^{13}C$ and $\delta^{15}N$ was used (Table 3, estimation method 2) (Phillips 2001). When no unique solution was possible, a distribution of possible mixing solutions was calculated with IsoSource (Phillips and Gregg 2003) (Table 3, estimation method 3). For each candidate food item assessed in IsoSource, we reported mean ± SD percent diet composition and also the "tolerance" for the estimate, which is the percent uncertainty in isotope ratios and fractionation factors accepted in the mixing estimates. Estimates with high tolerance tend to make less distinction between food sources, assigning a similar proportional diet contribution to each candidate food item. For each particular mixing problem, we began with low tolerance and chose successively higher values of 0.05, 0.1, 0.5, 1, 2, or 3 until IsoSource was able to calculate solutions. Less confidence should be placed in diet estimates that had high tolerance.

All statistics were performed in MS Excel and IsoSource.

Results

Gut Contents Guts of most mesograzer species contained a large proportion of amorphous material and sediment, with varying amounts of multicellular plants, single-celled algae, and crustacean parts (Table 1). The guts of *Elasmopus levis* and the ampithoid amphipods *Ampithoe longimana*, *Ampithoe valida*, and *Cymadusa compta* contained moderate to high proportions of macrophytes, with *A. valida* in May 2006 appearing to consume almost entirely macrophytes. However, the only mesograzer that clearly consumed significant amounts of eelgrass was the isopod *Idotea balthica*, in which an average of 27% of gut contents were coarse chunks of *Z. marina* in April 2005. *Caprella penantis*, the most abundant mesograzer at Goodwin Islands (Douglass et al. 2010), did not appear to consume macrophytes, but its gut contents included amorphous material, sediment, and microalgae in proportions similar to those in the gammaridean amphipod mesograzers (Table 1). Three mesograzer species, the isopod *I. balthica*, the amphipod *Elasmopus levis*, and the decapod shrimp *Hippolyte pleuracantha*, had some crustacean exoskeletal material in their guts, indicating possible intra-guild predation or zooplanktivory. Only for *I. balthica*, however, was a significant portion of crustacean material found in multiple individuals.

Predator gut contents varied among species, among size classes within species, and among dates sampled (Table 2). Blue crabs, *C. sapidus*, in size class one had diets similar to mesograzers, with guts full of sediment, unidentifiable material, and macrophytes, but they also included a few amphipods, crustacean parts, and polychaetes. Blue crabs in size class two had a greater proportion of crustacean parts in their guts, but this material appeared to come from other blue crabs or from barnacles rather than from mesograzers. Only five blue crabs in size class 3 were examined, and none contained mesograzers. Barnacles were the dominant component of blue crab diet in May 2006, suggesting opportunistic feeding on the heavy recruitment of barnacles to the eelgrass beds at that time (pers. obs.). In August 2006, one medium and one large crab each had a small, unidentifiable fish in its stomach. *Palaemonetes vulgaris* and *Crangon septemspinosa* shrimp were collected in large numbers, but with the exception of the *P. vulgaris* samples

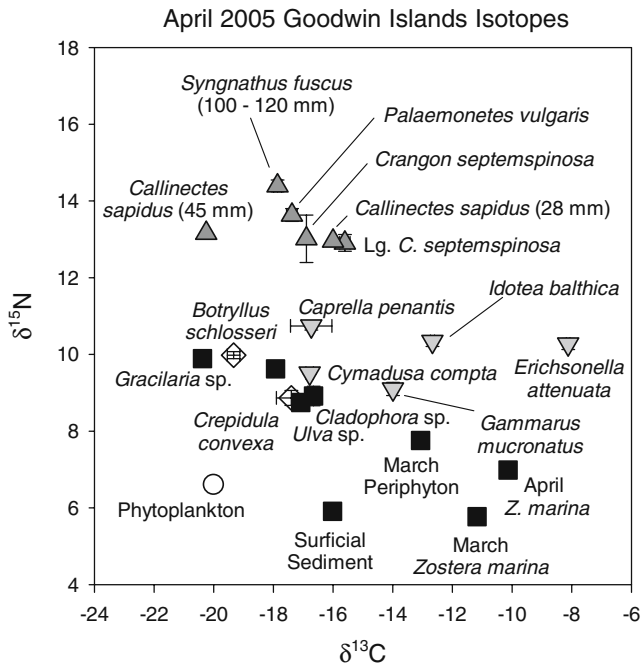

Fig. 1 Plot of $\delta^{15}N$ and $\delta^{13}C$ stable isotope signatures from biota collected at the Goodwin Islands eelgrass bed on 21 April 2005. Algae, plants, and sediment are shaded *black*, putative grazers are shaded *light gray*, putative predators are shaded *dark gray*, and filter feeders are *white with black outlines*. The inferred signature of phytoplankton, back-calculated from filter feeders, is indicated by a *white circle*. *Error bars* are calculated from procedural replicates of pooled tissue; not true replicates

taken in August 2006, most individuals had empty guts at the time of dissection. The *C. septemspinosa* individuals that did have full guts often contained polychaetes, along with a significant portion of sediment and unidentifiable material. *P. vulgaris* guts contained mostly sediment and amorphous material, but also some remains of epifaunal crustaceans (Table 2). A single *Penaeus aztecus* shrimp (rare in the Goodwin Islands eelgrass bed) had a gut full of polychaete segments. Relative to the shrimp and crabs, the small predatory fishes had less sediment and plant material in their guts, and more small crustaceans. Though the main components of fish gut contents were amphipod and isopod mesograzers, *Syngnathus fuscus* also preyed heavily on copepods, *Syngnathus floridae* consumed juvenile shrimp (though this was only seen in one individual with 17 *Palaemonetes* sp. individuals in its gut), *Gobiesox strumosus* ate ostracods, and *Gobiesoma bosci* guts contained more polychaetes than crustaceans.

Isotopic Signatures Z. marina tended to be more enriched in ^{13}C than other producers and basal food sources, with $\delta^{13}C$ around −10‰, versus approximately −18‰ for surficial sediment and −21‰ estimated for seston (Figs. 1, 2, and 3). However, periphyton was similarly enriched as *Z. marina* in May 2006 (−9.3‰), and the green macroalga *Ulva* sp. had a more enriched $\delta^{13}C$ signature in August 2006 (−6.8‰). $\delta^{15}N$ signatures for periphyton, *Z. marina*, and surficial sediment were usually more depleted than those of the mesograzers by 1–3‰, as would be predicted for mesograzer food sources (Figs. 1, 2, and 3). Macroalgae tended to be quite enriched in $\delta^{15}N$ ($\delta^{15}N$ 9–12‰), and in May and August some macroalgae had $\delta^{15}N$ signatures even higher than those of the predators (Figs. 2 and 3).

The isopod *Erichsonella attenuata* was enriched in $\delta^{13}C$ relative to the other mesograzers, with signatures similar to *Z. marina*. Most of the other consumer $\delta^{13}C$ signatures were more depleted than *Z. marina* and periphyton, indicating assimilation of depleted carbon from sources such as surficial sediments or phytoplankton. Depleted $\delta^{13}C$ signatures of known planktivorous organisms; the tunicate *Botryllus schlosseri* in April 2005, the hydroid *Hydractinia* sp. in May 2006, and an unidentified sponge species in August 2006, support our estimated positioning of phytoplankton as the most $\delta^{13}C$ depleted food source in each month (Figs. 1, 2, and 3). The large, $\delta^{13}C$ standard error bar for *Bittium varium* snails in May 2006 (Fig. 2) is probably due to incomplete acidification of some parts of

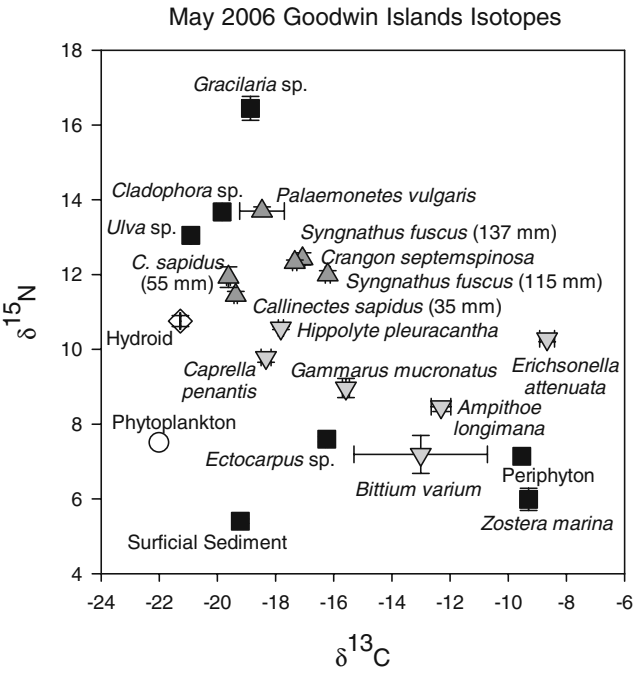

Fig. 2 Plot of $\delta^{15}N$ and $\delta^{13}C$ stable isotope signatures from biota collected at the Goodwin Islands eelgrass bed on 19 May 2006. Algae, plants, and sediment are shaded *black*, putative grazers are shaded *light gray*, putative predators are shaded *dark gray*, and filter feeders are *white with black outlines*. The inferred signature of phytoplankton, back-calculated from filter feeders, is indicated by a *white circle*. *Error bars* are calculated from procedural replicates of pooled tissue; not true replicates

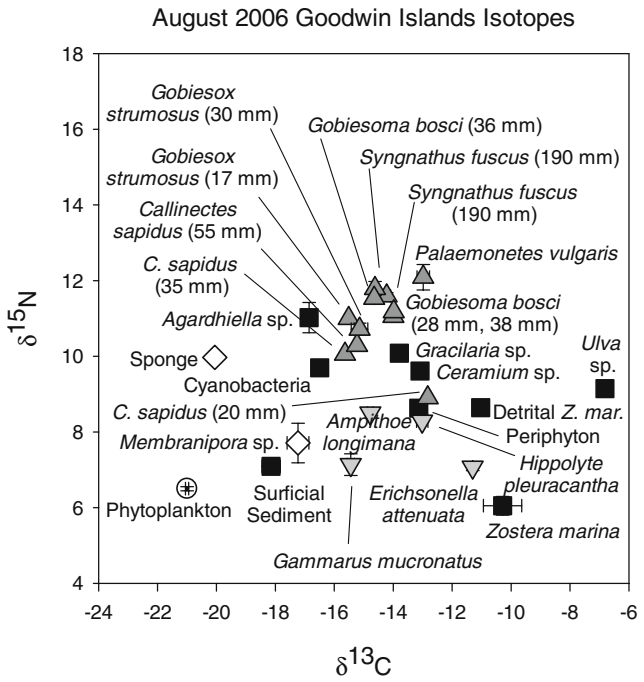

Fig. 3 Plot of $\delta^{15}N$ and $\delta^{13}C$ stable isotope signatures from biota collected at the Goodwin Islands eelgrass bed on 21 August 2006. Algae, plants, and sediment are shaded *black*, putative grazers are shaded *light gray*, putative predators are shaded *dark gray*, and filter feeders are *white with black outlines*. The inferred signature of phytoplankton, back-calculated from filter feeders, is indicated by a *white circle*. *Error bars* are calculated from procedural replicates of pooled tissue; not true replicates

the sample leading to inconsistency among procedural replicates.

The range in isotopic signatures among predator species ($\delta^{13}C$ −21 to −16, $\delta^{15}N$ 10 to 15) was less than that among basal food sources ($\delta^{13}C$ −22 to −6, $\delta^{15}N$ 6 to 18) and herbivore species ($\delta^{13}C$ −20 to −7, $\delta^{15}N$ 7 to 11). Predators were consistently about 3‰ $\delta^{15}N$ above the mesograzers. The only predator that deviated from this pattern was a very small (20 mm) blue crab in August 2006, which appeared to be at a lower trophic level closer to the mesograzers.

Diet Composition Estimates The mixing models indicated that surficial sediment was a major component in the diets of mesograzers (Table 3), a result that was also supported by the gut contents data. This suggests that benthic microalgae and detritus, whether on the bottom, resuspended, or loosely associated with eelgrass blades, complement firmly attached epiphytes as a main source of mesograzer nutrition in this system. Macroalgae appeared to be important for mesograzer diet in April 2005, particularly for *Cymadusa compta* and other ampithoid amphipods, which have often been documented feeding upon and living in association with macroalgae (Duffy and Hay 2000) (Table 3). *Z. marina* was apparently important in the diet of some mesograzers, particularly in August 2006 when it was the only food web component (of those we analyzed) that had a low enough $\delta^{15}N$ signature to match the mesograzer signatures after trophic enrichment. Sessile planktivores, represented by *Botryllus schlosseri*, *Hydractinia* sp. hydroids, and sponge for April 2005, May 2006, and August 2006, respectively, appeared important in the diet of predators. It is unlikely that the predators actually consumed these unpalatable sessile organisms, but they may have consumed zooplankton or infauna, which also feed on phytoplankton and phytodetritus and would have similar isotopic signatures to the sessile filter feeders. This inference is supported by the gut contents data for *Syngnathus* spp., which include copepods. Other items of apparent importance for predators were the mesograzers *Caprella penantis* and *Gammarus mucronatus* in spring, and ampithoid amphipods and *Erichsonella attenuata* in August.

Discussion

Our isotope and gut contents analyses paint a picture of a seagrass food web populated by a functionally diverse group of primary consumers, which exploit a wide range of available food sources: algal epiphytes, phytoplankton, seagrass, and benthic detritus, to varying degrees. The secondary consumers we examined were supported largely by epifaunal mesograzers, although they varied in the extent to which they also consumed infaunal prey, zooplankton, and sessile organisms.

Although our isotope analyses suggest a sizeable contribution of eelgrass itself to the diets of mesograzers, especially in August 2006 (Table 3), this finding is only supported by the gut contents analyses for a few mesograzer species like *Idotea balthica* (Table 1). Other mesograzers like *Erichsonella attenuata*, which isotopic evidence alone might identify as eelgrass consumers, had guts full of microalgal epiphytes and detritus, consistent with their benign influence on eelgrass in mesocosm experiments (Duffy et al. 2005). [Microalgal epiphytes sometimes had similar isotopic signatures to eelgrass, for example in May 2006 (Fig. 2), which could explain the discrepancy between the gut contents and isotopic diet estimates for *E. attenuata*.] Overall, our results support classification of the Goodwin Island eelgrass bed as a "seagrass detrital ecosystem" (Valentine and Duffy 2006) in which macrophytes provide structure and some trophic support, but detritus and microalgae on eelgrass blades, in the sediment, and in the water column are more important as food for most primary consumers.

Table 3 Diet composition estimates [mean % composition] for consumers based on $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures from organisms and resource pools at the Goodwin Islands eelgrass bed

Samples from March and April 2005														
Sample date	Sample date, consumer, size (mm)	Sediment	Periphyton	Phytoplankton	*Zostera*	Filter feeders	*Cladophora* sp.	*Ulva* sp.	*Caprella*	*Gammarus*	*Idotea*	Ampithoid	Esti. met.	Tol.
	22-Mar-05													
22-Mar-05	*Botr. schlosseri*			?										
	21-Apr-05													
21-Apr-05	*Crepidula* spp.	40		60									1	
21-Apr-05	*Caprella penantis*	2	65	33									2	
21-Apr-05	*Ga. mucronatus*	65	35										1	
21-Apr-05	*Erichs. attenuata*				?									
21-Apr-05	*Idotea balthica*	32, 4	36, 21		16, 7					17, 10			3	0.05
21-Apr-05	*Cyma. compta*	57, 1	0				41, 1	2, 2					3	1.00
21-Apr-05	Ampithoid amps.	50, 15	12, 8				22, 15	17, 12					3	2.00
21-Apr-05	*Palaem. vulgaris*					63, 7			29, 18	2,2	4, 3	3, 3	3	0.50
21-Apr-05	*C. septemspinosa*					27, 13			26, 18	14, 11	12, 9	21, 16	3	0.50
21-Apr-05	*C. septem.* (Lg.)					57, 17			16, 13	7, 6	6, 5	14, 11	3	0.50
21-Apr-05	*Callin. sapidus*, 28					35, 14			25, 18	12, 9	10, 7	19, 2	3	0.50
21-Apr-05	*Callin. sapidus*, 45					?								
21-Apr-05	*S. fuscus*, 100–120					48, 15			19, 16	9, 8	7, 6	17, 14	3	2.00
Samples from May and August 2006														
Sample date	Sample date, consumer, size (mm)	Sediment	Periphyton	Phytoplankton	*Zostera*	Filter feeders	*Ectocarpus* sp.		*Caprella*	*Gammarus*	*Erichsonella*	Ampithoid	Esti. met.	Tol.
	19-May-06													
19-May-06	*Hydractinia* sp.			?										
19-May-06	*Caprella penantis*	35, 2		35, 1			30, 1						3	0.05
19-May-06	*Ga. mucronatus*	67, 1	24, 1				9, 2						3	0.05
19-May-06	*Erichs. attenuata*		1		98		1						2	
19-May-06	*Ampithoe longimana*	32, 8	21, 15		35, 15		12, 9						3	1.00
19-May-06	*Hippo. pleuracantha*	87					13						1	
19-May-06	*Bittium varium*	44, 11	14, 10		33, 16		8, 6						3	2.00
19-May-06	*Palaem. vulgaris*					60, 8			26, 16	14, 9			3	0.50
19-May-06	*C. septemspinosa*					25, 10	23, 2		35, 20	17, 10			3	0.50
19-May-05	*Callin. sapidus*, 35	30				56			14				2	
19-May-05	*Callin. sapidus*, 55	28				67			4				2	
19-May-05	*S. fuscus*, 115					13, 8			34, 19	42, 17		11, 8	3	0.50
19-May-05	*S. fuscus*, 137					23, 12			47, 20	24, 15		7, 5	3	0.50
	21-Aug-06													
21-Aug-06	*Membranipora* sp.			?										
21-Aug-06	Porifera			?										
21-Aug-06	*Ga. mucronatus*	49	51										1	
21-Aug-06	*Erichs. attenuata*	27, 18	5, 3		69, 17								3	3.00
21-Aug-06	*Ampithoe longimana*	65, 15	5, 3		30, 14								3	2.00

21-Aug-06	*Hippo. pleuracantha*	42, 15	5, 4	53, 14				3	2.00
21-Aug-06	*Palaem. vulgaris*		25, 4		20, 12	26, 3	30, 17	3	0.10
21-Aug-06	*Callin. sapidus*, 20	19, 14			28, 21	31, 19	22, 16	3	2.00
21-Aug-06	*Callin. sapidus*, 35	61, 10			27, 16	11, 6	2, 1	3	0.05
21-Aug-06	*Callin. sapidus*, 56	49, 10			27, 16	11, 6	14, 2	3	0.05
21-Aug-06	*S. floridae*, 120				14, 8	2, 2	84, 8	3	0.50
21-Aug-06	*S. fuscus*, 190				25, 13	4, 3	71, 14	3	1.00
21-Aug-06	*Gobiosoma bosci*, 28				29, 2	0, 0	71, 3	3	0.05
21-Aug-06	*Gobiosoma bosci*, 36				37, 18	5, 4	58, 20	3	1.00
21-Aug-06	*Gobiosoma bosci*, 38				80, 12	2, 2	18, 13	3	1.00
21-Aug-06	*Gobx. strumosus*, 17				52, 26	8, 5	41, 27	3	2.00
21-Aug-06	*Gobx. strumosus*, 30				80, 12	2, 2	18, 13	3	1.00

Estimates assumed a fractionation of $+1\delta^{13}C$ and $+3\delta^{15}N$ per trophic level. Diet mixing estimates for two inferred sources used only $\delta^{13}C$ (estimation method 1). When possible, mixing between three sources was calculated with a linear mixing model based on both $\delta^{13}C$ and $\delta^{15}N$ (estimation method 2) (Phillips 2001). When a mixing problem could have multiple solutions, the distribution of possible sources was calculated with IsoSource (estimation method 3) (Phillips and Gregg 2003) and reported as [mean % composition, SD]. Tolerance is the minimum percent uncertainty value (from 0.05, 0.1, 0.5, 1, 2, or 3) that was necessary for the IsoSource program to calculate solutions. Question marks denote where there was only a single feasible food source and % composition could not be calculated

Esti. met. estimation method, *Tol.* tolerance, *Botr. Botrylllus* (tunicate), *Ga. Gammarus*, *Erichs. Erichsonella*, *Cyma. Cymadusa*, *Callin. Callinectes*, *amps.* amphipods, *Palaem. Palaemonetes*, *Gobx. Gobiesox*, *S. Syngnathus*, *Hippo. Hippolyte*

Some caveats to our interpretations merit discussion. Small sample sizes and limited spatial and temporal extent of sampling may exaggerate the importance of what could actually be rare or season-dependent trophic relationships. For example, the predominance of barnacles in the gut contents of blue crabs in May 2006 should not be interpreted as typical because barnacles are rare in the Goodwin Islands seagrass bed except during occasional high-recruitment events (pers. obs.). Other, well-known limitations of gut contents analysis could also affect our results. For example, it was impossible to determine if the amorphous detritus in mesograzer guts was a result of indiscriminate feeding on detritus, or if fine mastication and rapid digestion obscured the structure of ingested macrophytes and algal cells, as found by Zimmerman et al. (1979). The similar gut contents yet widely different carbon isotope ratios of *Gammarus mucronatus* and *Erichsonella attenuata* (Table 1, Figs. 1, 2, and 3) suggest that feeding is more selective than indicated by gut contents alone, and emphasize the general utility of complementing gut contents analysis with isotopic information.

Of course there are some limitations to isotopic analysis, as well, most notably the potential for incomplete representation of all possible resource pools. In this study, we did not sample phytoplankton, zooplankton, or infauna, and we did not separate microalgae from other types of organic material in our bulk samples of sediments and periphyton. Expanding the scope and the level of detail of sampling to include these resources in future studies would help to account for the connections between this eelgrass epifaunal food web and the pelagic and soft-bottom benthic food webs with which it is associated (Williams and Heck 2001).

Keeping these limitations in mind, there were nevertheless some interesting trends in our data, including the differences in diet between small and large *C. sapidus*. Our results support earlier findings that *C. sapidus*, though omnivorous and opportunistic at all life stages, undergo marked ontogenetic diet shifts (Tagatz 1968; Mansour 1992). Like Tagatz (1968) and Stoner and Buchanan (1990), we found that *C. sapidus* between 20 and 40 mm carapace width had a greater proportion of mesograzers in their diets than did larger and smaller crabs (Fig. 3, Table 3). This corresponds with the strong depression of mesograzer abundance by blue crabs in this size range in mesocosm experiments (Duffy et al. 2005) and field experiments (Douglass et al. 2007). No size group of *C. sapidus* preyed as strongly and selectively on mesograzers as did fishes, however, suggesting that fishes may be the primary top-down control on mesograzer abundance in this system, as they appear to be in some European *Z. marina* habitats (Bobsien 2006).

A noteworthy characteristic of the isotope data from May and August 2006 was the high $^{15}N/^{14}N$ isotope ratios

in some macroalgal taxa, especially *Gracilaria* sp. in May 2006 (δ^{15}N 16.4‰, Fig. 2). These opportunistic macroalgae probably exploited a different, more enriched, nitrogen source than did the other photoautotrophs in the system, though it is unclear whether this source was within our site or whether the macroalgae grew in another environment then drifted into the eelgrass bed.

The overall goal of this study was to characterize the middle trophic level connections in an eelgrass food web well enough to make testable predictions about the influence of top-down and bottom-up forces on the system. Based on our findings, we predict that a decrease in the production of periphyton or of benthic microalgae on the sediment surface would reduce mesograzer secondary production and, in turn, the production of predators. This would be consistent with negative correlations of turbidity with epiphyte and mesograzer abundance reported for this system (Douglass et al. 2010). We also predict, based on the relatively minor contribution of mesograzers to blue crab diet and the already low abundance of juvenile blue crabs in the Goodwin Islands eelgrass bed (Douglass et al. 2010), that a further reduction in juvenile crab abundance via overharvesting of adults (Lipcius and Stockhausen 2002) would have only slight effects on mesograzer abundance. On the other hand, an increase in the abundance of pipefish or another predator that preyed effectively on mesograzers might seriously reduce their abundance, releasing epiphytes from top-down control, with negative impacts on eelgrass growth and survival. Two factors that could potentially increase the abundance of fish that prey heavily on mesograzers are (1) warmer temperatures, which could hasten the spring advent of fish recruitment into eelgrass beds and allow southern species like pinfish (*Lagodon rhomboides*) to become more abundant in Chesapeake Bay (Nelson 1980), and (2) continuing overharvest of large, predatory fishes like spotted seatrout (*Cynoscion nebulosus*) and striped bass (*Morone saxatilis*), which may keep smaller predators like pipefish from becoming overly abundant, as has been shown for the overharvest of cod (*Gadus morhua*) in the Baltic Sea (Eriksson et al. 2009). It is therefore critical that future food-web studies in Chesapeake Bay, and other coastal ecosystems where seagrasses may be threatened, firmly identify the links between climate change, overfishing, and the small consumers of mesograzers.

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