

# Drivers of spatial and temporal variability in estuarine food webs

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**ABSTRACT:** A critical challenge to understanding the response of ecosystems to anthropogenic drivers is characterizing the spatial and temporal variability of controls on food web dynamics. We used a long-term (9 yr) isotope survey and a community metric isotope approach to determine the major physical factors influencing the source of energy to estuarine food webs. Overall, food web architecture was similar throughout the estuary, but there were some spatial differences. We observed greater overall variability in the primary production source to the food web in the upper estuary (which is more influenced by freshwater inputs) compared with the middle and lower estuary. The trophic level of one dominant species, mummichog *Fundulus heteroclitus*, was also highly correlated with tidal height, which controls high marsh access in the middle estuary. We also observed a strong influence of freshwater input on the benthic–pelagic coupling in the upper estuary. Our work demonstrates that the temporal and spatial variability of food webs in estuarine systems is highly coupled to physical drivers.

**KEY WORDS:** Stable isotopes · Food webs · Long-term · Estuarine

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## INTRODUCTION

Food webs describe the trophic interactions and the flow of carbon, nutrients, and energy among the organisms of an ecosystem (Lindeman 1942, Polis & Strong 1996). Typically, food webs combine descriptive measures of interaction strength with quantitative measures of community structure to provide a picture of energy flow (Elton 1927, Paine 1992). However, understanding how food webs might respond to ecosystem change has been difficult because traditional metrics do not provide quantitative measures of changes in resources, and are rarely studied in the context of physical drivers of environmental conditions (Pimm 2002, Polis et al. 2004, McCann 2012, Hussey et al. 2014).

Stable isotope analysis provides a time-integrated account of the material assimilated by organisms, and reflects resource use. As an inherent part of all biological material, the stable isotopes of carbon (<sup>13</sup>C)

and nitrogen (<sup>15</sup>N) have been most commonly employed in food web studies (Fry 2007). Carbon provides a useful indicator of the source of primary producer material because it changes little from the source to the consumer (DeNiro & Epstein 1981). Nitrogen is fractionated as it is assimilated from source to consumer by approximately 3 to 5‰, making it a useful indicator of trophic level (Minagawa & Wada 1984, Post 2002, Hussey et al. 2014). These isotopes in combination can reveal the primary sources of energy to food webs and some aspects of their trophic structure (Vander Zanden et al. 1999, Wilson et al. 2013). Layman et al. (2007) proposed a set of quantitative metrics based on stable isotope analysis to quantify community level characteristics such as niche space and trophic breadth (Cooper & Wissel 2012). Applying a Bayesian approach to these metrics allows for comparisons of trophic structure across ecosystems and over time (Jackson et al. 2012, Abrantes et al. 2014). This approach offers a powerful

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tool to describe energy flow patterns in food webs, and when coupled with physical drivers of ecosystem function, can identify potential drivers of energy flow change.

The primary production source and animal community that make up a food web can be significant factors that influence the total productivity and retention of nutrients in an ecosystem. For example, the physical and biological dynamics of the upper portion of an estuary, where nutrients and organic matter from the watershed first enter, can significantly affect the biogeochemical cycling and productivity of the entire estuarine ecosystem (Schuchardt et al. 1993, Holmes et al. 2000, Giblin et al. 2010). It is also habitat for a unique suite of species capable of tolerating extreme changes in environmental conditions (Jordan & Sutton 1984, Hines et al. 1987). Coupling between pelagic primary production and benthic secondary production can also increase the retention of allochthonous nutrients and organic matter inputs within the estuary (Hughes et al. 2000, Eyre & Ferguson 2006), potentially increasing secondary production and providing greater food web stability (Huxel & McCann 1998, Carpenter et al. 2005).

The geomorphic features of ecosystems can also create landscape energy flow patterns that benefit particular community structures (Pringle et al. 1988, Lake 2000, Christian & Allen 2014). Intertidal creeks are conduits that connect high marsh with low marsh and open bay habitats, thus increasing connectivity across the landscape (Kneib 1997, Christian & Allen 2014). Access to marsh areas increases the growth and survival of small fishes (Komarow et al. 1999, Haas et al. 2009). Knowing the proportion of energy acquired from the marsh and how these habitats respond to physical drivers, such as sea level rise, is critical to understanding how the total productivity of coastal ecosystems might change in the future.

Although we have a good understanding of how individual organisms function in food webs, our knowledge of how food webs as a whole respond to environmental drivers over longer periods is less well developed. Given the seasonal nature of allochthonous inputs of nutrients and the inter-annual variability in sources of production, it is necessary to evaluate systems over multiple years in order to understand the physical factors that drive food web energy flow (Fanklin 1989). However, most of the work on food web dynamics in estuarine systems has been done over short time periods (months to a year) (Hughes et al. 2000, Eyre & Ferguson 2006, Hoffman et al. 2008).

Our objective was to identify physical controls on food web structure by examining year-to-year varia-

tions in food web characteristics over nearly a decade. We used carbon and nitrogen stable isotopes in a 'community module' approach to identify controls on food web characteristics (Holt 1997, Gilman et al. 2010). A community module is a small group of species that share a characteristic configuration and interaction network that can be used to determine how whole communities respond to change (Gilman et al. 2010). We used carbon and nitrogen stable isotope ratios of the primary producers and secondary consumers that represent functional groups collected over a 9 yr period to determine the food web structure and resource use in the estuary. We compared the same set of primary producers and secondary consumers over multiple years to infer the relative changes in primary production sources to secondary consumers associated with physical drivers. The species we chose represent all of the primary functional groups, are the most abundant form of higher trophic level biomass, and accumulate most of their biomass over the summer, thus providing us with integrated isotope values during the growing season (Lotrich 1975, James-Pirri et al. 2001, Fry et al. 2008).

We used Bayesian stable isotope mixing models and community-wide metrics to compare trophic structure and organic matter source. We then used the metrics to identify food web controls. We hypothesized that the primary controls on the food webs would be strongly influenced by the prevailing physical dynamics in each part of the estuary.

## MATERIALS AND METHODS

### Study site and sampling locations

The study was conducted in the Plum Island Ecosystems Long-Term Ecological Research (PIE LTER) site near Newburyport, MA, USA (Fig. 1). PIE is a productive riverine salt marsh estuary, with a vertically well mixed water column and a horizontal salinity gradient of 0 at the head of the estuary to 32 at the marine end. The steepness of the gradient varies in space and time depending on the interplay between freshwater input via rivers and the spring/neap tidal cycle (Vallino & Hopkinson 1998). The Parker River (mean annual discharge:  $1.2 \text{ m}^3 \text{ s}^{-1}$ ; growing season [June to Sept]:  $0.5 \text{ m}^3 \text{ s}^{-1}$ ) has greatest influence on the salinity gradient in the system (Vallino & Hopkinson 1998, Holmes et al. 2000). The seasonal water temperature ranges from just below freezing ( $-1^\circ\text{C}$ ) in February to  $28^\circ\text{C}$  in July and August. The neap and mid-range tides (1.9 to 2.7 m) flood low and

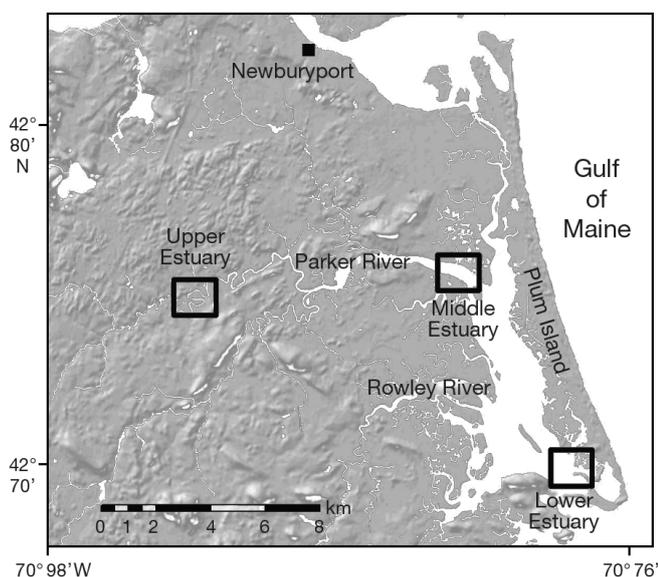


Fig. 1. Plum Island estuary; black boxes: sampling area

fringing marsh habitats, while spring tides ( $>2.8$  m) flood high marsh habitats.

Food webs were characterized from 1999 to 2008 in 3 regions: the upper ( $42.75^{\circ}$  N,  $70.92^{\circ}$  W), middle ( $42.76^{\circ}$  N,  $70.83^{\circ}$  W), and lower ( $42.70^{\circ}$  N,  $70.78^{\circ}$  W) regions of the estuary (Fig. 1). The upper estuary is characterized by a deep river channel with terrestrial inputs of pelagic particulate organic matter (PPOM), and is bordered by a brackish marsh dominated by C3 marsh plant species (*Typha* sp. and *Scirpus* sp. in tidal marshes near the upland edge, grading into C4 marsh plant species *Spartina* sp. along the river edge). The middle estuary is characterized by extensive saltmarsh habitats (C4 marsh plant species, low marsh *Spartina alterniflora* and high marsh *S. patens*). These saltmarshes produce about 600 to 700 g dry wt  $m^{-2}$   $yr^{-1}$  (Montagna & Ruber 1980, Morris et al. 2013). The lower estuary is an open, shallow (80% exposed at low tide) marine system dominated by sand flats with a steep shoreline that transitions quickly to upland, with little adjacent saltmarsh habitat.

### Food web analysis

Samples were collected in August and September to represent a time-integrated assessment of the growing season. Our functional groups were classified based on feeding mode as determined in Deegan & Garritt (1997) to provide examples of consumers at the various trophic levels and resource use types of the dominant species in the estuary (Wilson 1999). We used benthic diatoms and marsh macrophytes as

proxies for benthic primary producers and PPOM as a proxy for pelagic primary producers (see Table 1). The values for organic matter sources were collected annually in each section of the estuary and used to determine consumer source contributions from each region of the estuary. Our consumers were mixed zooplankton, ribbed mussels *Geukensia demissa*, soft-shell clams *Mya arenaria*, sandworms *Nereis virens*, blue mussels *Mytilus edulis*, mummichog *Fundulus heteroclitus*, and Atlantic silverside *Menidia menidia*. Zooplankton represented mobile pelagic primary consumers (McClelland & Montoya 2002) while the bivalve species represented sessile water column feeders (Gillikin et al. 2006).

The sandworm, mummichog, and Atlantic silverside are mobile omnivores that vary in their feeding strategies. Sandworms burrow in the sediment and represent benthic deposit feeders (Nielsen et al. 1995). Mummichogs represent benthic oriented generalists and consume a wide variety of predominantly benthic food items, from detritus and algae to small invertebrates such as copepods and insects (Kneib 1986). Atlantic silverside represent a pelagic oriented invertebrate feeder, but have been shown to feed on benthic prey items as well (Adams 1976, Fry et al. 2008).

Sample preparation included collecting individuals, isolating and cleaning the sample, compositing tissue of 3 to 20 individuals (depending on species), washing with deionized water, drying at 50 to 60°C for 48 h, and grinding to a powder with a Wig-L-Bug grinder (Crescent Dental). All filters were glass fiber (size F), pre-combusted at 500°C for 4 h. All dried samples were stored in glass vials. Stable isotope samples were processed at the Marine Biological Laboratory using a Europa ANCA-SL elemental analyzer-gas chromatograph preparation system attached to a continuous-flow Europa 20-20 gas source stable isotope ratio mass spectrometer ([www.mbl.edu/ecosystems/silab/](http://www.mbl.edu/ecosystems/silab/)).

Benthic algae were collected by placing a 210  $\mu$ m Nitex screen on the sediment surface for 30 min at low tide during daylight hours to collect diatoms that migrate up through the sediment to the surface of the screen. The material on the surface of the screen was rinsed with deionized (DI) water onto a filter and rinsed of carbonates using 10% HCl, then re-dried. Water column PPOM (a mix of live phytoplankton and dead organic matter) was vacuum filtered at low pressure from water collected at mid ebb tide and stored frozen ( $-20^{\circ}$ C). *S. alterniflora*, *S. patens*, *Typha* sp., and *Scirpus* sp. were collected for the macrophyte end-members. Live leaves were cleaned, rinsed with distilled water and dried. The leaves were collected at

multiple sites within the PIE marshes over the 9 yr sampling period.

Mixed zooplankton were collected non-quantitatively at mid ebb tide using a towed 150  $\mu\text{m}$  plankton net. Zooplankton were separated out by light migration and hand picking, then gently filtered onto filters and dried. Mummichog (35 to 50 mm total length) and Atlantic silverside (50 to 70 mm total length) were captured with a seine. A small sample of muscle tissue was taken from 15 to 20 individuals to create a composite sample. The abductor muscle from ribbed mussels, softshell clams, and blue mussels (4 to 6 individuals of each species) was dissected out, rinsed with DI water, composited by individual species, and dried. Sandworms ( $n = 3$  to 10) were held for 24 h in clean seawater to purge their guts, then rinsed with DI water and dried.

### Stable isotope and statistical analysis

All statistical analysis was done in R (R Development Core Team) v.3.1.2 ([www.R-project.org/](http://www.R-project.org/)). Stable isotope analysis in R (SIAR v.4.0) (Parnell et al. 2010) was used to estimate the percent contribution of each organic matter source to the functional groups. In the upper estuary we used 4 organic matter sources: PPOM, benthic algae, C4 saltmarsh, and C3 brackish marsh. In the middle and lower estuary, where there is no brackish marsh habitat, we used 3 sources; PPOM, benthic algae, and C4 saltmarsh (see Table 1). The model was corrected for elemental concentration of the organic matter sources using the mean elemental concentrations determined by mass spectrometry (Phillips & Koch 2002, our Table 1).

Three community metrics were used to describe general food web structure: (1) mean distance to centroid (CD), (2) total area (TA) and (3) the standard ellipse area (SEA). CD is the average Euclidian distance of each community component to the centroid and provides a measure of trophic variation. TA represents a measure of the total niche space occupied by the community and is strongly influenced by the most outlying values on both the x- and y-axis (Layman et al. 2007, Abrantes et al. 2014). The SEAs are drawn to constrain ~40% of the data and provide a more comparable representation of the trophic niche space than TA, which relies on the extreme values to calculate area (Batschelet 1981). To determine if consumer isotope values varied more than primary producer values, we compared the inter-annual variability in the consumer's isotope values with the primary producer's isotope values using Bartlett's test of

homogeneity of variances (Snedecor & Cochran 1989). Statistical comparisons of the trophic niche space were done using the stable isotope Bayesian ellipses in R (SIBER) package (Jackson et al. 2011) in SIAR. The trophic niche space was quantified using the small sample size corrected standard ellipse area ( $\text{SEA}_c$ ) calculated as  $\%^2$  (Batschelet 1981). The Bayesian approach propagates natural and sampling uncertainty through the derived ellipses allowing for comparison between community components (Jackson et al. 2011).

Trophic level was calculated using the nitrogen isotope values of the primary organic matter sources as the base line:

$$\text{TL} = (\delta^{15}\text{N}_{\text{cons}} - \delta^{15}\text{N}_{\text{base}}) / \Delta \delta^{15}\text{N} + 1 \quad (1)$$

where the  $\delta^{15}\text{N}_{\text{cons}}$  is the consumer's  $\delta^{15}\text{N}$  value,  $\delta^{15}\text{N}_{\text{base}}$  is the base value calculated from the mixture of organic matter sources that contribute to a given consumer at a particular site, and  $\Delta \delta^{15}\text{N}$  is 3.00 (Mina-gawa & Wada 1984, Post 2002, Hussey et al. 2014).

### Physical drivers

To explain the variability in our isotope data, we tested some hypotheses that have been put forward in the literature to explain food web variability in saltmarshes. Access to the high marsh habitat is an important factor for the growth of small nekton in saltmarshes, particularly mummichog, because it provides access to a greater abundance of prey items and dietary protein (Komarow et al. 1999, Currin et al. 2003, Haas et al. 2009). The high marsh habitat in PIE is approximately 2.7 m above mean sea level. We used the number of days during the growing season with tides greater than 2.8 m (which puts >10 cm water on the marsh platform) as an indicator of marsh access. Greater marsh access has the potential to provide greater access to invertebrate prey as well as decreasing the effective density of mummichog by providing access to much greater expanses of habitat and thus reducing intraspecific competition.

We used mean summer growing season (May to September) salinity as a measure of the influence of river discharge on the food web. Conductivity and temperature were recorded at 42.750° N, 70.901° W (approximately 3 km from the terminus of the Parker River) using a YSI 6600 every 15 min. Mean summer salinity was calculated using the practical salinity scale (Fofonoff 1985). The calculated salinity was then compared to the mean contribution of PPOM to mummichog isotope value.

## RESULTS

Primary producer isotope values varied little from year to year compared with the variation of the consumers (Table 1; Bartlett's  $K^2 = 8.33$ ,  $df = 1$ ,  $p = 0.003$ ). This indicates that the isotope values of the sources of carbon and nitrogen to the system did not vary significantly during the study. Therefore, the observed annual differences in isotope values of consumers were driven by changes in consumer use of primary organic matter sources and not annual variability in primary producer isotope values. Primary producer's values did vary along a spatial gradient with the position in the estuary relative to fresh and saltwater inputs. PPOM was highly depleted ( $-30.4\text{‰}$   $\delta^{13}\text{C}$ ) in the upper estuary and had typical marine values ( $-22.5\text{‰}$ ) in the lower estuary. Benthic diatom carbon isotope values varied only slightly across the estuary ( $CD = 1.2 \pm 1$ ), while C4 ( $-13.1\text{‰}$   $\delta^{13}\text{C}$ ) and C3 ( $-26\text{‰}$   $\delta^{13}\text{C}$ ) macrophytes had no systematic spatial differences in isotope values; therefore, we used a single mean macrophyte isotope value (Table 1). The nitrogen iso-

tope values for producers varied little from region to region and did not display a systematic spatial trend ( $CD = 0.9$ ). Zooplankton and mummichog  $\delta^{13}\text{C}$  values were variable across the estuary, while Atlantic silverside had similar isotope values at all sites (Table 1). The sessile invertebrates, which only occurred at the middle and lower estuary sites, showed a strong contribution from PPOM (Table 2, Fig. 2). Blue mussels had the highest contribution from PPOM ( $>61\%$ ) and sandworms, the least ( $57\%$ ) (Table 2).

The mean niche areas ( $SEA_c$  and TA) in the upper estuary were 3 times as large as those in the middle and lower estuary (Table 2, Fig. 3). Zooplankton had the greatest range in mean niche area across the estuary, from  $9.5\text{‰}^2$  in the upper bay to  $0.9\text{‰}^2$  in the middle estuary ( $\Delta = 8.6\text{‰}^2$ ). Mummichog mean niche area was intermediate in size and had the smallest range ( $8.4$  to  $2.6\text{‰}^2$ ,  $\Delta = 8.6\text{‰}^2$ ), while Atlantic silverside had intermediate values and the smallest mean niche values ( $7.7$  to  $0.4\text{‰}^2$ ,  $\Delta = 7.3\text{‰}^2$ ) of the 3 species that occurred in all 3 sites. The mobile invertebrate sandworm had low niche areas similar to the nekton

Table 1. Annual ( $n = 9$ ) mean and SD for the carbon and nitrogen isotope values for the primary producers and consumers in each region of the estuary. PPOM: pelagic particulate organic matter

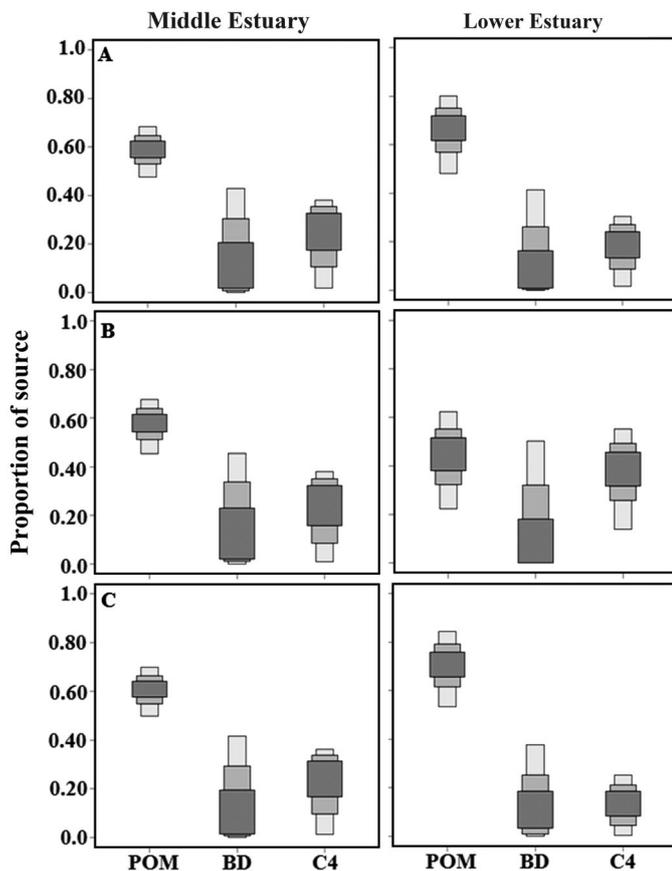
Site	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		% C		% N	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Upper estuary</b>								
PPOM	-30.2	0.7	5.7	1	48	5	8	1
Benthic diatom	-15.8	1.4	3	0.6	48	5	8	1
C4 marsh plants	-13.1	0.4	4.7	0.6	45	5	1.5	0.5
C3 marsh plants	-26	1.6	5	0.5	45	5	1.5	0.5
Zooplankton	-31.4	2.5	7.9	1.2	55	8	9	2
Mummichog	-22.9	2.5	10.4	1.4	53	6	13	4
Atlantic silverside	-25	1.1	12.2	2	52	4	15	3
<b>Middle estuary</b>								
PPOM	-24.6	1.5	3.4	1.5	48	5	8	1
Benthic diatom	-16.1	2.1	3.1	1.4	48	5	8	1
C4 marsh plants	-13.1	0.4	4.7	0.6	45	5	1.5	0.5
Zooplankton	-23.1	1.1	7.7	0.7	55	8	9	2
Mummichog	-15	1.2	8.2	0.8	53	6	13	4
Atlantic silverside	-19.6	0.4	11.1	0.3	52	4	15	3
Softshell clam	-19.0	0.5	10.3	0.5	57	4	13	2
Sandworm	-18.9	0.7	10.2	1.6	54	5	15	3
Blue mussel	-19.2	0.6	9.6	0.4	51	4	12	4
Ribbed mussels	-20.3	0.5	8.9	0.5	49	5	14	5
<b>Lower estuary</b>								
PPOM	-22.5	1.4	5.5	0.4	48	5	8	1
Benthic diatom	-17.9	2	4.5	1.5	48	5	8	1
C4 marsh plants	-13.1	0.4	4.7	0.6	45	5	1.5	0.5
Zooplankton	-21.9	1.5	8.5	0.5	55	8	9	2
Mummichog	-15.7	1.4	10.4	0.5	53	6	13	4
Atlantic silverside	-19.6	0.6	11.3	0.3	52	4	15	3
Softshell clam	-18.6	0.3	8.9	0.6	56	5	15	4
Sandworm	-16.7	1.4	7.9	2.7	52	4	13	3
Blue mussel	-19.1	0.3	8.7	0.5	50	3	13	5

in the site where they co-occurred, with a range of 2.9 to  $1.6\text{‰}^2$ . The sessile invertebrates generally had smaller niche areas than the mobile nekton or mobile invertebrates, with no niche areas greater than  $0.8\text{‰}^2$ . There was no overlap between functional groups in the upper estuary (Fig. 3). All functional groups had their smallest niche areas in the middle estuary (mean  $1.04\text{‰}^2$ ). Softshell clams had the smallest mean niche area for all functional groups ( $0.5\text{‰}^2$ ). Of the functional groups that occurred at all locations, the Atlantic silverside had the smallest niche space (mean =  $2.9\text{‰}^2$ ).

PPOM was the most significant organic matter source in the food webs across the estuary ( $58\%$ ; Figs. 2 & 4), while marsh macrophytes ( $18\%$ ) and benthic algae ( $15\%$ ) contributed almost equally to the food web. The relative dependence of consumers on different organic matter sources varied in a manner consistent with their life history and location in the estuary. Zooplankton were the most dependent upon PPOM ( $85\%$ ; Fig. 4). Zooplankton also occupied the lowest trophic level at each site (mean =

Table 2. Mean, lower and upper 95% contribution of each organic matter source (pelagic particulate organic matter, PPOM, benthic diatoms, and marsh macrophytes), the small sample size corrected standard ellipse area ( $SEA_c$ ), and total area (TA) community metric for each functional group

Site	PPOM		Benthic diatoms		C4 marsh plants		C3 marsh plants		$SEA_c$ (% <sup>2</sup> )	TA (% <sup>2</sup> )
	Mean	Lower/Upper 95%	Mean	Lower/Upper 95%	Mean	Lower/Upper 95%	Mean	Lower/Upper 95%		
<b>Upper Estuary</b>										
Zooplankton	0.43	0.21/0.65	0.06	0/0.19	0.24	0/0.31	0.27	0/0.52	9.5	15.1
Mummichog	0.54	0.31/0.78	0.06	0/0.15	0.07	0/0.15	0.33	0.01/0.59	8.4	13.7
Atlantic silverside	0.43	0.1/0.84	0.18	0/0.37	0.1	0/0.55	0.29	0/0.55	7.7	10.1
<b>Middle Estuary</b>										
Zooplankton	0.89	0.73/1.00	0.06	0/0.18	0.05	0/0.15	NA	NA	0.9	1.1
Sandworm	0.57	0.45/0.67	0.22	0/0.45	0.21	0/0.45	NA	NA	1.6	1.8
Ribbed mussel	0.71	0.61/0.80	0.14	0/0.31	0.15	0/0.27	NA	NA	0.6	0.9
Softshell clam	0.58	0.47/0.68	0.2	0/0.42	0.22	0.02/0.38	NA	NA	0.3	0.45
Blue mussel	0.61	0.49/0.70	0.19	0/0.41	0.2	0.02/0.36	NA	NA	0.8	1.21
Mummichog	0.25	0.14/0.36	0.19	0/0.45	0.56	0.34/0.74	NA	NA	2.7	5.2
Atlantic silverside	0.67	0.57/0.76	0.16	0/0.35	0.17	0.02/0.31	NA	NA	0.4	0.6
<b>Lower Estuary</b>										
Zooplankton	0.82	0.43/1.00	0.15	0/0.42	0.03	0/0.31	NA	NA	2.5	3.3
Sandworm	0.57	0.45/0.67	0.22	0/0.45	0.21	0/0.45	NA	NA	2.9	3.1
Softshell clam	0.58	0.47/0.68	0.2	0/0.42	0.22	0.02/0.38	NA	NA	0.7	0.8
Sandworm	0.57	0.45/0.67	0.22	0/0.45	0.21	0/0.45	NA	NA	2.9	3.1
Blue mussel	0.61	0.49/0.70	0.19	0/0.41	0.2	0.02/0.36	NA	NA	0.6	0.7
Mummichog	0.4	0.18/0.63	0.28	0/0.61	0.32	0.11/0.54	NA	NA	2.6	2
Atlantic silverside	0.84	0.68/0.98	0.11	0/0.37	0.05	0/0.17	NA	NA	0.7	1.1



2.09 ± 0.3) indicating that the zooplankton were generally herbivores, consistent with their high dependence on PPOM. Mummichog were most dependent upon marsh macrophytes (44%; Fig. 4). Mummichog and Atlantic silverside both received approximately 18% of their organic matter from benthic algae (Fig. 4). Atlantic silverside occupied the highest trophic level (mean = 2.80 ± 0.5), which did not vary significantly across the estuary, compared to mummichog (mean = 2.44 ± 0.7), which exhibited the highest degree of inter-annual trophic variability in the middle of the estuary (Figs. 2 & 5).

In the middle estuary, the trophic level of mummichog increased from 2.0 to 3.5 (linear regression;  $R^2 = 0.68$ ,  $p < 0.05$ ) with increased access to the high marsh habitat (Fig. 5), despite no change in the mean nitrogen isotope values of C4 macrophytes with increasing inundation (Fig. 6). An increased number of higher high tides allowing access to high marsh habitats did not significantly influence mummichog trophic level

Fig. 2. Stable isotope analysis in R (SIAR) output for the contribution of pelagic particulate organic matter (PPOM), benthic diatoms (BD), and marsh macrophytes (C4) to (A) softshell clams, (B) sandworms, and (C) blue mussels in the middle and lower estuary. The bands on the box plots represent the 50th, 75th, and 95th percentiles

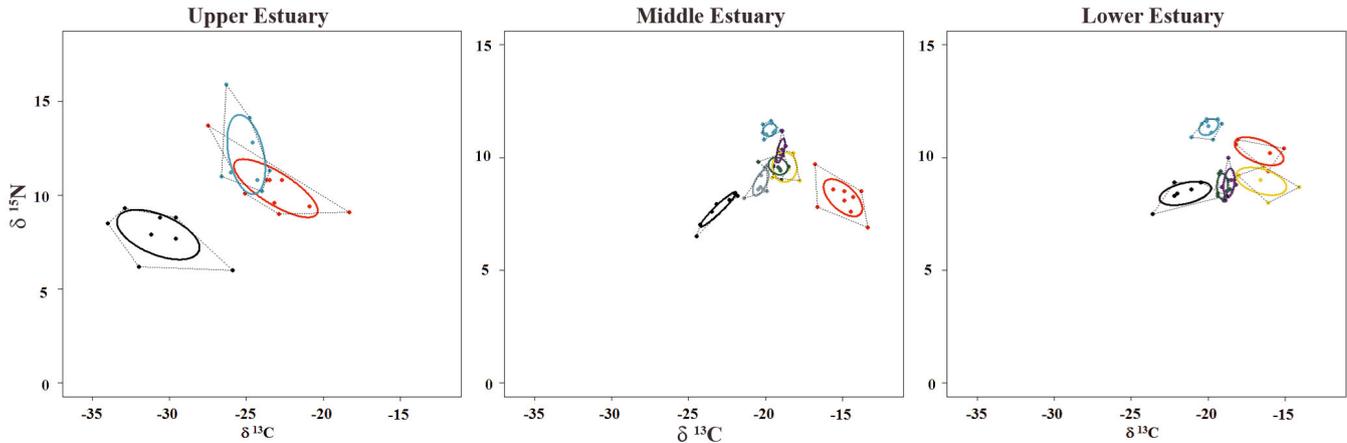


Fig. 3. Representation of niche areas in the upper, middle, and lower estuaries. Colored ellipses: small sample size corrected standard ellipse area ( $SEA_c$ ); dotted lines: total area (TA). In each figure, the color of the ellipse corresponds to a functional group: zooplankton (black), silverside (blue), mummichog (red), softshell clams (purple), ribbed mussel (gray), sandworm (yellow), blue mussel (dark green)

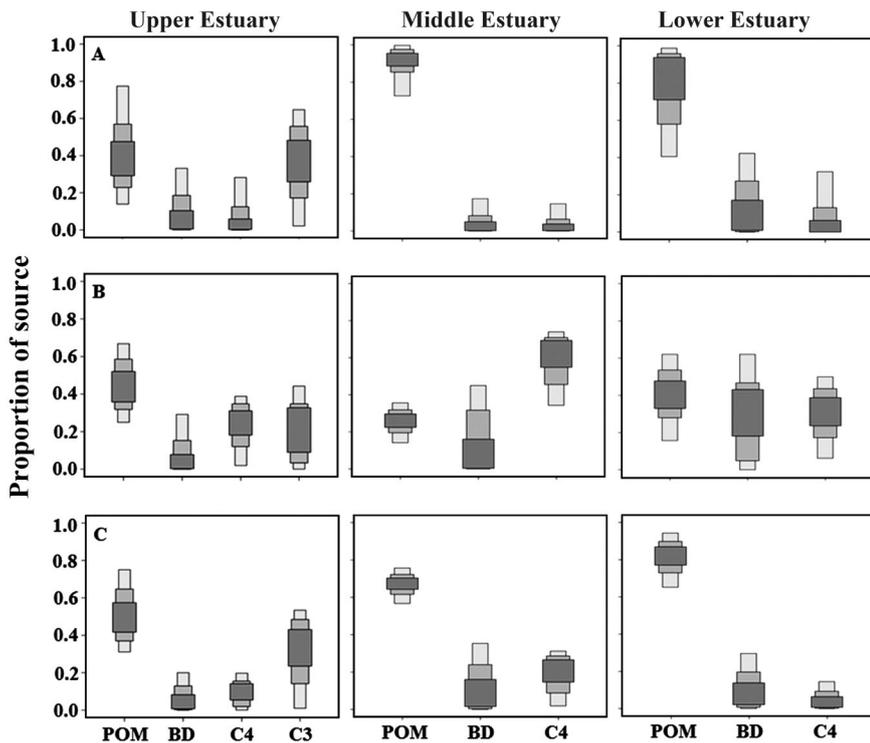


Fig. 4. Stable isotope analysis in R (SIAR) output for the contribution of particulate organic matter (POM), benthic diatoms (BD), C3 and C4 marsh macrophytes to (A) zooplankton, (B) mummichog, and (C) Atlantic silverside in the upper, middle, and lower estuary. The bands on the box plots represent the 50th, 75th, and 95th percentiles

in the upper or lower estuary (Fig. 5). Atlantic silverside trophic levels did not respond to increased high marsh inundation time at any site in the estuary (Fig. 5).

The higher degree of source variability in the upper portion of the estuary was driven by river discharge and riverine sources of organic matter (Figs. 2 & 7).

We observed a significant increase in the use of PPOM by mummichog with increased river flow (linear regression;  $n = 8, p < 0.5$ ) with a 3-fold increase in POM use from ~30 to 90% with a change of 10 in salinity.

### DISCUSSION

The functional groups varied across the estuary in their reliance on benthic or pelagic algal and macrophyte organic matter sources. The inter-annual variability in isotope values of the consumers was lowest in the lower estuary, indicating greater food web stability nearest the ocean. The variability of consumer isotope values was primarily due to changes in the proportion of organic matter source (i.e. diet) rather than fluctuations in primary producer isotope values (Tables 1 & 2). PPOM, a proxy for phytoplankton, was the most important organic matter source, which is counter to the prevailing view that estuaries with large expanses of salt-marsh such as Plum Island have

food webs fueled primarily by macrophyte detritus (Figs. 3 & 4).

In the upper estuary, mummichog reliance on PPOM sources was strongly controlled by the amount of freshwater input into the system. We did not observe a similar shift in either Atlantic silverside

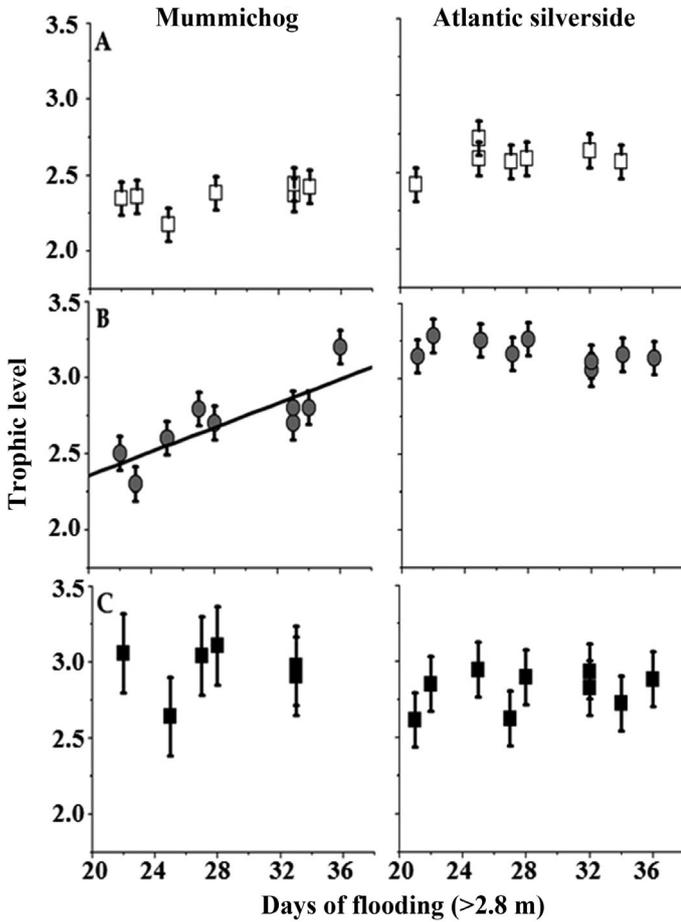


Fig. 5. Trophic level ( $\pm$ SE) of mummichog and Atlantic silverside in the (A) upper (oligohaline), (B) middle, and (C) lower estuary sites versus the number of days with tides  $>2.8$  m (i.e. that would provide nekton access to high marsh areas)

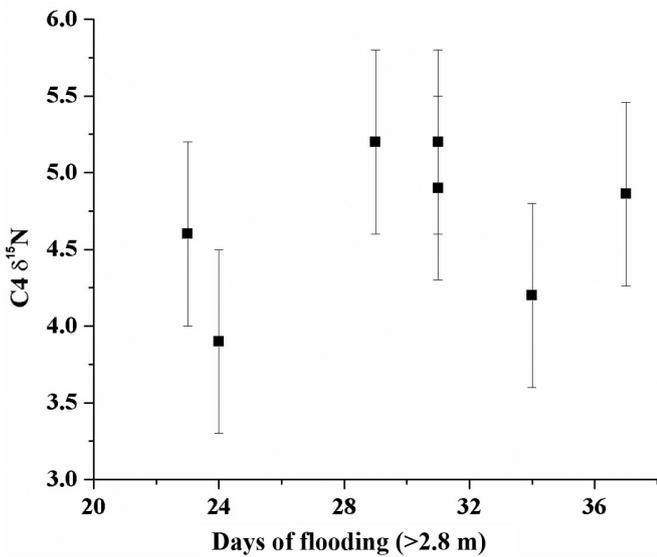


Fig. 6. C4 macrophyte nitrogen isotope values ( $\pm$ SE) versus the number of days with tides  $>2.8$  m used to calculate trophic level

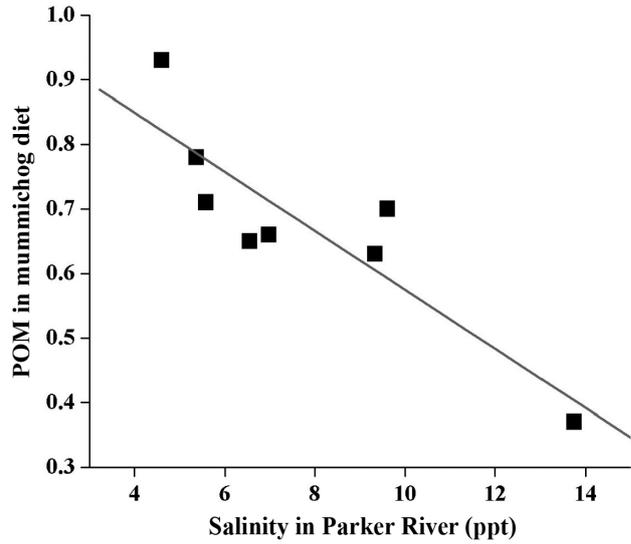


Fig. 7. Contribution of particulate organic matter (POM) to mummichog biomass versus the mean salinity of the upper estuary (linear regression;  $n = 8$ ,  $p < 0.5$ )

or zooplankton. Mummichog do not consume items directly from the water column, but feed exclusively in the benthos, consuming a wide variety of items from algae to small benthic invertebrates (Kneib & Stiven 1982, James-Pirri et al. 2001, Lockfield et al. 2013). Therefore, an increase in the PPOM signal in mummichog would indicate increased benthic-pelagic coupling in the upper estuary (Fig. 7). Hughes et al. (2000) conducted a tracer level addition of  $^{15}N$  enriched nitrate in the upper region of the Parker River and found that phytoplankton re-mineralization fueled the benthic food web. Our observations confirmed these findings, that increased contribution from PPOM is most likely due to *in situ* planktonic primary production sources.

The food webs in the middle and lower estuary were more stable in their reliance on a particular primary production source, but the middle estuary showed increased dependence on high marsh derived productivity and high variability associated with marsh flooding. The contribution of high marsh protein is reflected in the increased trophic level with increased high marsh inundation time (Fig. 5). This observation is consistent with prior observations of increased consumption of protein with increased access to high marsh habitat (Able et al. 2006, Haas et al. 2009). The increased connectivity to high marsh habitat also facilitates the flux of production from the marsh to the estuary because the protein is typically derived from terrestrial insects (Haas et al. 2009).

The sessile invertebrates (softshell clams, ribbed mussels, and blue mussels) had very similar contribution from organic matter sources and had the smallest mean niche widths of any functional group (Fig. 2). As expected, the filter feeders relied heavily on pelagic organic matter, but the small niche widths indicate that inter-annual variation in diet is quite small, demonstrating a reliance on particular food sources. Combined with annual estimations of biomass and growth, these organisms would be a good candidate for bioindicators of ecosystem health or at least indicators of the amount of pelagic productivity in the estuary.

The apex consumer in our community module, the Atlantic silverside, was the most consistent consumer in the module and consistently reflected the general proportions of the production sources in the estuary (Fig. 4, Table 2). Atlantic silverside feed on a variety of both benthic and pelagic prey items, most often small crustaceans and worms (Bayliff 1950, Fry et al. 2008). Atlantic silverside grow rapidly in the estuary during the summer months and can exceed 8 g m<sup>-2</sup> biomass at the end of the growing season (Conover & Ross 1982). Many economically important species prey on Atlantic silverside, including striped bass *Morone saxatilis* and bluefish *Pomatomus saltatrix* (Bayliff 1950). By coupling both benthic and pelagic production sources in estuaries, Atlantic silverside is an important link between estuarine primary productivity and the higher trophic levels of the coastal food web. A recent study on small coastal fish demonstrated that a single estuarine fish species exported ~20% of the total production available to offshore predators (Nelson et al. 2013). It is likely that Atlantic silverside perform a similar function, and quantifying their contribution to coastal secondary productivity should be a priority of future research.

Our data provides some of the first long-term information on the stability of food webs in estuaries. It shows that the most physically dynamic portion of the estuary, the upper estuary, also has the most variable food web, while the middle and lower estuary are stabilized by the more constant tidal input from the ocean. Our ability to quantify the variability in the food webs allowed us to determine some of the primary drivers of the observed changes in resource use. These factors can be used to infer how the food web may respond to future change or as parameters in ecosystem models.

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