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Position of horseshoe crabs in estuarine food webs: N and C stable isotopic study of foraging ranges and diet composition

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Abstract

To discern the position of horseshoe crabs as a potentially important predator in estuarine food webs, we determined where they foraged and what they ate. We used N and C stable isotopes to link adult horseshoe crabs to their foraging locations and potential food sources in Pleasant Bay, Cape Cod. The δ^{15}N in tissues of horseshoe crabs and their potential foods suggest crabs were loyal to local foraging sites and did not forage substantially in subestuaries receiving >110 kg N ha^{-1} year^{-1}. Among locations where crabs foraged, δ^{13}C values in potential foods showed that food webs in subestuaries subject to higher N loads were supported by algal producers, while food webs in subestuaries with lower N loads were also supported by *Spartina*. δ^{13}C values in horseshoe crab tissue did not change with load, suggesting they ate a mixed diet, regardless of N load. N and C isotopes in horseshoe crab feces were similar to signatures of estimated diet, suggesting low assimilation efficiency, perhaps due to ingestion of low quality organic matter. Although horseshoe crabs were relatively opportunistic in foraging habits, conservation or culture of horseshoe crabs may require habitats with higher water quality, ample particulate organic matter, and supporting a variety of prey.

Keywords: Bivalve; Crustacean; Gastropod; *Limulus polyphemus*; Organic matter; Polychaete

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1. Introduction

The Atlantic horseshoe crab (*Limulus polyphemus*) is a potentially highly mobile benthic consumer whose adult habitat ranges from the continental shelf to inshore estuaries where they congregate in the spring to spawn (Shuster, 1982; Sekiguchi, 1988). Adults are capable of migrating great distances and do not necessarily return to the same beaches each year (Shuster, 1955, 1982, 1996; Baptist et al., 1957; Sekiguchi, 1988).

Horseshoe crabs are thought to have decreased in abundance in recent decades (Rudloe, 1982; Michels, 1996; Swan et al., 1996; Widener and Barlow, 1999; Berkson and Shuster, 1999), raising interest in protecting natural horseshoe crab populations and culturing crabs to restore declining populations (Tanacredi, 2001; Botton, 2001). These efforts have prompted concerns that protecting horseshoe crabs will, in turn, reduce abundance of other commercially important species such as bivalves (MA Division of Marine Fisheries staff, personal communication). To address these concerns we need a more detailed understanding of where horseshoe crabs forage and what they eat.

Many animal species, sediment, and vascular plants have been found in the guts of adult horseshoe crabs (Botton and Haskin, 1984; Botton, 1984a; Botton and Ropes, 1989). Gut content analysis, however, may be biased toward detection of species such as bivalves since shells or exoskeletons are not readily digested and tend to remain in the gut (Sutela and Huusko, 2000; Alexander et al., 1996; Hyslop, 1980). Gut content analysis is also biased toward food items recently consumed and cannot provide information regarding longer-term assimilation or provide direct links to foraging locations.

Measurement of stable isotopes has become a widely used method for defining relationships between consumers and their food sources (Peterson and Fry, 1987; Michener and Schell, 1994; Cabana and Rasmussen, 1994), determining sources of N and C to these food webs, and have been applied to study foraging, migration, and other life history phenomenon (Hesslein et al., 1991; Alisauskas and Hobson, 1993; Walker et al., 1999; Best and Schell, 1996; Hansson et al., 1997; Kline et al., 1998; Griffin and Valiela, 2001; McGinnis and Emslie, 2001). Hence, stable isotope data may offer valuable evidence to define horseshoe crab foraging ranges and diet composition.

N isotopes can be used to link consumers to foraging sites because freshwater delivered to different estuaries bear specific signatures derived from N sources on land (McClelland et al., 1997; McClelland and Valiela, 1998a). Heavier N isotopic signatures are typically found in more eutrophied estuaries where wastewater is the primary source of N mediating isotopic signatures (McClelland et al., 1997; McClelland and Valiela, 1998a). These land-derived isotopic signatures are conveyed to producers and ultimately transferred to consumers (Peterson and Fry, 1987; Griffin and Valiela, 2001; Evgenidou and Valiela, 2002; Weiss et al., 2002; Shriver et al., 2002). Location-specific isotopic signatures have been used to link relatively sedentary estuarine consumers to their foraging sites (Evgenidou and Valiela, 2002; Weiss et al., 2002; Shriver et al., 2002). Here we apply stable isotopes to identify the foraging sites of a highly motile invertebrate benthic predator.

In addition to their utility in identifying sources of N and C to estuaries, stable isotope ratios are useful in defining food webs because isotopes are relatively

consistently fractionated by biological and physical processes as they pass through food webs. Isotopic ratios become heavier by 2–4‰ for N and by 1–2‰ for C with each trophic transfer (Peterson and Fry, 1987; Cabana and Rasmussen, 1994). This fractionation from potential food sources to tissues in consumers has been used to identify trophic relationships. Similarly, fractionation of isotopic signatures from potential food sources to feces, which are comprised of unassimilated food and by-products of metabolism, can be used to identify foods that may be consumed but not assimilated (Klein Breteler et al., 2002).

Trophic relationships between motile consumers like horseshoe crabs and their food sources may depend on size of consumers as well as foraging patterns. Larger animals are able to feed higher in the food web and often acquire heavier isotopic signatures in their tissues (France et al., 1998; Gaines et al., 2002; Harvey et al., 2002). Hence, sizes of consumers and food sources need to be considered when comparing isotopic signatures across different locations. Foraging patterns also may uncouple isotopic relationships between consumers and food sources if tissue turnover rates in consumers are slower than changes in location (Schmidt et al., 2003), and such data have not been collected for horseshoe crabs. Since juvenile horseshoe crabs remain within intertidal areas near natal beaches for the first years of life (Rudloe, 1979, 1981; Shuster, 1982), they acquire the isotopic signature of food sources in their natal estuaries (Gaines et al., 2002). Comparing isotopic signatures in different sized resident juveniles with those in tissues of adults may discern whether differences in isotopic signature among crabs from different areas are related to body size or foraging location.

In this study, we used $\delta^{15}N$ and $\delta^{13}C$ signatures of adult horseshoe crabs and their prey in Pleasant Bay on Cape Cod to (1) determine whether crabs foraged throughout Pleasant Bay or fed locally in certain subestuaries by sampling horseshoe crabs and their potential food sources from subestuaries of the Bay receiving different N loads and different isotopic ratios, and (2) define the diet composition and trophic position of adult horseshoe crabs foraging in subestuaries of Pleasant Bay by comparing isotopic values in available food sources to those in horseshoe crab tissue and feces.

2. Methods

2.1. Determination of foraging locations

To determine whether horseshoe crabs within Pleasant Bay foraged in specific subestuaries or throughout the Bay, we compared isotopic signatures of adult crabs from sites in seven subestuaries and at Nauset Beach in the main portion of Pleasant Bay (Fig. 1) to food sources available in each location. Watershed delineations as well as relationships between land cover on watersheds and resulting N inputs to the subestuaries of Pleasant Bay are described elsewhere (Carmichael et al., in press). From these data, we selected sampling sites within subestuaries of Pleasant Bay and in the main portion of the Bay at Nauset Beach that receive the widest range of land-derived N loads to the Bay (Table 1) and where N loads convey estuary-specific isotopic signatures to producers and consumers (Carmichael et al., in press).

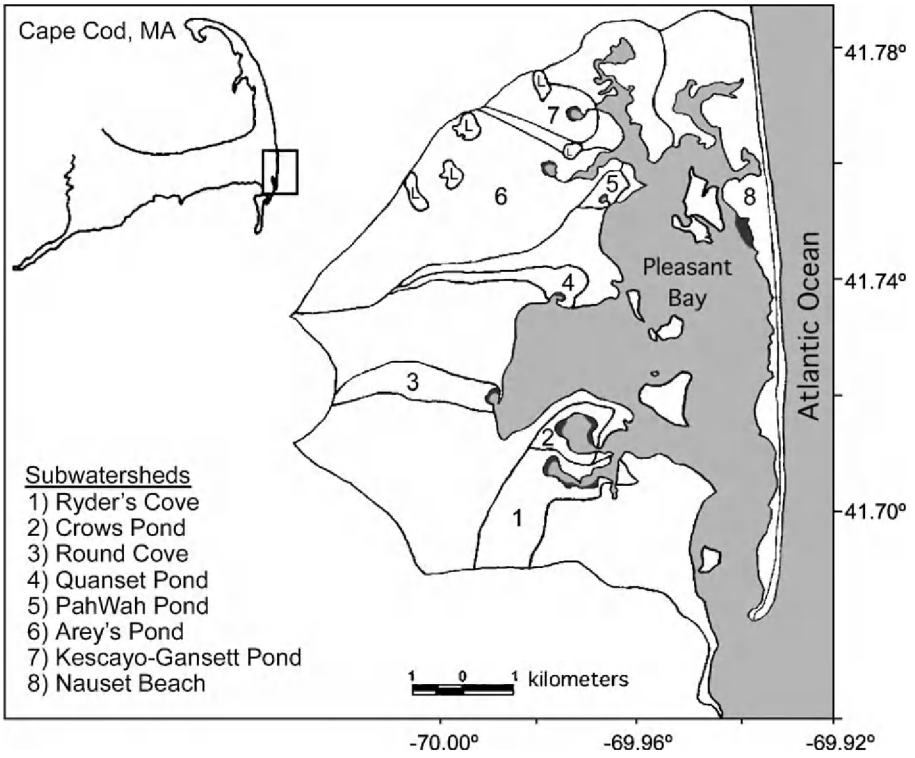


Fig. 1. Location, subwatershed delineations, and associated subestuaries of Pleasant Bay. Black areas indicate general locations from which horseshoe crabs, seston, sediment, and biota were collected for stable isotope analyses. Samples collected at Nauset Beach represent the entire Pleasant Bay system (Carmichael et al., in press). L = intercepting lakes.

To further link adults to foraging locations and assess changes in isotopic signatures with size, we compared the isotopes in tissues of adult crabs to those of juveniles. We obtained juvenile and adult horseshoe crabs from intertidal sand flats at Nauset Beach (Fig. 1), where juveniles of various sizes were available. Juvenile and subadult crabs ranged in size from 7 to 168 mm, representing the majority of sizes of immature crabs in Pleasant Bay (Carmichael et al., 2003). We determined maturity and sex of sampled crabs by the presence or absence of monodactylus pedipalps and the structure of gential pores (Shuster, 1982; Sekiguchi, 1988) and determined size by measuring prosomal width (Shuster, 1955; Riska, 1981) of crabs to the nearest 1 mm.

2.2. Horseshoe crab sampling

To obtain horseshoe crab tissue samples for isotope analysis, we clipped tissue from the last two segments of the second or third walking leg of adults and juveniles greater than 20 mm. This sampling procedure was non-lethal, and crabs were released to the water immediately after sampling. No crab was sampled more than once. For smaller juveniles,

Table 1
Embayment areas, total annual nitrogen load, and nitrogen loading rates[a] normalized to embayment area for the entire Pleasant Bay system and subwatersheds (cf. Fig. 1) from which we sampled horseshoe crabs, prey species, seston, and sediment

	Area (ha)	N load (kg $year^{-1}$)	N load (kg ha^{-1} $year^{-1}$)
Ryder's Cove	43.1	4701	109
Crows Pond	47.1	1450	31
Round Cove	5.4	1076	199
Quanset Pond	4.6	498	108
Pahwah Pond	2.6	195	75
Arey's Pond	24.0	3301	138
Kescayo-Gansett Pond	8.3	519	62
Nauset Beach (Pleasant Bay)	2752	67443	25

[a] N loads include direct deposition to the water body (Carmichael et al., in press).

we collected whole animals. Tissues from 3 to 10 crabs of either sex were aggregated for each sample.

2.3. Food source sampling

To assess the signatures of potential prey of adult horseshoe crabs, we collected biota from each of the seven subestuaries and at Nauset Beach by hand and by sieving sediment from up to four grabs of 0.3×0.3 m to 0.1 m depth. Sediment was washed through a series of sieves ranging in size mesh from 0.63 μm to 2 mm. Since isotopic signatures of a species may vary with size, we collected prey species of similar size at each site.

To obtain signatures of suspended or sedimented organic matter that may be ingested by horseshoe crabs or their prey, we collected seston by filtering 2 l of water onto a pre-ashed 0.7 μm Whatman GF/F filter, and we sampled the top 3 cm of sediment using a 1-cm diameter modified syringe corer. To capture some of the variation within each estuary, we collected cores from three locations at each site and pooled these samples to obtain an aggregate isotopic value.

2.4. Feces sampling

To compare assimilated foods represented by horseshoe crab tissue to recently consumed but unassimilated foods, we sampled feces. We placed eight crabs from Nauset Beach in holding tanks in the laboratory, and collected feces immediately after they were deposited and within 1 h of horseshoe crab transport to the laboratory.

2.5. Stable isotope sample preparation and analysis

Samples were collected from May–Aug 2001 and 2002. Samples were dried at 60 °C, tissues and sediments were ground using a mortar and pestle, and all samples were sent to the University of California-Davis Stable Isotope Facility to determine $\delta^{13}C$ and $\delta^{15}N$

Table 2
Mean (± standard error) of $\delta^{15}N$ and $\delta^{13}C$ signatures (‰) in tissue from adult horseshoe crabs, prey species, and from organic matter in seston and sediment collected at eight locations in the Pleasant Bay system

	Estuary															
	Nauset Beach		Crows Pond		Kescayo-Gansett Pond		Pahwah Pond		Quanset Pond		Ryder's Cove		Arey's Pond		Round Cove	
	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C
Adult horseshoe crabs	9.1 (0.1)	−15.9 (0.3)	8.9 (0.3)	−16.4 (0.5)	10.2	−16.8	9.6	−15.7	10.1	−16.2	9.6	−16.5	8.1 (0.1)	−16.9 (0.6)	8.4	−16.1
Bivalves																
Ensis directus	7.5	−17.8	–	–	–	–	–	–	–	–	–	–	–	–	8.3	−18.6
Geukensia demissa	7.2	−18.1	–	–	8.0	−19.6	7.5	−19.6	–	–	–	–	8.3 (0.3)	−20.4	–	–
Gemma gemma	7.3 (0.3)	−17.5 (0.1)	–	–	7.6	−16.6	–	–	–	–	–	–	7.7	−17.9	–	–
Mya arenaria	7.0 (0.3)	−18.0 (0.3)	7.3	−19.8	8.6 (0.3)	−20.9 (0.6)	7.2	−17.7	8.4 (0.2)	−18.9 (0.5)	8.7 (0.2)	−18.7 (0.2)	8.9	−19.2	8.4 (0.6)	−18.8 (0.9)
Mytilus edulis	7.7	−18.3	–	–	–	–	–	–	7.3 (0.3)	−20.7	–	–	–	–	–	–
Mercenaria mercenaria	8.6 (0.4)	−17.4	9.2	−18.8 (0.2)	9.6 (0.2)	−20.9	9.4	−16.9	9.6 (0.1)	−18.3	10.0 (0.5)	−18.3 (0.4)	–	–	9.8 (0.4)	−18.6
Tagelus plebius	–	–	–	–	–	–	–	–	–	–	7.3	−17.6	–	–	–	–
Mean	7.5 (0.2)	−17.8 (0.1)	8.3 (1.0)	−19.3 (0.5)	8.4 (0.4)	−19.5 (1.0)	8.1 (0.7)	−18.1 (0.8)	8.4 (0.7)	−19.3 (0.7)	8.7 (0.8)	−18.2 (0.3)	8.3 (0.4)	−19.2 (0.7)	8.8 (0.3)	−18.7 (0.1)
Crustaceans																
Byblis serrata	–	–	–	–	–	–	–	–	–	–	7.6	−15.5	–	–	–	–
Caprella spp.	7.0	−15.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chiridotea coeca	7.9	−11.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cirolana polita	–	–	–	–	–	–	9.0	−14.6	–	–	–	–	11.1	−13.6	–	–
Edotea triloba	4.7	−11.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Gammarus mucronatus	6.3	−12.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Gammarus oceanicus	5.9	−12.2	–	–	–	–	–	–	–	–	–	–	7.2	−14.4	–	–

Haustorius spp.	–	–	–	–	–	–	–	–	–	–	7.8	−13.8	–	–	–	–
Idotea baltica	6.5	−12.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Orchestia spp.	–	–	–	–	–	–	–	–	–	–	10.4	−13.8	–	–	–	–
Orchomonella spp.	–	–	–	–	–	–	–	–	–	–	10.3	−14.9	–	–	–	–
Mean	6.4	−12.5	–	–	–	–	9.0	−14.6	–	–	9.0	−14.5	9.2	−14.0	–	–
	(0.4)	(0.6)									(0.8)	(0.4)	(1.9)	(0.4)		
Gastropods																
Littorina littorea	6.1	−16.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	(0.2)	(0.6)														
Lunatia heros	7.6	−17.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	(0.3)	(0.1)														
Nassarius spp.	–	–	–	–	9.8	−20.9	7.0	−13.3	–	–	–	–	9.8	−13.7	9.2	−11.3
Mean	6.9	−17.0	–	–	9.8	−20.9	7.0	−13.3	–	–	–	–	9.8	−13.7	9.2	−11.3
	(0.5)	(0.2)														
Polychaetes																
Ampharete spp.	9.5	−14.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Eteone longa	9.8	−14.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Glycera spp.	–	–	11.0	−14.9	10.8	−16.8	–	–	–	–	10.0	−15.3	–	–	11.3	−15.5
Neanthes succinea	6.7	−12.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nephtys spp.	–	–	9.4	−15.5	–	–	–	–	–	–	–	–	–	–	11.9	−15.4
Nereis spp.	9.5	−14.3	9.3	−15.9	11.8	−16.8	8.0	−15.4	9.4	−14.7	11.9	−19.0	8.7	−14.9	10.8	−17.1
Ophelia spp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11.9	−14.1
Orbinia ornata	7.0	−12.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pectinaria gouldii	6.8	−13.1	–	–	8.2	−14.5	–	–	–	–	9.6	−14.3	–	–	–	–
Mean	8.2	−13.7	9.9	−15.4	10.3	−15.6	8.0	−15.4	9.4	−14.7	10.5	−16.2	8.7	−14.9	11.5	−15.5
	(0.6)	(0.4)	(0.5)	(0.3)	(1.1)	(0.7)					(0.7)	(1.4)			(0.3)	(0.6)
Cnidarian																
Haloclava producta	–	–	–	–	–	–	–	–	–	–	11.3	−13.9	–	–	–	–
Organic matter																
Sediment	3.37	−13.7	4.2	−15.9	4.1	−18.9	4.6	−17.9	5.6	−15.7	5.2	−21.8	5.2	−18.0	5.3	−19.5
Seston	5.02	−15.8	6.4	−18.6	6.5	−20.9	6.1	−22.0	6.0	−19.2	6.4	−22.0	6.1	−22.5	6.7	−21.2
	(0.1)			(0.7)	(0.1)	(0.2)	(0.1)	(0.1)	(0.2)	(0.5)			(0.2)	(1.5)		(0.5)
Mean	4.2	−14.8	5.3	−17.3	5.3	−19.9	5.4	−20.0	5.8	−17.5	5.8	−21.9	5.6	−20.3	6.0	−20.4
	(0.8)	(0.9)	(1.1)	(1.4)	(1.2)	(1.0)	(0.7)	(2.1)	(0.2)	(1.7)	(0.6)	(0.1)	(0.4)	(2.2)	(0.7)	(0.8)

signatures by mass spectrometry. Aggregated samples were analyzed one or two times, with individual samples randomly chosen for quality control replication to test homogeneity of aggregates and spectrometer function.

3. Results and discussion

3.1. Foraging within Pleasant Bay

To determine whether adult horseshoe crabs in Pleasant Bay preferred foraging in certain subestuaries, we needed to link horseshoe crabs to the locations from which they were collected. To do this, we first determined whether the isotopic signatures of food sources available to crabs in each area (Table 2) were related to estuary-specific N loads (Table 1) and then discerned whether these signatures were, in turn, conveyed to horseshoe crabs (Table 2).

Isotopic signatures of potential foods: The δ^{15}N values of organic matter in seston and sediment and in tissue of animal taxa that may be food for horseshoe crabs increased significantly as land-derived N loads to the subestuaries increased (Table 3, Figs. 2 and 3). Each group of potential food items responded similarly to increasing N load, having common slopes [test for homogeneity of slopes (Sokal and Rohlf, 1981) $F = 1.94$ ns] (Figs. 2 and 3). The differences in land-cover on watersheds of the different subestuaries of Pleasant Bay exposed the producers and consumers in the receiving subestuaries to different N loads, and their isotopic signatures accordingly varied with load (Figs. 2 and 3). These responses are consistent with earlier studies that found heavier isotopic signatures in estuaries receiving higher N loads (McClelland et al., 1997; Voss and Struck, 1997; McClelland and Valiela, 1998b; Waldron et al., 2001; Evgenidou and Valiela, 2002; Weiss et al., 2002; Shriver et al., 2002; Mayer et al., 2002; Cole et al., in press).

The magnitude of response to increasing N load across estuaries differed among food groups. Sediment δ^{15}N values were significantly lighter than seston (Fig. 2 and Table 3), and mean δ^{15}N values of animal taxa were an average of 3.5 ‰ heavier than δ^{15}N in organic matter from sediment and seston (Figs. 2 and 3). This difference is consistent with expected mean N fractionation from food sources to consumers for whole food webs

Table 3
P-values from Fishers's PLSD ($\alpha = 0.05$) analysis of covariance post-hoc test comparing the regressions (Figs. 2 and 3) of δ^{15}N isotopic signatures of consumers, seston, and sediment against N load (ANCOVA: $F = 39.87$, $P < 0.0001$)

	Taxa						Organic matter	
	Crustaceans	Gastropods	*M. arenaria*	*M. mercenaria*	Other bivalves	Polychaetes	Seston	Sediment
Crustaceans		−0.84	0.57	<0.0001	0.64	<0.0001	<0.0001	<0.0001
Gastropods	–	–	0.74	0.0005	0.54	0.0001	<0.0001	<0.0001
M. arenaria	–	–	–	0.002	0.33	<0.0001	<0.0001	<0.0001
M. mercenaria	–	–	–	–	<0.0001	0.45	<0.0001	<0.0001
Other bivalves	–	–	–	–	–	<0.0001	<0.0001	<0.0001
Polychaetes	–	–	–	–	–	–	<0.0001	<0.0001
Seston	–	–	–	–	–	–	–	0.0009

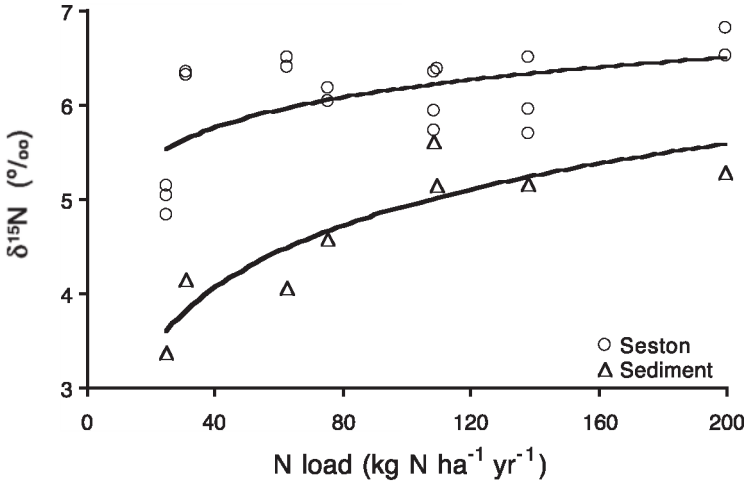


Fig. 2. δ^{15}N of organic matter from seston and sediment samples collected at Nauset Beach and from seven subestuaries of Pleasant Bay compared to total N loading rate to each area. For seston, each point represents a single sample, and each site was sampled two to three times. Sediment points represent a composite of three samples from each site (seston: $y=0.46\ln(x)+4.07$, $R^2=0.36$, $F_{reg}=8.98$, $P<0.01$; sediment: $y=0.94\ln(x)+0.59$, $R^2=0.80$, $F_{reg}=24.32$, $P<0.01$).

(Vander Zanden and Rasmussen, 2001), suggesting organic matter was food to many animal taxa in Pleasant Bay.

There was considerable scatter in the regressions of δ^{15}N in animal tissue with N load (Fig. 3). One source of variation was that relationships between δ^{15}N and N load were taxon-specific. Among bivalves there were sufficient data on individual species across locations to allow regression of individual species against N load. These regressions show that mean δ^{15}N signatures of *Mercenaria* mercenaria were consistently heavier than those of *Mya arenaria* or other bivalves (Table 3 and Fig. 3, top left). These findings are consistent with data from other Cape Cod estuaries of different N load (Carmichael et al., unpublished), and suggest species-specific differences in trophic fractionation. Similar variation was found among crustaceans, with *Gammarus oceanicus* lighter than other crustaceans and *Cirolana polita* having among the highest δ^{15}N values (Fig. 3, bottom left).

Some of the differences in δ^{15}N values between species were likely related to differences in trophic position of taxa, with primary consumers having lighter signatures than predators. For example, the polychaete, *P. gouldii*, a deposit-feeding herbivore (Gordon, 1966), had substantially lighter δ^{15}N signatures than the predatory (Redmond and Scott, 1989) polychaete *Nephtys* spp. (Fig. 3, bottom right). Despite this variation, each regression curve in Fig. 3 describes a significant relationship between δ^{15}N of potential food items for horseshoe crabs and the specific subestuaries where they were collected. These relationships, in turn, provide a tool to potentially link horseshoe crabs to their foraging locations in Pleasant Bay through their food supply.

Isotopic signatures of horseshoe crabs: Horseshoe crabs collected from different subestuaries bore different isotopic signatures (Table 2 and Fig. 4). The δ^{15}N of horseshoe

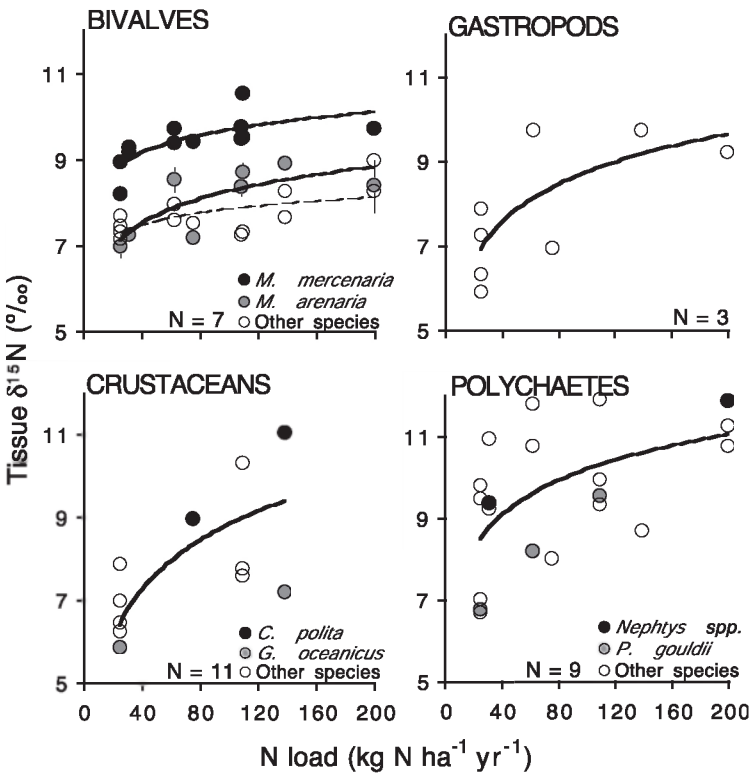


Fig. 3. δ^{15}N of tissue from bivalves, gastropods, crustaceans, and polychaetes collected from Nauset Beach and seven subestuaries of Pleasant Bay compared to total N loading rate to each area. Each point represents a single sample composed of many individuals, except for *M. arenaria*, where mean values are shown for clarity. N = number of species. Error bars show standard error. Where no error bars are visible, error was smaller than the symbol. (– –) represents regression for other bivalve species combined. (*M. mercenaria*: $y = 0.58\ln(x) + 7.02$, $R^2 = 0.54$, $F_{reg} = 11.96$, $P < 0.05$; *M. arenaria*: $y = 0.81\ln(x) + 4.53$, $R^2 = 0.60$, $F_{reg} = 8.88$, $P < 0.05$; other bivalves: $y = 0.39\ln(x) + 6.08$, $R^2 = 0.38$, $F_{reg} = 6.61$, $P < 0.05$; gastropods: $y = 1.29\ln(x) + 2.81$, $R^2 = 0.54$, $F_{reg} = 6.96$, $P < 0.05$; crustaceans: $y = 1.73\ln(x) + 0.89$, $R^2 = 0.52$, $F_{reg} = 12.05$, $P < 0.01$; polychaetes: $y = 1.15\ln(x) + 4.90$, $R^2 = 0.33$, $F_{reg} = 9.86$, $P < 0.01$).

crab tissue increased significantly with increasing N load to estuaries receiving up to 109 kg N ha^{-1} year^{-1} (Table 2 and Fig. 4). In subestuaries subject to higher N loads, δ^{15}N values in tissue of crabs were comparable to or lower than δ^{15}N of crabs caught in the lowest load estuaries (Fig. 4). These findings suggest that horseshoe crabs were either specifically selecting lighter foods at higher N loads or that crabs did not feed enough within these highest load estuaries to acquire signatures of foods from those estuaries.

We can further assess the plausibility of these two options by comparing the mean fractionation from potential food sources to horseshoe crab tissue across sites (Fig. 5). To calculate fractionation, we subtracted the mean δ^{15}N value of each taxonomic group from the mean δ^{15}N value in horseshoe crab tissue (data from Table 2) for each location in Pleasant Bay (Fig. 5). Since consumers are typically enriched 2‰ to 4‰

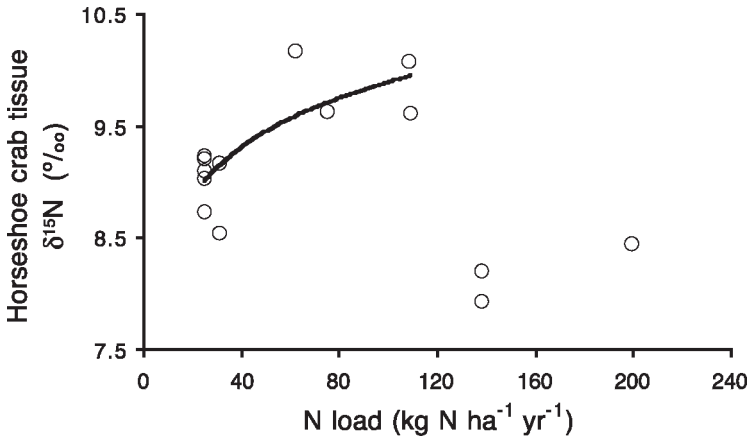


Fig. 4. δ^{15}N signatures of tissue sampled from adult horseshoe crabs at Nauset Beach and seven subestuaries of Pleasant Bay compared to total N loading rate to each area. Each point represents a composite of three or more crabs from each area, $y = 0.63\ln(x) + 6.97$, $R^2 = 0.60$, $F_{reg} = 13.66$, $P < 0.01$.

compared to their food sources, fractionation outside this range indicates crabs fed on different foods or in different locations. The dotted lines in Fig. 5 show this range of expected fractionation for food items consumed by horseshoe crabs across locations in Pleasant Bay. As already suggested by the data in Fig. 4, horseshoe crabs fed in a fashion consistent with known fractionation within locations in Pleasant Bay receiving <110 kg N ha^{-1} year^{-1}, but seemed to avoid feeding on most prey from estuaries exposed to higher N loads, indicated by negative mean fractionation relative to isotopic signatures of animal taxa (Fig. 5, top) and lower fractionation from organic matter in seston and sediment (Fig. 5, bottom) in these locations.

These data further suggest it is not likely that crabs fed selectively on isotopically lighter foods at higher N loads. First, horseshoe crabs did not show preferences for specific foods among other estuaries (Fig. 5). Second, to obtain the δ^{15}N in tissues acquired by crabs in the highest loaded estuaries, these animals would have had to forage almost exclusively on bulk sediment and seston particles (Fig. 5, bottom). This type of foraging would require a level of selectivity and dexterity, for example, to separate other food items from sediment particles, that is unrealistic and unlikely given horseshoe crab anatomy and behavior (Shuster, 1982; Botton, 1984a,b). Third, gut contents corroborate that crabs feed broadly and, if selective, would tend to choose animal prey that would be heavier than seston or sediment (Botton, 1984a,b; Botton and Haskin, 1984). Hence, the most reasonable explanation of our findings is that crabs sampled in the two estuaries receiving the highest N loads did not substantially forage in those estuaries.

We cannot be certain why crabs did not prefer the most highly N loaded locations in Pleasant Bay. Increased N loads are associated with reduced water clarity, increased hypoxia, and loss of commercial fisheries species (Valiela et al., 1992, 1997; Smith et al., 1999; Breitburg, 2002). Changes in water quality associated with increased N loads could, therefore, make more highly loaded estuaries less hospitable to horseshoe crabs. The highest load estuaries in Pleasant Bay were not significantly different from other

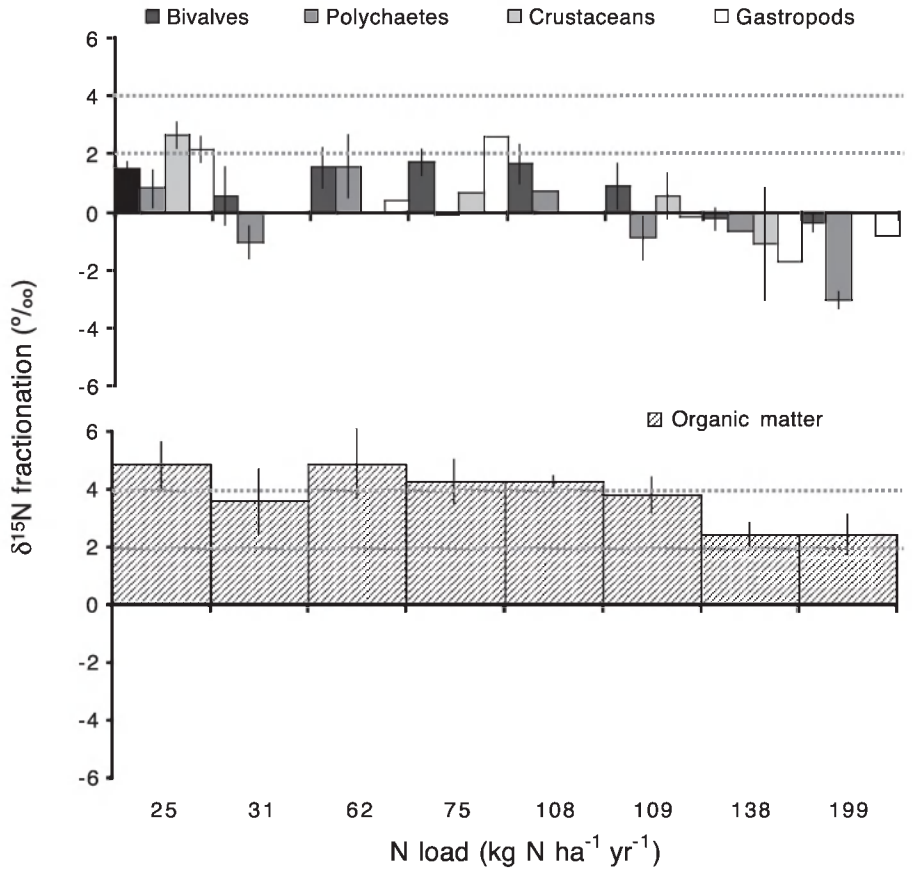


Fig. 5. Mean ± standard error of δ^{15}N fractionation from food sources to horseshoe crab tissue across locations of different N loading rates in Pleasant Bay. Fractionation was calculated by subtracting the mean δ^{15}N signature of each food group from the mean δ^{15}N signature of horseshoe crab tissue from each location, using data from Table 2. Food sources include a variety of species of bivalves, polychaetes, crustaceans (top panel), and organic matter from seston and sediment (bottom panel). Dotted gray lines delineate the range of expected δ^{15}N fractionation (+2–4‰) for one trophic step.

subestuaries in salinity, temperature, depth, or proximity to salt marsh, mooring areas, and shoreline structures such as docks (RMP, 1998; Carmichael, unpublished). Highly loaded sites may have other features that parallel N load and deter horseshoe crab foraging.

To verify that the increase in δ^{15}N signatures in tissue of adult horseshoe crabs collected from estuaries receiving <110 kg N ha^{-1} year^{-1} was indeed related to N load and not differences in crab size, we compared horseshoe crab size to δ^{15}N values in resident juveniles and adults (Fig. 6). Although the mean δ^{15}N values in crab tissue differed among estuaries (Fig. 6 and Table 2), the mean size of adult crabs sampled was not different (ANOVA: $F=0.44$, $P=0.81$), indicating that the differences in δ^{15}N values in crabs caught in different subestuaries did not result from differences in their size. The size

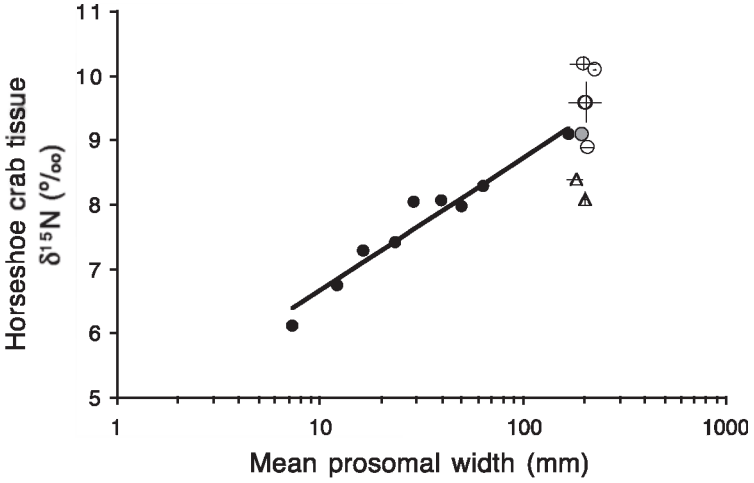


Fig. 6. Change in δ^{15}N signatures of horseshoe crabs with increasing prosomal width. Data points correspond to juveniles ~ 7–168 mm in size (●) and adults (●) collected at Nauset Beach, and adults collected from 7 other locations in the Pleasant Bay system (open symbols), separated to show where crabs foraged (○) and where foraging did not occur (△). Error bars show standard error. Model II regression yielded a best-fit line to the Nauset Beach juvenile data, $y = 0.89\ln(x) + 4.62$, $R^2 = 0.95$ $F_{reg} = 162.08$, $P < 0.001$.

range of adult crabs measured in this study (170–242 mm) also was representative of the size range of most adult crabs in Pleasant Bay (Carmichael et al., 2003).

The δ^{15}N signatures of young crabs that were actively feeding and molting at Nauset Beach increased as they aged (Fig. 6), suggesting they sought larger prey and moved up trophic steps as they grew (Gaines et al., 2002). Adult crabs sampled from the same location had δ^{15}N values (Table 2) that closely matched values expected by extrapolating δ^{15}N signatures of juveniles to adults (Fig. 6). This finding verifies that adult crabs collected from Nauset Beach likely foraged there as well. A key difference among these data is that δ^{15}N values in tissues of adults from other estuaries did not match the value expected for Nauset Beach adults (Fig. 6). If adult horseshoe crabs were foraging broadly among different estuaries throughout the Bay, δ^{15}N values in their tissues would likely not differ, but deviate toward a mean value representative of Pleasant Bay as a whole. Furthermore, we would expect juveniles from other locations in Pleasant Bay to have signatures producing regressions that would parallel those observed among Nauset Beach crabs, but reflecting the heavier isotopic signatures in foods available in those estuaries (Table 2, Figs. 2 and 3). An adequate number of juveniles of different sizes were not found in other subestuaries, however, to make these comparisons possible. These data suggest that although adult horseshoe crabs may travel great distances, they tend to remain and forage in a circumscribed area if conditions are favorable.

There are several possible explanations for the relatively light isotopic signatures in crabs from the two highest load estuaries (Table 2, Figs. 4 and 6). These crabs may have foraged indiscriminately in lower N load locations throughout the Bay since their δ^{15}N values are comparable to those of some crabs sampled from Nauset Beach (Fig. 4). They may also have foraged from a location in the Bay that we did not sample, but that supports

foods with even lighter isotopic signatures. It is also possible that these crabs migrated into Pleasant Bay from other locations. It seems implausible, however, that the majority of horseshoe crabs in Pleasant Bay foraged substantially offshore or even from areas outside the local subestuaries from which they were collected since the isotopic signatures of most crabs we sampled paralleled food sources available in individual subestuaries, with the appropriate trophic fractionation.

The isotopic link between horseshoe crabs and local subestuaries suggests two possible foraging habits. First, adult crabs may do most of their foraging in inshore estuaries in the spring, rather than foraging substantially offshore during the winter months. Gut contents of adult crabs sampled from spawning beaches have been found to hold the largest quantity of food in the fall and early winter (Botton, 1984a), with the quantity of food in the gut decreasing as crabs moved offshore (Botton and Ropes, 1989). These observations suggest adult crabs that migrate offshore may forage heavily from near-shore areas before leaving for the winter. A similar pattern of foraging is reported in lobsters (Lawton and Lavalli, 1995). Second, adult crabs may remain in the Bay throughout the year. Adult crabs may bury themselves shallowly in sediments of local estuaries during winter months, rather than migrate offshore (Widener and Barlow, 1999). Most importantly, regardless of strategy, most adult horseshoe crabs foraged substantially within local subestuaries to allow assessment of their trophic position and diet composition.

3.2. Food web position and diet composition

To compare $\delta^{13}C$ and $\delta^{15}N$ signatures of crabs and potential prey species within Pleasant Bay, we focused our analysis on the estuaries in which the $\delta^{15}N$ signatures of horseshoe crabs increased significantly with N load (Fig. 4). We stratified our analysis by comparing signatures of horseshoe crabs and prey collected within the two estuaries subject to the lowest loads separately from those in the four remaining higher load estuaries (Table 4). We chose this stratification because the $\delta^{15}N$ signatures of crabs collected from the two estuaries of lowest N load were not significantly different from each other ($t=0.94$, $P=0.39$), but the mean $\delta^{15}N$ signature of crabs from the combination

Table 4
Mean (± standard error) of $\delta^{15}N$ and $\delta^{13}C$ signatures (‰) in tissue, estimated diet[a], and feces of crabs from estuaries that we stratified[b] into lower and higher N load categories

	Estuary							
	Nauset Beach	Crows Pond	Kescayo-Gansett Pond	Pahwah Pond	Quanset Pond	Ryder's Cove	Arey's Pond	Round Cove
N load	Lower		Higher				Excluded	
	N	C	N	C				
Tissue	9.0 (0.2)	−16.1 (0.5)	9.9 (0.2)	−16.3 (0.2)			–	
Diet	6.0	−17.1	6.9	−17.3			–	
Feces	5.7 (0.6)	−16.1 (2.2)	–	–			–	

[a] Signatures of diet were calculated by subtracting 3‰ for N and 1‰ for C from the mean isotopic signatures of horseshoe crab tissue.

[b] N load stratification was based on relationships between N load (Table 1) and $\delta^{15}N$ of horseshoe crabs in Fig.

of these two estuaries was significantly different from the mean δ^{15}N value of crabs collected among the four higher load locations ($t=5.05$, $P \leq 0.0001$) (Table 2).

To be certain that we weighted the contribution of each site equally when calculating mean signatures of potential prey and horseshoe crab tissue in our stratified groups, we calculated mean δ^{13}C and δ^{15}N signatures for each site where prey and crabs were sampled more than once. We then averaged values among sites for the two lower load locations and the four higher load locations separately to create our stratified groups (Table 4).

Comparing isotopic signatures of horseshoe crabs to available foods: Isotopic signatures in tissue of horseshoe crabs differed in N but not in C between lower and higher N load groups. Mean δ^{15}N signatures in horseshoe crab tissue were significantly different ($t=4.00$, $P=0.02$) between stratified lower and higher N load sites (Table 4). After stratification, the magnitude of difference in δ^{15}N signatures in potential prey between lower and higher N load sites was reduced, but still generally heavier at higher N load sites (Table 5), suggesting that differences in N isotopic signatures related to N load were transferred to horseshoe crabs through their diet. Mean δ^{13}C signatures of crab tissue did not differ between lower and higher load sites (Table 4) ($t=-0.50$, $P=0.67$), suggesting that the source of carbon supporting their food supply did not change significantly across foraging sites.

The δ^{13}C and δ^{15}N signatures of *Littorina littorea* in lower load areas and *Tagelus plebius* and *Gemma gemma* among higher load sites were most similar to signatures predicted for horseshoe crab diet (Table 4 and Fig. 7), but few other prey species had isotopic values close to those expected for crab diet (Fig. 7). These findings imply that horseshoe crabs in Pleasant Bay ate a mixed diet, regardless of N load.

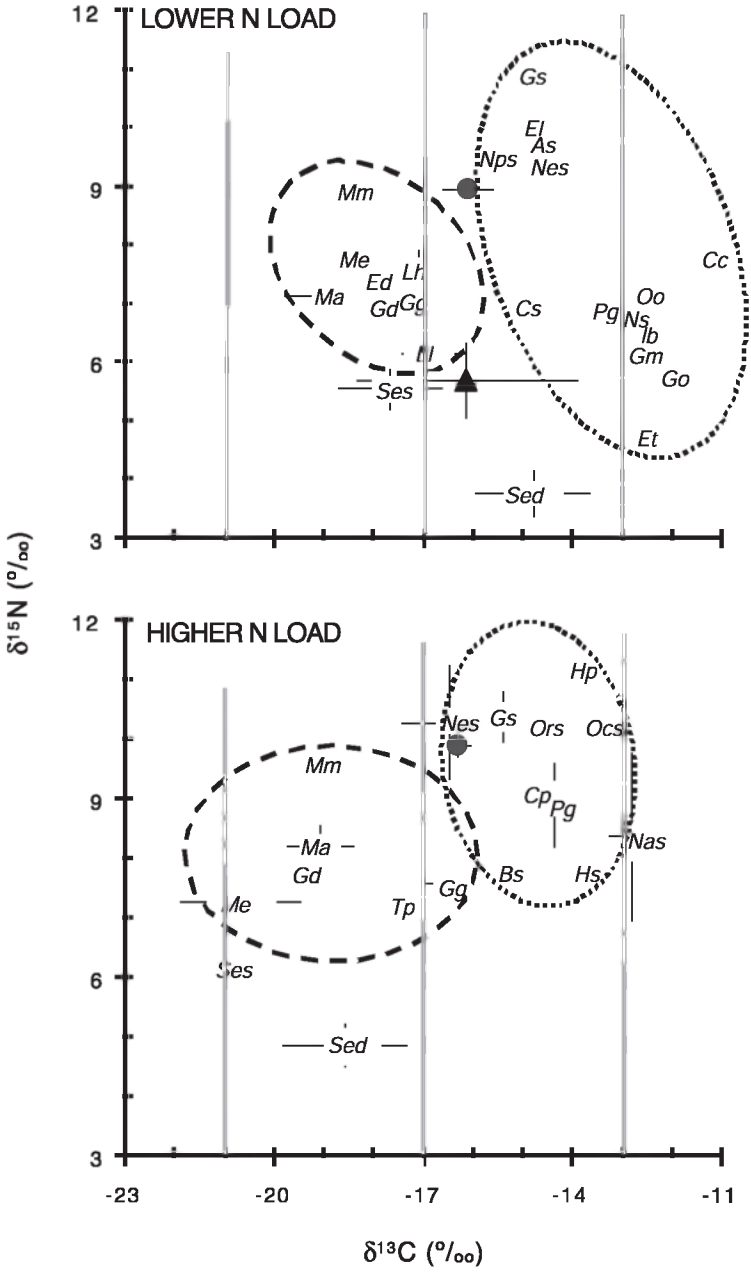
The vertical grey lines at $-21‰$, $-17‰$ and $-13‰$ δ^{13}C in Fig. 7 represent established mean carbon signatures of marine phytoplankton, macroalgae, and *Spartina alterniflora* (salt marsh cord grass), respectively (Peterson and Fry, 1987; McClelland and

Table 5
t-Statistics and *P*-values from comparisons of δ^{15}N and δ^{13}C signatures of individual and groups of potential foods for horseshoe crabs between sites of lower and higher N loads in Pleasant Bay

	Comparison between higher and lower N load groups			
	δ^{15}N		δ^{13}C	
	t[a]	P	t[a]	P
Individual prey taxa				
M. arenaria	2.04	0.11	−0.19	0.86
M. mercenaria	2.92	0.02*	−0.92	0.38
Nereis spp.	0.63	0.57	−0.92	0.41
Food groups				
Bivalves	1.20	0.24	−1.30	0.20
Crustaceans	3.66	0.005**	−2.86	0.02*
Polychaetes	1.61	0.13	−2.14	0.13
Seston	2.31	0.04*	−2.95	0.02*
Sediment	1.97	0.12	−1.89	0.13
All foods	2.21	0.03*	−1.73	0.09

[a] Positive and negative *t*-statistics indicate heavier and lighter isotopic signatures, respectively, at higher N load sites. Significant (*) and highly significant (**) differences are indicated for clarity.

Valiela, 1998b; Kang et al., 1999). The $\delta^{13}C$ values of horseshoe crabs suggest that crabs in lower and higher load sites could have either consumed a diet supported largely by macroalgal biomass (Fig. 7) or by a mixture of phytoplankton and *Spartina* since either



option could result in a $\delta^{13}C$ signature near −17‰. To determine which option was more likely, we further assessed the $\delta^{13}C$ composition of potential food sources.

The $\delta^{13}C$ values in organic matter from seston and sediment shifted toward more depleted signatures at sites receiving higher N loads. $\delta^{13}C$ values of organic matter in seston from lower and higher load areas fell in the range of $\delta^{13}C$ in marine phytoplankton and macroalgae, but had a significant shift closer to phytoplankton signatures at higher loads (Fig. 7). The $\delta^{13}C$ signature of sediment organic matter also shifted, but from a value reflecting *Spartina* in lower load areas (Fig. 7, top) to one representative of phytoplankton and macroalgae at higher N loads (Fig. 7, bottom). This shift may result from increased algal production in the water column and at the sediment surface, typically associated with increased N loads because N is the primary nutrient limiting production in marine coastal waters (Goldman, 1975; Valiela et al., 1992; Prins et al., 1999; Smith et al., 1999). Similar shifts in $\delta^{13}C$ signatures of organic matter have been observed between sites of different N load (McClelland and Valiela, 1998b). This shift may be propagated up the food web to prey species of horseshoe crabs at higher loads since relatively more food is available from phytoplankton and macroalgae compared to *Spartina*.

Isotopic signatures of available food items sorted into groups based primarily on $\delta^{13}C$ signatures (Fig. 7). Mollusks (bivalves and gastropods) from lower and higher N load areas had $\delta^{13}C$ signatures indicating they primarily assimilated phytoplankton and macroalgal food sources (Fig. 7, dashed ovals), while $\delta^{13}C$ values of polychaetes and crustaceans in both areas clustered more near the signature of *Spartina* (Fig. 7, dotted ovals). The $\delta^{13}C$ signature of the gastropod *Nassarius* spp. did not fall in the range of $\delta^{13}C$ values of other mollusks, having a $\delta^{13}C$ signature similar to *Spartina* (Fig. 7, bottom). These findings are largely consistent with known feeding habits of these species (Gordon, 1966; McDermott, 1987; Kamermans, 1994; Ruppert and Barnes, 1994; Creach et al., 1997; Redmond and Scott, 1989; Kang et al., 1999). These findings demonstrate that prey available to horseshoe crabs in Pleasant Bay were supported by a combination of phytoplankton, macroalgae, and *Spartina*, and perhaps explain why $\delta^{13}C$ values of estimated horseshoe crab diet (Table 4) were intermediate among $\delta^{13}C$ of these producers.

$\delta^{13}C$ signatures of consumer groups also generally shifted toward lighter values (those more representative of macroalgae and phytoplankton) at higher load sites (Fig. 7,

Fig. 7. Mean $\delta^{15}N$ and $\delta^{13}C$ signatures of horseshoe crab tissue (●) and feces (▲) compared to mean signatures of potential food items from locations stratified into groups of lower (top) and higher (bottom) N loads in Pleasant Bay. No feces were obtained from crabs in higher load sites. Solid gray bars show $\delta^{13}C$ values of marine phytoplankton (−21‰), macroalgae (−17‰), and Spartina (−13‰), respectively. Food items are labeled by abbreviation (Table 2). Each point is a composite of several individuals. Error bars show standard error, and where no error bars are visible, error is smaller than the symbol or only one sample is represented. For clarity, dashed ovals indicate groupings of gastropods and bivalves, and dotted ovals show crustaceans and polychaetes. Each potential food item is given a two-letter abbreviation (in parentheses): *Ensis directus* = *Ed*, *Geukensia demissa* = *Gd*, *Gemma gemma* = *Gg*, *Mya arenaria* = *Ma*, *Mytilus edulis* = *Me*, *Mercenaria mercenaria* = *Mm*, *Tagelus plebius* = *Tp*, *Byblis serrata* = *Bs*, *Caprella* spp. = *Cs*, *Chiridotea coeca* = *Cc*, *Cirolana polita* = *Cp*, *Edotea triloba* = *Et*, *Gammarus mucronatus* = *Gm*, *Gammarus oceanicus* = *Go*, *Haustorius* spp. = *Hs*, *Idotea baltica* = *Ib*, *Orchestia* spp. = *Ocs*, *Orchomonella spp.* = *Ors*, *Littorina littorea* = *Ll*, *Lunatia heros* = *Lh*, *Nassarius* spp. = *Nas*, *Ampharete* spp. = *As*, *Eteone longa* = *El*, *Glycera* spp. = *Gs*, *Neanthes succinea* = *Ns*, *Nephtys* spp. = *Nps*, *Nereis* spp. = *Nes*, *Ophelia* spp. = *Os*, *Orbinia ornata* = *Oo*, *Pectinaria gouldii* = *Pg*, *Haloclava producta* = *Hp*, *Sediment* = *Sed*, *Seston* = *Ses*.

bottom), following the $\delta^{13}C$ shift in organic matter at the base of the food web. Although there were not a sufficient number of species common between sites to make statistical comparisons for most individual species, six of the eight common species showed a shift in $\delta^{13}C$ toward lighter values at higher N loads (Fig. 7, *Me*, *Mm*, *Gd*, *Nes*, *Gs*, *Pg*). This shift was also significant for crustaceans as a group (Table 5). These results suggest that an important effect of N inputs to subestuaries of Pleasant Bay was to change food web structure of available prey by prompting a change in carbon source through increased algal primary production.

Although the source of carbon in horseshoe crab food sources appears to have shifted toward primarily algal sources at higher N loads, signatures of horseshoe crab tissue, however, differed only in $\delta^{15}N$ (Fig. 7). There are several possible reasons why $\delta^{13}C$ signatures of horseshoe crab tissue did not shift to follow $\delta^{13}C$ values of available food sources. First, horseshoe crab diet may be so mixed as to represent the mean isotopic signature of most available food sources, rather than individual or groups of similar species. Comparing between stratified lower and higher N load sites, the mean $\delta^{15}N$ value of all available prey was heavier at higher N loads (8.5 ± 0.4‰ compared to 7.4 ± 0.3‰ in lower load sites, Table 4), and this difference was apparently transferred up the food web to horseshoe crabs. In contrast, mean $\delta^{13}C$ of all prey did not differ significantly between sites (-16.5 ± 0.6‰ at high loads compared to -15.2 ± 0.5‰ at low load sites, Table 5) and, in turn, $\delta^{13}C$ did not differ in horseshoe crab tissues across stratified locations. Hence, the composite of available foods in each location may have been more important to assimilated diet of horseshoe crabs than individual species or groups of similar species. Second, there may be additional rare or patchily distributed food items that have not been accounted for because they were not encountered during sampling.

Comparing horseshoe crab feces to estimated diet: Isotopic signatures in horseshoe crab feces were similar to estimated diet (Table 4 and Fig. 7, top), suggesting that horseshoe crabs may not have assimilated a large fraction of the food they ingested. Values for fractionation from horseshoe crab diet to feces fell within the range reported for other

Table 6
Mean ± standard deviation of fractionation (enrichment or depletion) of $\delta^{15}N$ and $\delta^{13}C$ signatures in feces compared to diet for horseshoe crabs sampled during this study and for several other species

Class	Species	Fractionation from diet to feces		Source
		$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	
Merostomata	*L. polyphemus*	-0.3 ± 1.9	$+0.9 \pm 2.2$	This study
Bivalve	*M. mercenaria*	-2.7 ± 0.5	–	Carmichael, unpubl.
Bivalve	*M. arenaria*	-3.5 ± 1.6	–	Carmichael, unpubl.
Crustacean	*Euphausia superba*	-2.1 ± 0.6	-0.6 ± 1.6	Schmidt et al., 2003
Crustacean	*Temora longicornis*	+8.0	−4.3 to −11.3	Checkley and Entzeroth, 1985; Klein Breteler et al., 2002
Crustacean	*Mysis mixta*	+3.4	+1.4	Gorokhova and Hansson, 1999
Crustacean	Various zooplankton	$+2.2 \pm 0.2$	–	Altabet and Small, 1990
Gastropod	*Helix adspersa*	–	± 0.5	DeNiro and Epstein, 1978
Mammal	*Bos taurus*	$+1.9 \pm 0.3$	–	Steele and Daniel, 1978
Teleost	*Dicentrarchus labrax*	-5.4 ± 2.4	-2.0 ± 1.3	Franco-Nava et al., 2002

species but were most similar to those reported for a herbivorous (Schmidt et al., 2003) euphausiid (Table 6). Fractionation of isotopes from diet to feces differs among species (Table 6) and is likely due to differences in assimilation and metabolism (Montoya et al., 1992; Gorokhova and Hansson, 1999; Vander Zanden and Rasmussen, 2001; Klein Breteler et al., 2002). Similarity in fractionation among species, therefore, may represent similarity physiological processing of foods. Since plant matter and detritus are assimilated relatively inefficiently (Valiela, 1995), our data on feces suggest the diet of horseshoe crabs sampled in Pleasant Bay included a substantial amount of relatively poor quality organic matter. Correspondingly, vascular plant material and large quantities of sediment have been found among gut contents of horseshoe crabs (Botton, 1982; 1984a), which bioturbate sediments while foraging (Rudloe, 1985; Kraeuter and Fegley, 1994; Commito et al., 1995). Although not efficiently assimilated, organic matter should not be disregarded as a potentially important food source since it includes amino sugars that are a component of chitin (Benner and Kaiser, 2003), a major portion of horseshoe crab carapace (Lafon, 1941).

Variation in $\delta^{13}C$ signatures of horseshoe crab feces spanned $\delta^{13}C$ values of tissue from bivalves, gastropods, polychaetes, and crustaceans (Fig. 7, top). Since changes in horseshoe crab diet would be reflected in feces more quickly than in tissue (Schmidt et al., 2003), this finding suggests that recent diet (represented by feces) was varied and supported by marine phytoplankton, macroalgae, and *Spartina*-based food webs, similar to longer-term diet (represented by tissues). This similarity between $\delta^{15}N$ values of horseshoe crab feces and estimated diet is also consistent with our assessment that these horseshoe crabs foraged primarily in localized areas.

These data provide a better understanding of where horseshoe crabs forage and how they may use locally available food sources. Our findings are novel in demonstrating that horseshoe crabs may be loyal to specific foraging sites within estuaries and in providing an assessment of foods actually assimilated by horseshoe crabs. Adult horseshoe crabs loyal to local foraging sties consumed a mixed diet regardless of differences in composition of prey species and producers supporting the food webs in the different locations. These results agree with gut content analyses that suggest horseshoe crabs are dietary generalists (Botton, 1984a; Botton and Haskin, 1984; Botton and Ropes, 1989), but further suggest the composite of available foods were more important to assimilated diet of horseshoe crabs than individual taxa and that horseshoe crabs may consume foods that are poorly assimilated. Successful conservation and culture of horseshoe crabs, therefore, may depend on assuring high water quality, ample sources of particulate organic matter, and a range of habitats that support phytoplankton, macroalgae, and salt marsh production to sustain horseshoe crabs and the variety of prey they consume.

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