Contrasting Trophic Niche and Resource Use Dynamics Across Multiple American Horseshoe Crab (*Limulus polyphemus*) Populations and Age Groups

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Abstract

Horseshoe crabs (*Limulus polyphemus*) exhibit ontogenetic and geographic variability in migratory patterns, yet the implications of movement on their resource use dynamics remain poorly understood. Here, we evaluate horseshoe crab resource use across ontogeny (instars 14–18 and adults), sex, seasons, and between two spatially distinct populations that exhibit different migratory patterns. Multi-tissue stable isotope analyses of carbon, nitrogen, and sulfur were used to examine (1) ontogenetic and sex-specific variability in the reliance of resource pools; (2) variation in isotopic niche breadth within and among populations; and (3) seasonal variability in resource use within juvenile life stages. We found evidence for subtle ontogenetic shifts in resource use, with increased contributions of phytoplankton with ontogeny, but all age groups predominantly relied on sedimentary organic matter (SOM) pools. Sex-specific differences in resource use were observed with SOM serving as the predominant source assimilated in males, while females exhibited a higher dependence on phytoplankton resource pools, indicating foraging or spatial preferences may be different between sexes. Low trophic niche overlap between adult and juvenile horseshoe crabs was evident, likely arising from differences in migratory patterns and size constraints. Within juveniles, no seasonal differences in resource use were observed, indicating that dietary patterns may remain static across temporal scales, consistent with limited dispersal of that life stage. Spatial differences in resource use were, however, observed between adult crabs likely reflecting the migratory strategies of different populations. Our results are consistent with previous evidence that horseshoe crabs are dietary generalists but provide novel insights into the linkages between movement and trophic patterns.

Keywords Horseshoe crabs · Trophic ecology · Space use · Ontogeny · Stable isotopes · *Limulus polyphemus*

Introduction

The Atlantic horseshoe crab (*Limulus polyphemus)* is a commercially important benthic invertebrate that is widely distributed throughout the eastern coast of North America (Maine, USA, to Mexico). Their ongoing coastwide decline has led to their "vulnerable" conservation status (Smith et al. 2016). Despite the decline, there is a limited understanding of critical subtidal habitats that support the horseshoe crabs throughout their range. Variability in movement, dispersal, and access to habitat composition among conspecifics can often engender disparities in resource use dynamics (Rosenblatt et al. 2015). For example, differences in trophic characteristics and foraging habits have been observed in organisms with moderate biogeographic extents due to spatiotemporal variability in resource availability (Correa and Winemiller 2014) and disparities in spatial mobility (Shipley

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et al. 2019). Therefore, evaluating the complexity of the trophic ecology and movement characteristics of a species is crucial for functional roles (Twining et al. 2020) and subsequently determining their plasticity to environmental perturbations (Korotkevich et al. 2018). This information is particularly important for motile consumers, such as horseshoe crabs, whose habitat use may vary across space and time (Polis et al. 1997). Examining the intricacies of trophic dynamics within this exploited species is critical to identify resource requirements and bolster conservation strategies (Cury et al. 2005).

Horseshoe crabs are generalist foragers, but their diet varies with ontogeny and location (Botton 1984; Botton and Haskin 1984; Botton and Ropes 1989; Carmichael et al. 2004). For example, stable isotope analysis has indicated that juvenile horseshoe crabs from instars 2–11 presumably rely on small crustaceans, polychaetes, and particulate organic matter (benthic and newly pelagic) in salt marsh habitats in Pleasant Bay, MA, USA (Carmichael et al. 2004). In contrast, adults primarily foraged on larger benthic macrofauna in adjacent, local estuaries and occupied a higher trophic level relative to the juveniles, and even relied on a mixture of organic matter pathways (phytoplankton and salt marsh production) indirectly through prey (Gaines et al. 2002; Carmichael et al. 2004; 2009). For larger immature (14+ instars) and mature crabs of both sexes residing on the continental shelf in the Mid-Atlantic, gut content analysis demonstrated that bivalves (< 7 mm in size) appeared to be the primary resource consumed by weight with secondary components comprised of a mixture of gastropods, polychaetes, crustaceans, and echinoids (Botton 1984; Botton and Ropes 1989). However, the contribution of sedimentary organic matter pathways assimilated across multiple life history stages remains to be addressed and may be an important energy source given that it is found in the guts from juveniles to adults (Botton and Ropes 1989; Carmichael et al. 2004). While these studies have provided great insight into the foraging habits of horseshoe crabs, the relationships between resource use and the spatial ecology of horseshoe crabs remain limited across temporal scales and ontogeny, particularly with respect to older juvenile stages (instars 12–18).

Horseshoe crab migratory structure varies throughout the NW Atlantic, notably between Mid-Atlantic and New England populations, and among life history stages. In the Mid-Atlantic, nearly half of adult horseshoe crabs migrate seasonally from the continental shelf to spawning beaches in local estuaries in the spring, and then subsequently migrate from spawning estuaries to the continental shelf in the fall (Smith et al. 2009; Bopp et al. 2021). In northern New England, adult horseshoe crabs are predominately resident in local estuaries year-round, with limited estuarine-shelf exchange (James-Pirri 2010).

Movement patterns also contrast among horseshoe crab life history stages in the same region. For instance, an acoustic telemetry study demonstrated that < 10% of older juvenile (12–18 instars) horseshoe crabs migrated between the continental shelf and local estuaries in the New York region; in contrast, adults exhibited 5-fold higher migration rates relative to juvenile counterparts within the same estuary (Bopp et al. 2021). Given these migratory differences, it may be appropriate to assume that intraspecific resource partitioning is occurring among demographics with disparate migratory modes (i.e., migratory, resident) (McCarthy and Waldron 2000).

Within conspecifics, ontogenetic shifts in resource use and space use may result in different trophic niche width characteristics among life history stages, and subsequently, engender variability in intraspecific trophic niche overlap (Hammerschlag-Peyer et al. 2011). Increased intraspecific competition can also arise when trophic niche breadth overlap coupled with space use overlap is high among age groups and can have profound impacts within populations, such as alterations in growth rates and amenability to environmental change (Sánchez-Hernández et al. 2019; Maguire et al. 2018). Quantifying the trophic niche patterns across ontogeny and among populations of horseshoe crabs could contribute towards an enhanced understanding of their ecological roles and habitat requirements, especially when coupled with previous knowledge of their space use patterns.

Here, we examined the horseshoe crab resource use patterns and trophic ecology between two populations in Long Island, New York (Moriches Bay adjacent to the Atlantic Ocean and West Meadow Beach connected to Long Island Sound), across ontogeny and between seasons using bulk stable isotope analysis of carbon, nitrogen, and sulfur. Specifically, we addressed the following hypotheses: 1) Horseshoe crabs will exhibit an increased reliance on phytoplankton energy pathways with age given their higher propensity to migrate onto the continental shelf with ontogeny; 2) Isotopic niche breadth will be higher for adults compared to juveniles because of their ability to access more resource pools via movement among estuarine and continental shelf habitats and thus, we predict low isotopic niche overlap will be present among age groups; 3) Mature females will exhibit contrasting resource use 142 patterns because of their larger body size relative to males; 4) Dietary shifts will not be present among seasons within juveniles; and 5) Adult horseshoe crabs in Moriches Bay will exhibit broader isotopic niche breadths relative to West Meadow crabs given their seasonal access to the Atlantic Ocean.

This work provides novel insight into the trophic characteristics of horseshoe crabs in the context of space use and ontogeny while building on previous efforts (Carmichael et al. 2004).

Fig. 1 Map of sampling locations on the north (West Meadow Beach, NY, left bottom panel) and south (Moriches Bay, NY, right bottom panel) shores of in Long Island, NY. Atlantic Ocean samples were collected within 3 nautical miles on the continental shelf just south of Moriches Bay and Fire Island, NY (denoted by dark blue box)
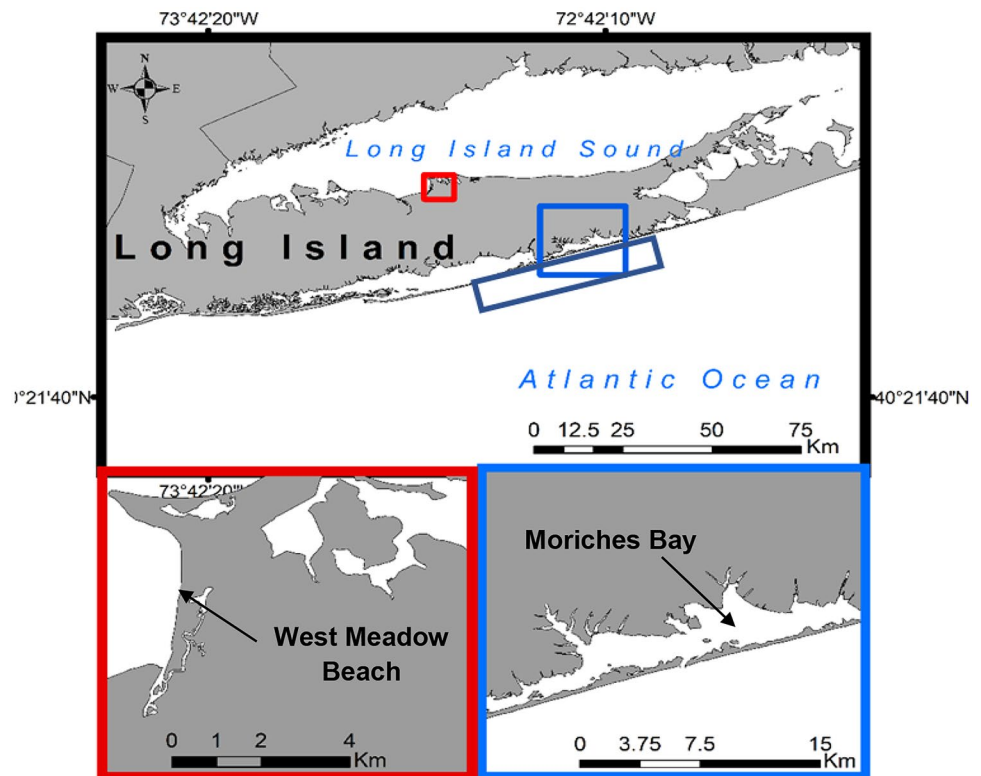
Methods

Study Site

Long Island, New York (40.8°N, 73.3°W; Fig. 1), is a heavily urbanized coastal island in the Northwestern Atlantic Ocean that extends 193 km and has > 500 km of coastline. Our study sites were Moriches Bay, which is a part of an interconnected series of lagoonal estuaries on Long Island's South Shore, and West Meadow Beach adjacent to Long Island Sound on the North Shore of Long Island (Fig. 1). Both Long Island Sound and Moriches Bay are highly productive estuaries primarily comprised of salt marsh, blue mussel beds, and intertidal sand/mud flat habitats (Carpenter and Brinkhuis 1991; Lopez et al. 2014).

Tissue Sampling and Preparation

Whole blood and muscle tissues were collected from adult horseshoe crabs and juvenile crabs by hand in Moriches Bay and West Meadow Beach (adults only). Several crabs were also sampled from the Atlantic Ocean opportunistically during an ongoing bottom trawl survey aboard the R/V Seawolf in June 2018 (Table 1). Upon capture, prosoma width (PW) was measured to the nearest 1.0 mm, weight was measured to the nearest 1.0 g, and sex was identified by the presence of modified pedipalps in adults (Table 1). In juveniles, sex was identified via gonopores underneath the operculum.

Whole blood (4–5 mL) was extracted from the pericardial membrane joint in between the prosoma and opisthosoma with luer-lock needles (18-guage and 24-gauge) and placed into BD Vacutainer vials (Franklin Lakes, New Jersey) lined with the blood anticoagulant sodium heparin (SH). Muscle tissues were obtained by removing soft tissue from all leg segments below the tibia on the third pair of walking legs (chitin exoskeleton was excluded). We sampled both whole blood and muscle because in many invertebrate taxa, these tissues typically have different turnover rates, and thus, reflect different temporal perspectives of a consumer's diet (VanderZanden et al. 2015). Whole blood comprises a faster turnover rate (1–4 months) relative to muscle (4–6 months) in several marine invertebrate arthropods (Suring and Wing 2009; DeVries et al. 2015). Therefore, we assumed that whole blood would serve as a seasonal proxy of diet compared to muscle.

To assess dietary components assimilated into horseshoe crab tissues, potential diet species of horseshoe crab were also collected and included invertebrate taxa from Gastropoda, Crustacea, Bivalvia, and Polychaeta and primary producers such as *Zostera marina*, *Spartina alterniflora*, *Ulva* spp., and *Rhodophyta* within the estuaries and Atlantic Ocean (See Table S1). Sedimentary organic matter (SOM) was also collected with a corer that collected the top 3 cm of sediment. Collected material was pushed through a 0.5-mm sieve to remove benthic macrofauna. Immediately following collection, all samples were

Table 1 Summary of the sample size, average prosoma width (PW) in millimeter, prosoma width range (mm), and sex ratios of each age group sampled within each location in 2018. Samples in May and June were collected < 4 weeks apart across all locations. M and F symbols denote males and females, respectively

Age group	Location	Months collected (n)	Mean PW (±SE)	PW range (mm)	Sex ratio (M:F)
Adult	Atlantic Ocean	June (M = 2, F = 5)	M = 217 F = 255 ± 42	M = 198–236 F = 205–309	1:3
	Moriches	May (M = 34, F = 26)	M = 197 ± 2.4 F = 258 ± 5.0	M = 173–232 F = 231–353	1.3:1
	West Meadow Beach	June (M = 34, F = 35)	M = 197 ± 2.1 F = 258 ± 2.0	M = 169–228 F = 229–282	0.97:1
Juvenile	Atlantic Ocean	June (M = 7, F = 4)	M = 153 ± 3.7 F = 255 ± 2.4	M = 151–176 F = 150–160	1.75:1
	Moriches	May + October (M = 36, F = 34)	M = 145 ± 3.5 F = 145 ± 4.4	M = 106–188 F = 104–192	1.05:1
	West Meadow Beach	-	-	-	-

transported on ice to Stony Brook University and were frozen at −20 °C until further analysis.

Samples for CNS analysis were rinsed with de-ionized water (except for whole blood), dried for 48 h at 60 °C, ground to a fine powder with a mortar and pestle, and weighed to the nearest 0.01 mg and were encapsulated in tin capsules (Elemental Microanalysis, Okehampton, UK). Prior to rinsing, drying, and grinding, SOM samples were treated with 0.1 M HCl to remove inorganic carbonates. Most individual diet items were processed separately; however, when the weight of individual organisms was insufficient, multiple individuals of the same species type were pooled in the sample. All samples were analyzed on a PDZ Europa 20–20 continuous flow isotope ratio mass spectrometer coupled with a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility at the University of California-Davis. Carbon, nitrogen, and sulfur isotope ratios are represented in delta notation (δ) and measured in permille (‰), as the final ratio values were relative to the international standards of Vienna Pee Dee Belemnite, air, and Vienna-Canyon Diablo Troilite, respectively (Peterson and Fry 1987). QA/QC was conducted by using the following in-house reference samples for C and N, amaranth flour, bovine liver, caffeine, chitin, glutamic acid, enriched alanine, keratin, and nylon powder, and for S, cysteine, hair, mahi-mahi muscle, taurine, and whale baleen. Machine error did not exceed 0.2‰, 0.3‰, and 0.4‰ for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively. The average difference (± standard error) for repeat samples was 0.05‰ (±0.009‰), 0.05‰ (±0.008‰), and 0.29‰ (±0.073‰) for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively.

To account for lipids being depleted in ^{13}C relative to carbohydrates or proteins, samples with C:N ratios that exceeded 3.5 were mathematically corrected for lipids following Post et al. (2007). C:N ratios were also evaluated in SOM to determine the sources (e.g., bacteria, algae, C_4 terrestrial plants, detritus) of its composition (Khan et al. 2015). SOM C:N values ranging from 2 to 10 suggest algal/ bacteria are the primary contributing sources, while C:N ratios > 40 usually indicate detrital sources are dominant in the sediment (Khan et al. 2015).

Statistical Analyses

Resource Use and Isotope Trends: Tissues, Size, and Location

To compare muscle isotope (C and N only) values between all locations, a Kruskal–Wallis (K-W) test was performed. If the K-W test was significant, a pairwise post-hoc Conover test was used to determine which locations were different. A Bonferroni correction for multiple comparisons was employed assuming a familywise error rate of $\alpha = 0.05$. Wilcoxon rank tests were used to compare muscle isotope values between juveniles and adults in Moriches Bay.

To determine if resource use trends varied across ontogeny, we employed linear regression models for all isotope values vs. prosoma width. Shapiro–Wilk tests and qqplots were used to ensure the data had a normally distributed error structure. If the assumptions of linear regression models (e.g., homogeneity of variance) were violated, non-linear regressions were performed.

To determine if isotope values differed between tissues within individual crabs, we used paired Wilcoxon rank-sum tests using the *PairedData* R package for each element analyzed (Champely 2018). Separate tests were conducted for juveniles and adults to determine if tissue differences were consistent within age groups. We also employed Spearman rank correlation tests between muscle and blood tissue stable isotope values within each geographic population to measure the strength of the relationship between the two

tissues. Separate correlation tests were employed for each geographic population to account for potential differences in isotope values from the combination of differences in vagility (Bopp et al. 2019), habitat availability, and isotopic baselines among regions throughout Long Island (Watson et al. 2018). If strong positive correlations were present between whole blood and muscle CNS values, we assumed tissue discrimination factors were nearly similar between tissues or there was lack of seasonal differences in resource use through time (Matich et al. 2010). When strong inter-tissue correlations were present, isotope values from muscle tissues were used in the estimation of trophic metrics given that muscle provides a long temporal perspective of assimilated resources in most animal taxa (VanderZanden et al. 2015).

Estimating the Dietary Contributions of Organic Matter

Bayesian mixing models were employed to estimate the proportional contribution of basal production sources assimilated into horseshoe crab diet across age and season. Bayesian mixing models incorporate stable isotope values to quantify the proportion of diet items that are assimilated into a consumer (Jackson et al. 2011; Parnell et al. 2013; Stock et al. 2018). Given that dietary trophic discrimination factors' (DTDFs) uncertainty can have profound effects on parameter estimation (Phillips et al. 2014), we conducted model sensitivity analysis by comparing parameter outputs of multiple model runs with different DTDFs applied to similar consumers (Cresson et al. 2016). DTDF values were derived from marine arthropod taxa, such as red rock lobster (*Jasus edwardsii*) and spiny lobster (*Panulirus cygnus*). Two DTDF scenarios were employed for carbon and nitrogen. For the first combination (DTDF scenario 1), mean and standard error values derived from red rock lobster for carbon and nitrogen fractionation were 0.84‰ ± 0.30 and 3.28‰ ± 1.0, respectively (Suring and Wing 2009). The second combination (DTDF scenario 2), derived from spiny lobster, was carbon and nitrogen fractionation values of 2.08‰ ± 0.30 and 3.54‰ ± 0.65, respectively (Waddington and MacArthur 2008). In all DTDF scenarios, the $\delta^{34}S$ fractionation values were set at 0.50‰ ± 0.56‰ (McCutchan et al. 2003).

One major assumption of mixing models is that every source sampled adequately explains a consumer's diet and adheres to mass balance, so the model can find a logical solution for estimating dietary proportions for each consumer. To determine if this assumption was violated, we employed the "point-in-polygon" approach outlined in Smith et al. (2013) on horseshoe crabs sampled. This approach tests whether mass balance has been established for each individual by using MCMC simulation of mixing polygons that incorporate the distributions of sources and DTDFs. If each individual falls within 95% of the mixing polygon space, then a solution is possible for explaining a consumer's

isotope values (Smith et al. 2013). Individuals outside of the 95% mixing space were excluded from the analysis.

Since only juvenile horseshoe crabs were sampled across seasons, we created separate Bayesian mixing model candidate sets using them to explore if seasonal effects influence resource use. The representative source groups included in the mixing models were phytoplankton (bivalves served as proxy), seagrass, cordgrass, macroalgae, and SOM. Given a strong correlation and standard deviation overlap between cordgrass and macroalgae in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values, these groups were aggregated into one source group in models that will be hereafter referred to as "macrocord" (Parnell et al. 2010; Mavraki et al. 2020). Isotope values were averaged between macroalgae and cordgrass in all isotope tracers (Table S1). In the model, it was assumed that horseshoe crabs would assimilate energy indirectly from basal resource pools (primary producers) with the exception of SOM and bivalves (proxy for phytoplankton), and thus, isotopic trophic discrimination factors were corrected for two trophic levels of energy transfer for seagrass, and the "macrocord" aggregated source. Horseshoe crabs were assumed to assimilate energy from bivalves and SOM directly in both the mixing models, and thus, were assigned one level of trophic transfer in the discrimination factors (Botton and Ropes 1989; Carmichael et al. 2004). Only four production sources were incorporated to adhere to recommendations from Moore and Semmens (2008) in efforts to improve the model's discriminatory power and accuracy.

We also employed a mixing model analysis for juvenile and adult crabs with potential prey groups and SOM as sources to compare results with previous studies and understand the key prey groups that are assimilated. This analysis included gastropods, bivalves, crustaceans, polychaetes, and SOM as the prey source groups. Crustaceans and gastropods were combined as an aggregate source (referred to as "crustopod") in the model due to their overlap in standard deviation for CNS isotope values (Phillips et al. 2005). Isotope biplots of all isotope combinations are presented in Fig. S1 and denote the mean and standard deviation of all horseshoe crab and organic matter source groups.

In both the adult and juvenile mixing models, we were interested in how intrinsic covariates explained the variation behind the mixture (Ogle et al. 2014). Therefore, we included the covariates of sex and size as fixed effects. Since juveniles were sampled across two seasons, juvenile mixing model candidate sets also included season (fixed effect) as a covariate. Given the limited sampling of prey items and lack of $\delta^{34}S$ values derived from West Meadow Beach and the Atlantic Ocean ($n < 20$), mixing models were only constructed for Moriches Bay crabs. Model selection inference was conducted using Watanabe-Akaike information criterion (WAIC) (Watanabe and Opper 2010), and relative model support was assessed with Akaike weights (Burnham and Anderson 2002; Stock
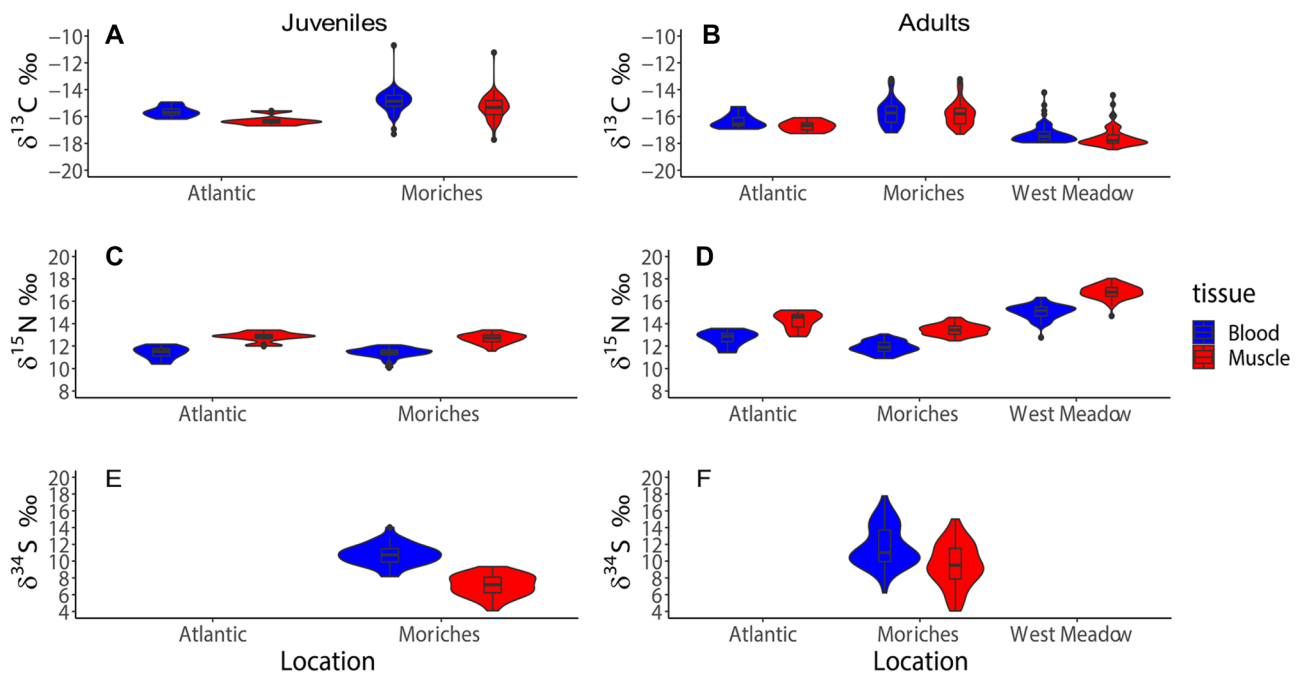
Fig. 2 Boxplots inset within violin plots that represent the distribution of stable isotope values of C (**A** and **B**), N (**C** and **D**), and S (**E** and **F**) between adult (left column) and juvenile (right column) crab tissues in the respective waterbody locations. Whole blood and muscle tissues are denoted in blue and red, respectively. No juveniles were sampled in West Meadow

et al. 2018). In cases where Akaike weights exceeded 0.9 and ΔWAIC > 2, we assumed model selection uncertainty was minimal within the candidate set (Gelman et al. 2014; McElreath 2015). Given that muscle tissue was successfully collected from all individuals, we only incorporated muscle tissues into the Bayesian mixing model analysis.

All Bayesian mixing models were analyzed in the *MixSIAR* R package (Stock et al. 2018). Each model had 3 chains with 50,000 iterations with a burn-in of 25,000, and a thinning rate of 25. A Dirichlet distribution was chosen as an uninformative prior (Parnell et al. 2013). Model convergence was assessed with the Gelman-Rubin diagnostic for each parameter (Gelman and Rubin 1992) and visually with trace plots with the *MCMCvis* R package (Youngflesh 2018).

Isotopic Niche Overlap and Ellipse Characteristics

To test the niche breadth hypotheses between age groups and among populations, we estimated the total isotopic niche (95% of the data) overlap from all isotope values (CNS), which incorporates natural variability of a species' realized niche by estimating the Bayesian standard elliptical area (SEA_B) (Swanson et al. 2015; Shipley et al. 2018). SEA_B was also calculated to determine core isotopic niche area for each group because it is more robust than total ellipse area (TA) due to its ability to estimate uncertainty from the sampling process (Jackson et al. 2011; Syväranta et al. 2013). Given that $\delta^{34}S$ values were only

measured from horseshoe crabs in Moriches Bay, SEA_B estimates were comprised of $\delta^{13}C$ and $\delta^{15}N$ when comparing West Meadow Beach and Moriches Bay populations; otherwise, all stable isotope combinations of C, N, and S were used to estimate multiple SEA_B estimates. To test isotopic niche overlap between age groups, the R package *nicheRover* was used to probabilistically draw overlap estimates from the posterior distribution of the SEA_B (Swanson et al. 2015) of each age group. It is important to note that isotopic niche width is not an absolute, but relative measure of trophic niche breadth given that isotope fractionations can be influenced by other mechanisms outside of metabolic fractionation (Shipley and Matich 2020).

Results

Population and Age Group Differences in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$

Kruskal–Wallis tests revealed that average $\delta^{13}C$ ($\chi^2 = 77.8$, df = 2, $p < 0.01$) and $\delta^{15}N$ ($\chi^2 = 102.5$, df = 2, $p < 0.01$) values were different among horseshoe crab populations. Average $\delta^{13}C$ and $\delta^{15}N$ values were different between Moriches Bay and West Meadow Beach (Fig. 2; Table S2). Atlantic adult crabs had different $\delta^{13}C$ values between Moriches Bay and West Meadow Beach (Fig. 2; Table S2). $\delta^{15}N$ also differed between Atlantic adults and both Moriches Bay and

Fig. 3 Linear regression relationships between prosomal width (mm) and $\delta^{13}C$ and $\delta^{15}N$ in West Meadow (blue) and Moriches Bay (gold). Adults and juveniles are included in the Moriches models. Standard error is shown in gray shading. Linear model formulas, R^2 coefficients, and p values are included on each plot. West Meadow $\delta^{13}C$ values had non-normally distributed errors; therefore, a second-order polynomial equation is presented
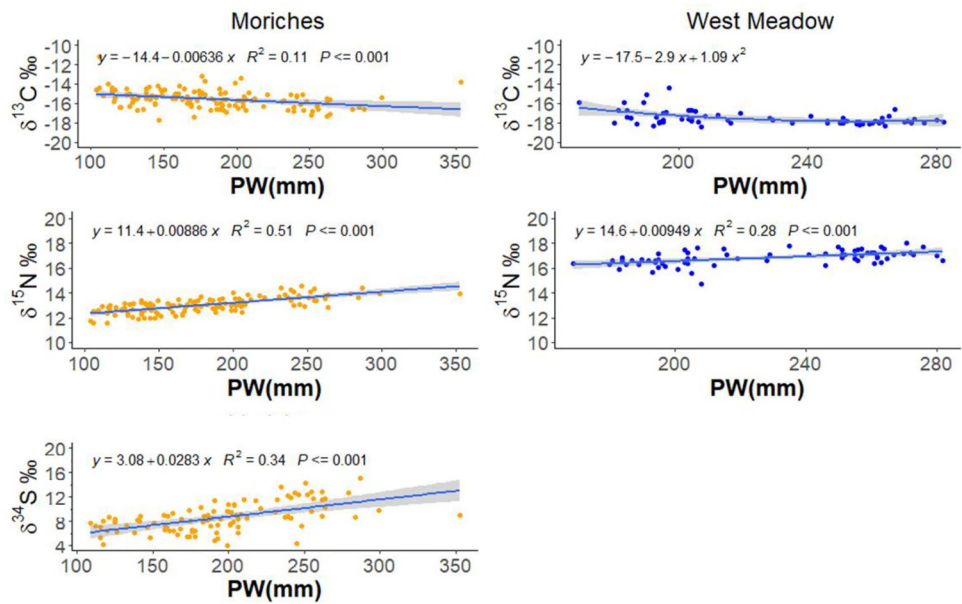
West Meadow Beach populations (Fig. 2; Table S2). $\delta^{13}C$ (WRT, W = 1329, $p < 0.01$), $\delta^{15}N$ (WRT, W = 3789, $p < 0.01$), and $\delta^{34}S$ (WRT, W = 1875.5, $p < 0.01$) values in muscle tissue differed between juveniles and adults within Moriches Bay.

Size Trends in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$

$\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in muscle tissue varied with body size (Fig. 3). $\delta^{13}C$ decreased with body size (juveniles included in Moriches Bay) in both the Moriches Bay (df = 125, R^2 = 0.11, $p < 0.01$) and West Meadow populations (Fig. 3). Evidence for increasing $\delta^{15}N$ values (i.e., more enriched) with increasing body size (juveniles included in Moriches Bay) was supported in both Moriches Bay (df = 125, R^2 = 0.50, $p < 0.01$) and West Meadow (df = 69, R^2 = 0.28, $p < 0.01$) (Fig. 3). $\delta^{34}S$ increased with body size within adults (R^2 = 0.21, $p = < 0.001$; Fig. S2). No relationship between $\delta^{34}S$ and body size (R^2 = 0.01, $p = 0.65$) was evident within juveniles (Fig. S2).

Tissue Differences in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$

$\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values for adults differed between whole blood and muscle tissues within the all populations (Fig. 4A–C). $\delta^{13}C$ (Wilcoxon paired test (WPT); $V = 0.345$, $p < 0.001$) and $\delta^{34}S$ (WPT; $V = 4719$, $p < 0.001$) values in muscle were slightly more deplete relative to whole blood. Conversely, whole blood was more deplete relative to muscle in $\delta^{15}N$ values (WPT; $V = 0$, $p < 0.001$). Across all populations, the average absolute difference (±standard error) in $\delta^{13}C$ ($n = 213$), $\delta^{15}N$ ($n = 213$), and $\delta^{34}S$ ($n = 96$) between whole blood and muscle tissue was $0.37 \pm 0.03‰$ (SE), $1.51 \pm 0.02‰$ (SE), and $2.86 \pm 0.15‰$ (SE), respectively. Strong correlations were evident between muscle and blood tissues across all locations (Fig. 4). Juveniles (Moriches Bay only) exhibited similar correlation trends between muscle and blood tissues to adults, with the exception of $\delta^{34}S$ values (Fig. 4D–F). SH did not appear to influence $\delta^{34}S$ values in adult whole blood samples (WPT; $V = 81$, $p = 0.84$), and thus, no $\delta^{34}S$ correction for SH was required in blood samples.

Bayesian Mixing Model and Isotopic Niche Metrics

SOM was the primary resource pool relied upon for both adult and juvenile crabs in Moriches Bay with SOM contributing a higher average proportion to diet of juveniles ($72.2 \pm 2.9\%$ SD) relative to adults ($56.0\% \pm 3.1\%$ SD). The average contribution of phytoplankton, macroalgae, and cordgrass organic matter sources contributed < 30% (each) to the assimilated diet of adults and juveniles (Table 2). Mixing model parameter estimates were not sensitive to DTDFs, because the parameter difference between each DTDF scenario was < 15%, and the order of the primary dietary component contributions did change. However, the model was somewhat sensitive to the secondary and third source contributions given that bivalves switched from being the third to second highest dietary component in both age groups.

In the mixing model with prey items, SOM comprised the highest source contribution assimilated in juvenile crabs (> 70%), with no clear secondary prey group assimilated given overlap in 95% credible intervals (Table S3). In adults, SOM contributed nearly 50% to their diet, while ~30% of their
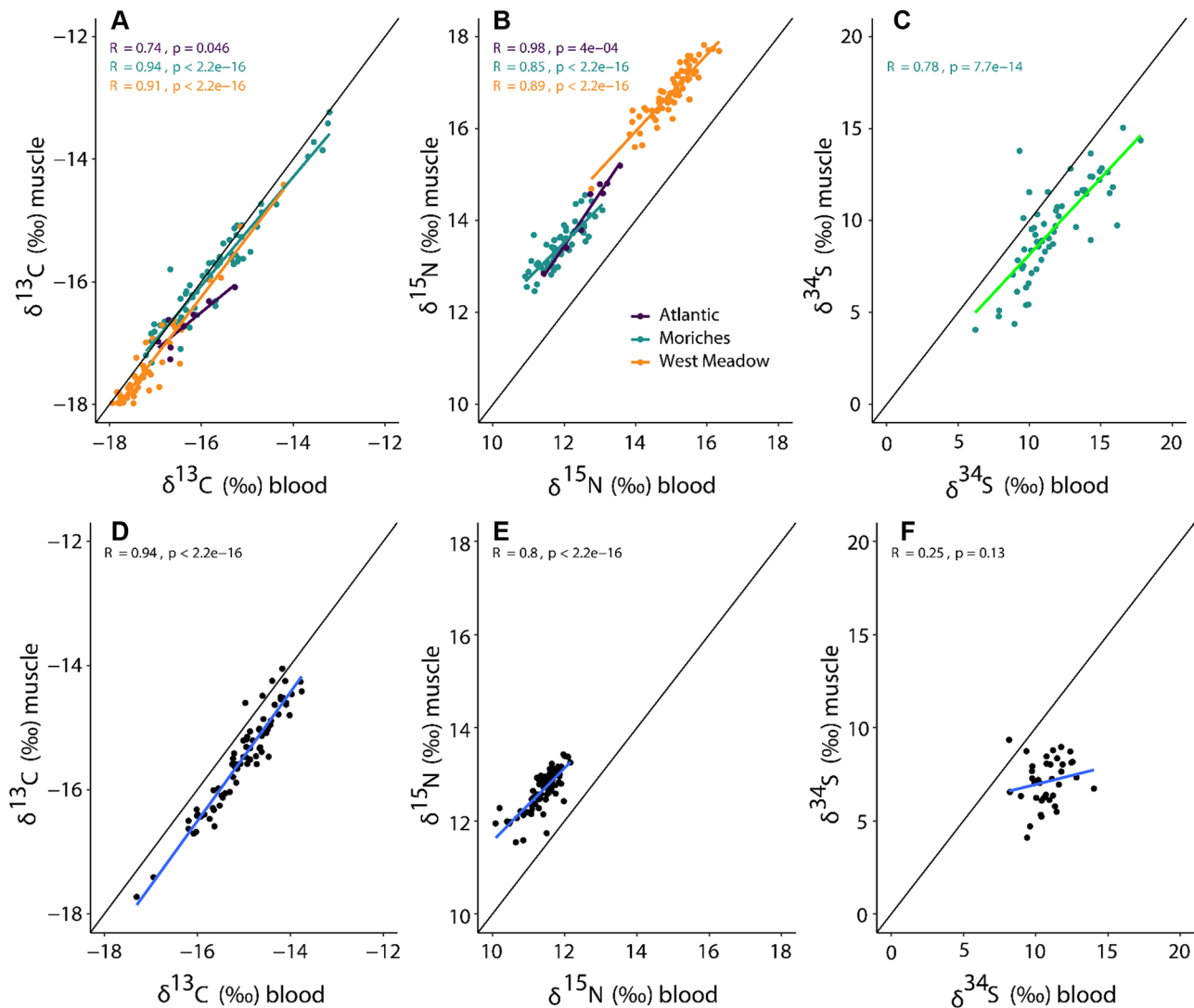
Fig. 4 Scatterplots showing all stable isotope values between muscle vs. blood tissues in horseshoe crabs across all sampling locations. $\delta^{13}C$ (**A**), $\delta^{15}N$ (**B**), and $\delta^{34}S$ (**C**) values denote adult tissue comparisons (top row). Juvenile $\delta^{13}C$ (**D**), $\delta^{15}N$ (**E**), and $\delta^{34}S$ (**F**) inter-tissue comparisons from Moriches Bay only are also presented (bottom row). Spearman rank correlation test results for each location are denoted in the upper left-hand corner. Black line denotes 1:1 relationship

Table 2 Average estimated contribution of organic matter sources contributing to adult and juvenile horseshoe crabs within DTDF scenario 1 from the model. 95% credible intervals are presented in parentheses. Macrocord group represents the aggregated source of macroalgae and cordgrass

Group	DTDF scenario 1	
	Adult	Juvenile
Phytoplankton	14.8 (3.0–28.7)	5.9 (0.10–16.8)
Macrocord	27.1 (13.7–38.5)	17.2 (4.0–27.6)
Seagrass	2.2 (0.0–7.3)	4.8 (0.30–14.0)
SOM	56.0 (49.2–62.3)	72.2 (66.4–78.2)

diet consisted of polychaetes (Table S3). Gastropods, crustaceans, and bivalves comprised ~20% of adult horseshoe crabs' resource use profile (Table S3).

Strong support for sex and size effects on the contribution of dietary proportions in adult horseshoe crabs given model 1 (~PW + sex) and model 2 (~PW) comprised > 0.99 of the Akaike weight (Table 3). While both adult sexes relied substantially on SOM, males exhibited a higher reliance (> 16%) on SOM relative to females, and SOM was their predominant resource utilized. In contrast, adult females had no clear primary organic matter source contributing to diet, but collectively relied on mixtures of SOM, macroalgae, cordgrass, and bivalve production (Table 3; Fig. 5B). The contributions of seagrass

Table 3 Candidate model results from Bayesian mixing model variants to determine which covariates influence the estimated proportional dietary contributions in adult and juvenile horseshoe crabs. Sex and season covariates are factors. PW denotes prosomal width (mm) and is a continuous covariate. Models are ranked by WAIC (widely applicable information criterion) and AIC weight. ΔWAIC represents the difference of WAIC scores between all models and the model with the lowest WAIC. SE ΔWAIC denotes the standard error of the difference between model and the model with the lowest WAIC. AIC weight denotes the relative support for each model

Juveniles (n = 39)

Model number	Model	WAIC	SE WAIC	ΔWAIC	SE ΔWAIC	AIC weight
1	~PW	−21.3	14.4	0	-	0.373
2	~Season	−20.3	16.4	1	4.5	0.226
3	~1	−20.2	15.5	1.1	3.5	0.215
4	~PW + sex + season	−18.7	15.2	2.6	3.3	0.102
5	~Sex + PW	−17.2	14.4	4.1	1	0.048
6	~Sex	−16.6	15.6	4.7	3.7	0.036
7	~Season + PW	−12.5	9.5	8.8	6.1	0.005

Adults (n = 61)

Model number	Model	WAIC	SE WAIC	ΔWAIC	SE ΔWAIC	AIC weight
1	~PW + sex	69.9	16.9	0	-	0.599
2	~PW	70.7	17.6	0.8	3.3	0.401
3	~Sex	96.9	21.3	27	10.9	0
4	~1	122	21.5	52.1	12.5	0

assimilated into each adult sex were low (< 3.0% average). SOM was the predominant organic matter pool comprising adult horseshoe crab diets for crabs < 240 mm in PW (mostly males). The average C/N ratios of our SOM samples were 6.9 (±0.31 SE).

For juvenile crabs, AIC weights were distributed among five candidate models and no model variant received a weight > 0.40. Overall, there was moderate support for season, size, and sex effects on the dietary proportions of juvenile crabs (Fig. 5A, Tables 3 and S4).

Isotopic Niche Overlap and Width Estimates

The core and total isotopic SEA_B in blood and muscle of adults from West Meadow did not overlap in $\delta^{15}N$ space with their Moriches Bay counterparts (Fig. 6A, B), but no other differences were observed between populations. In $\delta^{13}C$ space, West Meadow adults were shifted relative to Moriches, but while core ranges differed, there was overlap in the total isotopic niche range. Bayesian standard ellipse area (SEA_B) from muscle and blood derived from $\delta^{13}C$ and $\delta^{15}N$ did not differ between Moriches Bay and West Meadow
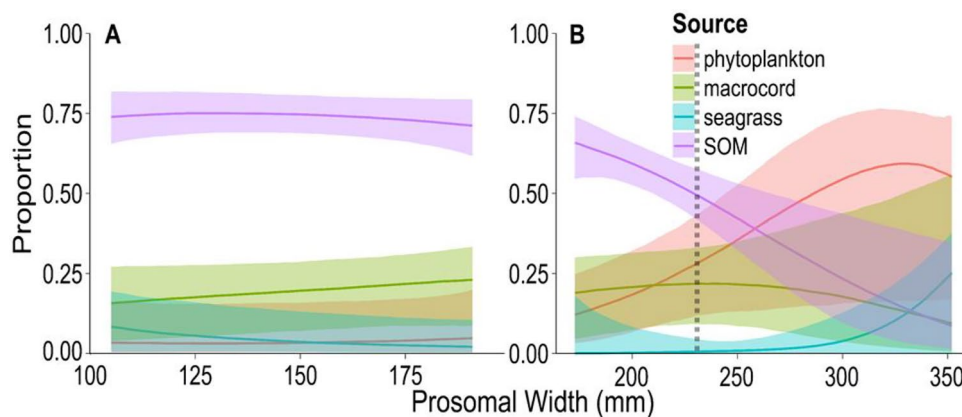

Fig. 5 The average proportional contribution of each organic matter source across prosomal width (mm) in juvenile (**A**) and adult (**B**) horseshoe crabs from the mixing model in Moriches Bay (Table 2). 95% credible intervals are in the shaded region around the solid lines (means) of each source. The gray vertical line represents the prosoma width cutoff between adult males and females sampled in this study. The maximum male prosomal width was 232 mm and minimum female width was 231 mm
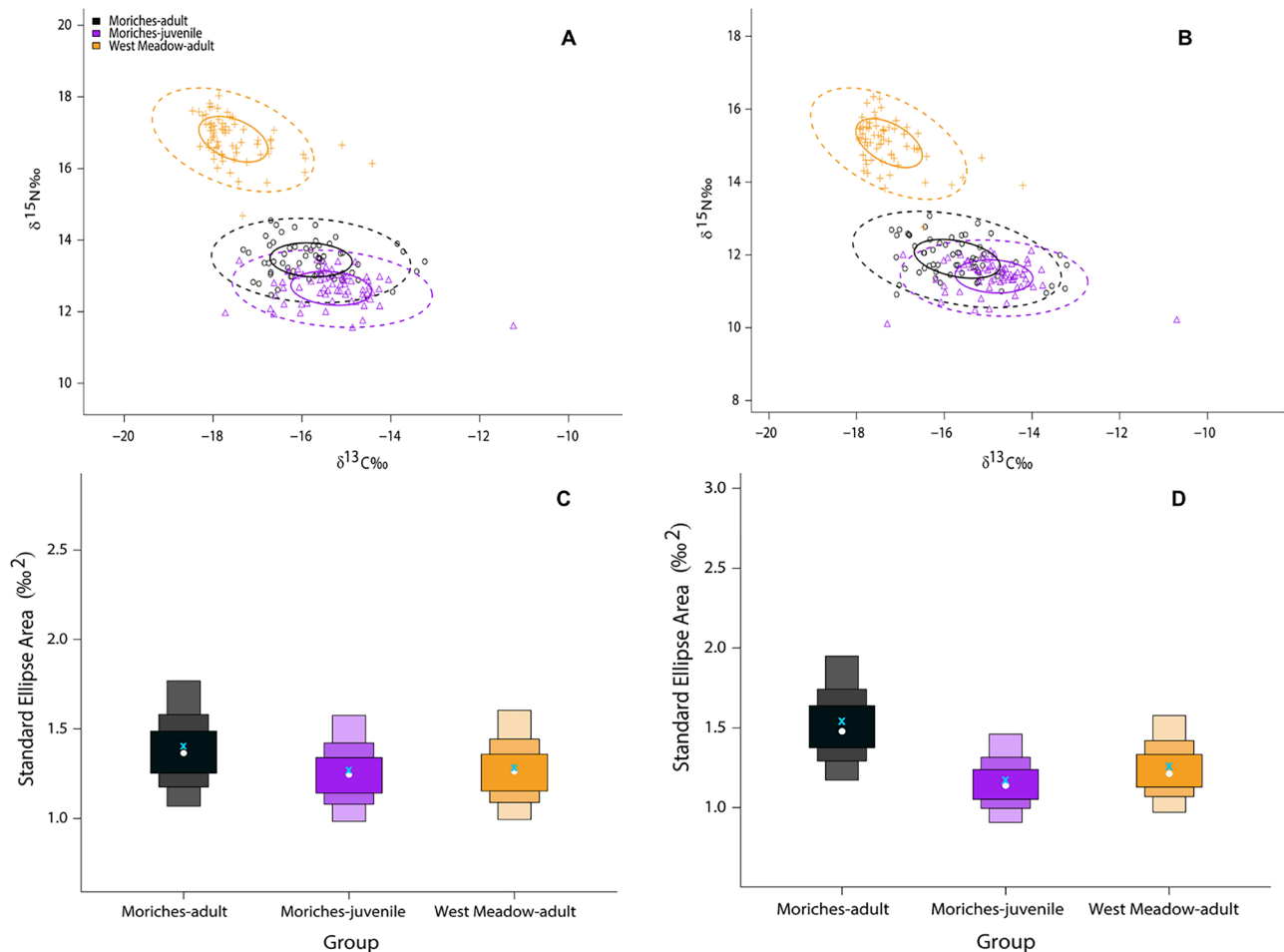
Fig. 6 Top row: core isotopic niche (40% of data (SEA$_B$), solid lines) and total isotopic niche (95% of data, dashed lines) estimates for horseshoe crabs in Moriches Bay (juvenile and adult) and West Meadow beach for muscle (**A**) and blood tissues (**B**). Bottom row:

SEA$_B$ estimates with credible intervals (50%, 75%, and 95%) with turquoise x representing maximum likelihood estimates and white dots representing median Bayesian estimates for muscle tissue (**C**) and whole blood (**D**)

adult populations with considerable overlap in 95% credible intervals (Fig. 6C, D).

Within Moriches Bay, adults exhibited ~2.3-fold larger average SEA$_B$ area values derived from both the combination of $\delta^{13}C/\delta^{34}S$ and $\delta^{15}N/\delta^{34}S$ (Fig. 7C, D), relative to juveniles. Bayesian standard ellipse area from muscle and blood derived from $\delta^{13}C$ and $\delta^{15}N$ did not differ between adult and juveniles with considerable overlap in 95% credible intervals (Fig. 6C, D). Core isotopic niche and total isotopic niche ranges were similar for Moriches adults and juveniles in N vs. C space (Fig. 6A, B) but reflected differences in $\delta^{34}S$ space in the S vs. C and S vs. N plots (Fig. 7A, B).

There was considerable shifting in niche overlap with ontogeny (Fig. 8). Total isotopic niche overlap (CNS isotopes) was moderate between instars 14–15 with both 16–18 (> 55%) and adults (> 45%). Instars 16–18 exhibited high niche overlap between 14–15 (> 75%) and adults (> 85%).

Conversely, adults exhibited relatively low average overlap with instars 14–15 (< 25%) and 16–18 (< 35%). No seasonal differences in isotopic niche overlap were present within juveniles when calculated from whole blood (Fig. S3).

Discussion

In this study, bulk stable isotope analysis has provided insights into the complexity of horseshoe crab resource use dynamics across populations with different levels of migratory exchange, across ontogeny, and between sex groups. Importantly, our study suggests SOM is a major diet resource utilized by both juvenile and adult crabs, and it contrasts with gut content studies that report bivalves as the primary diet source (Botton and Ropes 1989). Our resource use findings, however, are similar to those reported for juvenile Asian horseshoe crabs, including
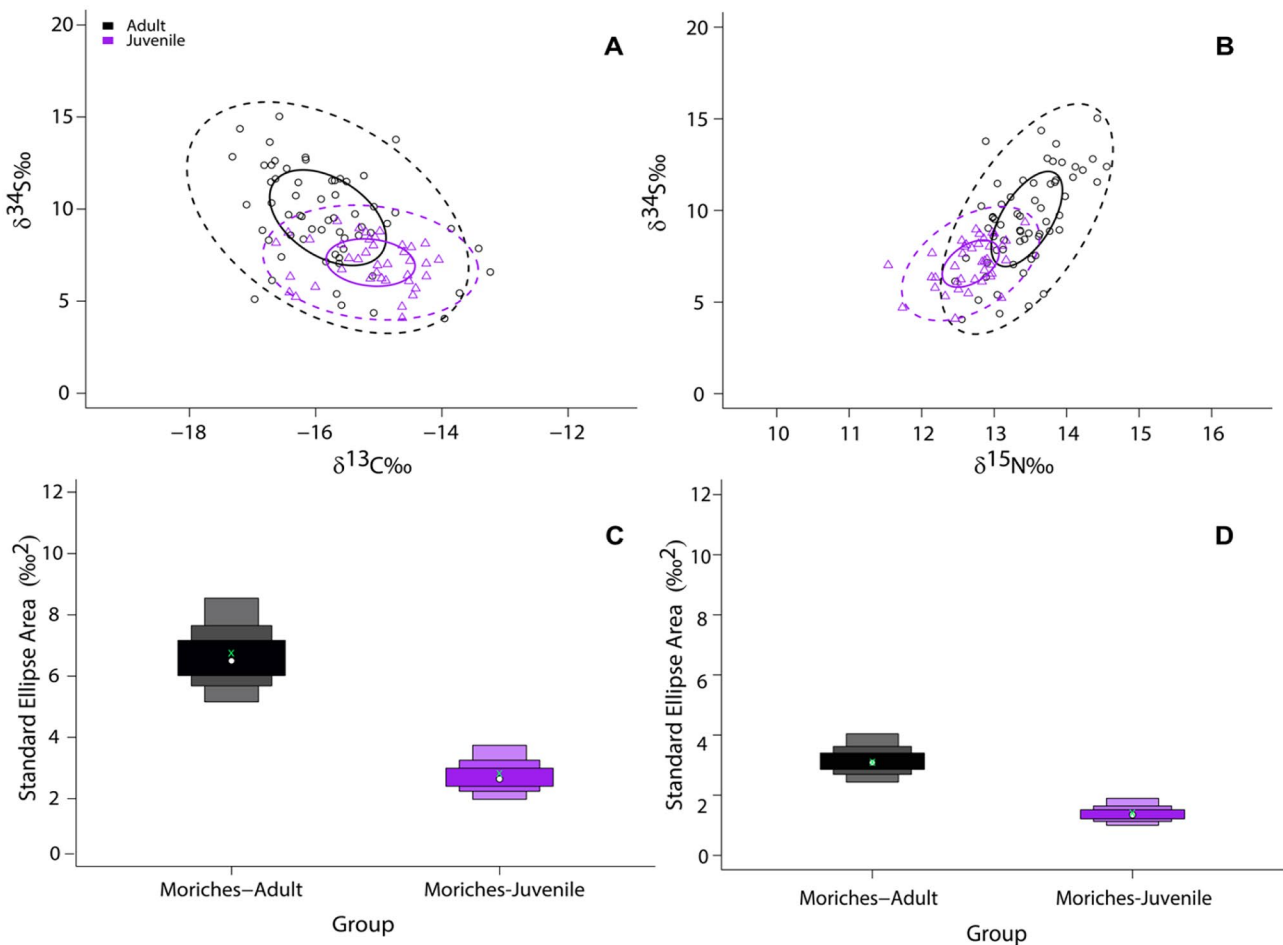
Fig. 7 Core isotopic niche (40% of data (SEA$_B$), dashed lines) and total isotopic niche (95% of data, solid lines) estimates for juvenile and adult horseshoe crabs from Moriches Bay (top row) with stable isotopes of S vs. C (**A**) and S vs. N (**B**). Stable isotope values derived from muscle tissues. SEA$_B$ estimates with credible intervals (50%, 75%, and 95%) with turquoise x representing maximum likelihood estimates and white dots representing median Bayesian estimates. Niche breadth of stable isotope of S with C (**C**) and S with N (**D**) is denoted in the bottom left and right panels, respectively

tri-spine (*Tachypleus tridentatus*) and mangrove (*Carcinoscorpius rotundicauda*) horseshoe crabs, where contributions of SOM comprised > 30% of all sources assimilated into tissues (Fan et al. 2017; Kwan et al. 2021). Although our study only sampled juvenile instars 14–18, the moderate isotopic niche overlap that juveniles expressed with adults coupled with a high reliance of SOM between these instar groups and adults indicates that ontogenetic shifts may be more subtle than previously realized between younger and older juvenile cohorts. Surprisingly, there was strong support for sex-specific differences in mature horseshoe crab resource use patterns, indicating that foraging preferences or locations may differ between sexes. Interestingly, juveniles and adult males exhibited a stronger reliance on SOM compared to adult females despite that all adult horseshoe crabs exhibited more enriched δ^{34}S values overall. Taken together, these results indicate that

horseshoe crab resource use may be mediated primarily by size but also partially by disparate ontogenetic movement and space use patterns because adults exhibit higher migratory exchange between estuaries and coastal oceanic environments compared to juveniles (Bopp et al. 2021). Future studies should examine the resource use characteristics across ontogeny in populations with different migratory strategies to identify broader ontogenetic and sex-specific dietary patterns in this species.

Sex-Specific Differences in Resource Use

Previous studies have demonstrated that adult male and female horseshoe crabs have similar dietary (Botton 1984) and resource use patterns (Carmichael et al. 2004); however, we found support for contrasting dependencies on basal production sources between mature sex groups. SOM
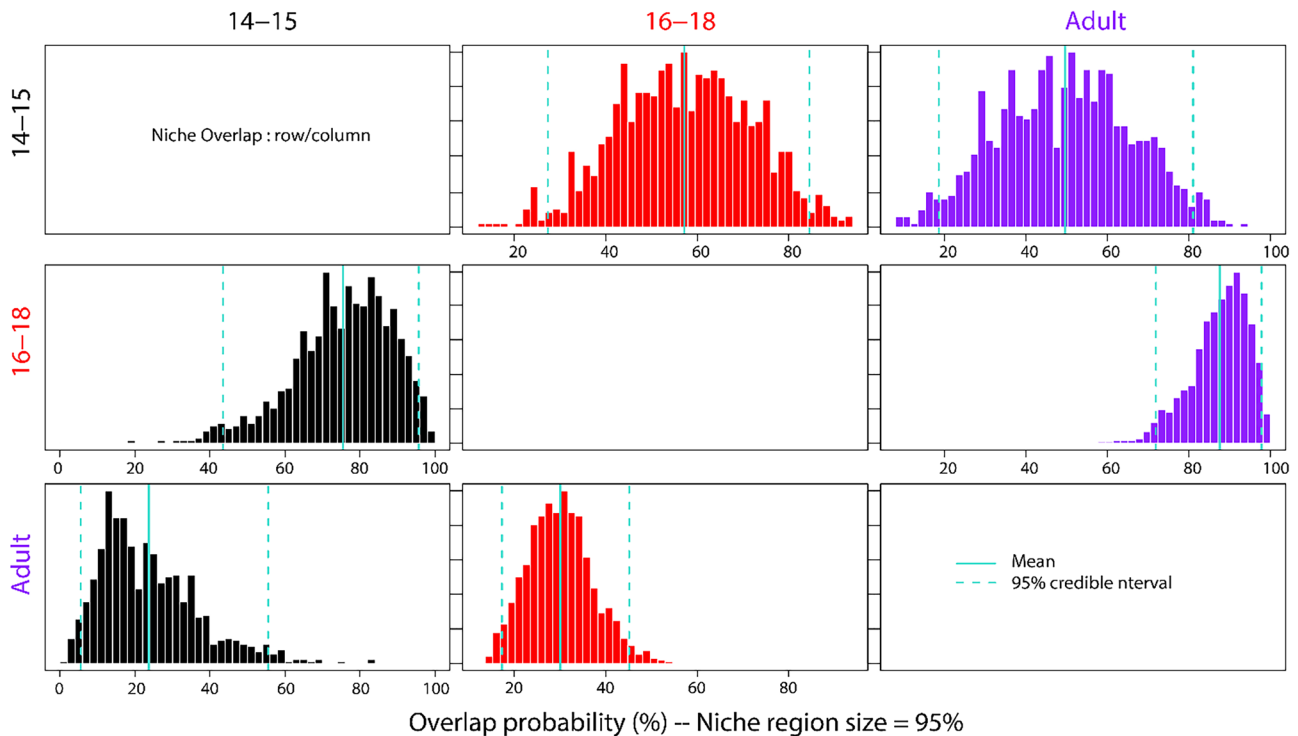
Fig. 8 Posterior density distributions from Bayesian isotopic niche overlap estimates for horseshoe crab age groups within Moriches Bay given that sulfur isotope analysis was not conducted from adults in West Meadow Beach. Dashed vertical blue lines represent 95% cred- ible intervals and solid lines denote mean overlap estimates. Isotopic niche estimates incorporate all stable isotopes of C, N, and S in mus- cle tissue

was the dominant basal production source assimilated into male tissues, while females assimilated a more homogenous mixture of basal sources. Potential underlying mechanisms, such as size and energetic requirements, may explain these differences between sexes. In mature horseshoe crabs, size is a sexually dimorphic trait with larger females, and thus females may have the capacity to consume larger or harder bodied prey such as bivalves more efficiently than males (Vögler et al. 2009). Within conspecifics, sexes may also exhibit diverging dietary shifts when experiencing size increases. In mature southern elephant seals (*Mirounga leonina*) for instance, each sex experiences dietary shifts differently with size; adult females broaden their resource use by foraging across multiple trophic levels with no change in trophic level, while males increase their trophic level with increasing size (Chaigne et al. 2013). In several taxa, differ- ent energetic requirements or space use patterns between sexes can lead to resource partitioning (Lee and Severing- haus 2004; Bearhop et al. 2006). Female horseshoe crabs are hypothesized to exhibit higher migratory propensity between local estuaries and the continental shelf in some populations relative to males, presumably to forage on energy-rich prey resources offshore to facilitate oocyte production (Smith et al. 2009). However, a recent acoustic telemetry study in Moriches Bay, New York, found no difference in migratory

proportions between sexes (Bopp et al. 2021). Therefore, we postulate that females may require the utilization of resources across a broader organic matter base to maintain their larger body size relative to males. It is common for the larger sex within a species to consume different dietary con- stituents compared to their smaller counterparts (the sexual size-dimorphism hypothesis) to maximize fitness strategies and to satisfy their greater metabolic needs (Clutton-Brock et al. 1982; Beck et al. 2007). Evaluating other dietary com- ponents, such as dietary energy density, within horseshoe crabs may resolve the underlying factors contributing to different dietary patterns between sexes (Beck et al. 2007).

It is important to note that sex-specific differences in the dependence of organic matter pathways could partially reflect spatial variation in organic matter composition along the estuarine-continental shelf gradient. SOM is an aggregate source of microphytoplankton, macrophyte and animal detritus, bacteria, and meiofuana (Marchand et al. 2003), and the proportional contributions of each constitu- ent can differ considerably across small (< 10 km) spa- tial scales (Pusceddu et al. 1999). In coastal estuaries, the biomass of macroalgae production sources (e.g., macroal- gae, seagrass, and cordgrass) may exceed phytoplankton production (MacIntyre et al. 1996), while the proportional contribution of phytoplankton in sediment organic matter

is often higher with distance from the coast and has been demonstrated increase concomitantly with rising salinity along temperature estuarine-oceanic gradients (Kim et al. 2020). Therefore, it is plausible that adult female horseshoe crabs' higher reliance on phytoplankton production pathways could be a byproduct of them spending more time offshore than their male counterparts in the spring months. For example, adult males spend more time near spawning beaches, and in some cases, arrive at estuaries earlier than females during the spring (Brockmann and Penn 1992; Smith et al. 2010), and therefore, might rely more heavily on estuarine production sources for nutritional needs relative to females. This could potentially explain why male tissues reflect δ^{34}S values closer to signatures of estuarine production sources relative to more enriched δ^{34}S values that are reflective of marine phytoplankton production on continental shelf (Rubenstein and Hobson 2004). Alternatively, females might have enriched δ^{34}S values because they require more energy-rich food source (e.g., SOM, bivalves) offshore that are dominated by phytoplankton-based pathways, while males may rely heavily on benthic SOM-based pathways, which are typically more deplete in δ^{34}S from higher sulfide concentrations in relation to other estuarine and oceanic production sources (Fry et al. 2008).

General Foraging Patterns

SOM is the predominant organic matter source assimilated into horseshoe crab muscle tissue with little contributions from other basal organic matter sources. For example, phytoplankton and macroalgae/cordgrass production pathways contributed < 30% to adult horseshoe crab diet (depending on DTDF scenario). The moderate dependence of adult horseshoe crabs on polychaetes and crustaceans/gastropods (> 14% contribution) indicated they require the consumption of other food web components outside of SOM to meet energetic demands. Juveniles depended less on other resources outside of SOM compared to adults (Tables 2 and S3). Previous linear mixing model analysis from δ^{13}C and δ^{15}N indicated that once juveniles reached their 8–9 instar, their diet composition shifted towards a higher reliance on benthic invertebrate groups, such as crustaceans and polychaetes (Carmichael et al. 2009). Carmichael et al. (2009) also demonstrated that during controlled feeding experiments (120-day duration), juvenile horseshoe crab survival rates were highest when diet compositions comprised of 40% protein (mixture of bivalves and polychaetes) and 60% macroalgae (mixture of *Ulva* spp., *Enteromorpha* spp., and *Tetraselmis* spp.). Interestingly, our prey mixing model results suggest that animal groups (bivalves, polychaetes, crustaceans, and gastropods) that are rich in protein comprised ~30% of overall juvenile crab diet (Table S3), while SOM comprised ~70% the remainder of their diet.

Although determining the composition of SOM was not a major objective in this study, we desired to examine the C:N ratios in SOM because it can facilitate the delineation of organic matter sources that accumulate in the sediments (Khan et al. 2015). In our study, the average C:N ratio of SOM samples was 6.9 indicating that algal and bacterial sources comprised most of the organic matter in the sediments compared to detrital sources which typically consist of C:N ratios of 25–50 in estuaries (Khan et al. 2015; Dalu et al. 2016; da Costa et al. 2021). This suggests that *Spartina* may not be a major component of SOM despite being prevalent in Moriches Bay. In saltmarsh-based estuaries, benthic edaphic microalgae (e.g., diatoms, cyanobacteria) and epiphytic algae can represent a major production source in estuarine sediments (Sullivan and Moncreiff 1990, Hasegawa et al. 2007). For instance, epiphytic algae growth on macroalgae and seagrass blades can exhibit higher production compared to its host given their higher turnover rates (Macreadie et al. 2014) and can be integrated into SOM from weather events resulting in uprooting of submerged and emergent vegetation. Discriminating the algal contributions to SOM is often challenging with stable isotopes (Sullivan and Moncreiff 1990) and is beyond the scope of this study, but should be addressed in future research to better understand specific pathways that support horseshoe crabs in the region. Nonetheless, our findings provide additional support for the assertion that horseshoe crabs may require the direct acquisition of organic matter sources, such as algae and bacteria, outside of macroinvertebrate prey sources to maintain their metabolic needs.

Juvenile resource use patterns did not differentiate between sex, across size classes or seasons. The static nature of juvenile resource use through time indicates that SOM is a reliable and consistent organic matter source for juveniles year-round (Table S4). The lack of seasonal changes in resource use is not surprising for this life history stage given their localized movement patterns among estuarine habitats and low emigration from coastal estuaries (Bopp et al. 2021). Juveniles have been observed to have a strong preference for subtidal mud substrates (Cheng et al. 2021), which are richer in organic matter compared to coarser sediments (Serrano et al. 2016), and may explain their assimilatory preferences for SOM. However, the constituents and quality of SOM often vary seasonally in temperate estuaries (Lesen 2006) and should be examined to determine its bioenergetic implications on horseshoe crab metabolism. For example, macronutrients (carbohydrates, proteins, and lipids) have been observed to be higher in SOM in the spring compared to the remainder of the year within some temperate estuaries (Danovaro and Fabiano 1997).

Unlike their adult counterparts, dietary shifts across body size were not apparent in juveniles despite exhibiting increasing ^{15}N trends with size (Fig. 5), which is typically indicative of increasing trophic position (Jennings et al. 2002). Outside

of ontogenetic differences in movement patterns, differing nutritional requirements between age groups may explain the lack of dietary shifts with size. For example, SOM may be sufficient for juvenile growth, but as animals reach sexual maturity, the energy demand may shift to accommodate gonad or egg development (Morehouse et al. 2010) and thus, larger adults shift their foraging habits to maintain their body size, reproductive organs, and other energetic needs (Sasakawa 2009; Lucifora et al. 2009). Gape-size limitation may also be an important factor that may preclude juveniles from foraging on larger prey items and could also explain their high reliance on more easily ingestible materials, such as SOM, coupled with lower $\delta^{15}N$ values. In many predator marine fishes for instance, 75% of their diet was comprised of prey that was < 20% of body size, indicating that gape-size limitation is common (Scharf et al. 2000). Additional work is needed to evaluate the tradeoffs in energy demand for growth, reproduction, and mass-specific metabolic rates to identify potential mechanisms that drive these observed dietary trends in horseshoe crabs among sex and age groups.

Although moderate isotopic niche overlap estimates between juveniles and adults in Moriches Bay were observed (Table 3; Figs. 6, 7, 8), we suggest that foraging competition is low between these age groups when coupling the knowledge of their movement and isotopic niche breadth patterns. A recent acoustic telemetry study demonstrated juveniles exhibit reduced habitat linkages (> 10-fold less), 5-fold lower migration rates, and higher site fidelity relative to adults in Moriches Bay, New York (Bopp et al. 2021). Additionally, adults exhibited broader isotopic niche breadth and more enriched $\delta^{34}S$ values compared to juveniles, suggesting that adults may assimilate energy from other resource pathways that are not assimilated to the same extent by juveniles, such as oceanic phytoplankton production sources on the continental shelf (Oczkowski et al. 2016). Furthermore, Lee (2010) revealed that adults intensively foraged in intertidal flats within estuaries, areas largely unoccupied by older juveniles, following the spawning season during the summer months. $\delta^{34}S$ values measured in the current study further corroborate differences in movement, as adults appeared to have more enriched $\delta^{34}S$ values compared to juveniles, indicating that adults migrate and access prey (e.g., bivalves) or SOM offshore that is more enriched in $\delta^{34}S$ compared to the $\delta^{34}S$ signatures of some estuarine organic matter sources (Rubenstein and Hobson 2004). Depleted $\delta^{34}S$ values within juveniles could also be attributed to their tendencies to occupy mud-based habitats (Cheng et al. 2021) that have higher sulfide concentrations from bacterial sulfate reduction (Brüchert and Pratt 1996). When considering these ontogenetic differences in space use, it appears that adults and juveniles exhibit moderate resource partitioning across space and time, which may be beneficial to reduce intraspecific foraging competition and may optimize fitness (Svanbäck and Bolnick 2007). Although minimal spatial overlap between age groups has been observed in the Moriches Bay study area, it is important to consider that spatial overlap among these age groups may be higher in other Mid-Atlantic horseshoe crab populations such as in coastal New Jersey, where Able et al. (2019) observed high space use overlap of both juveniles (instars 12+) and adults on the continental shelf from April to October. Therefore, it is imperative to define both spatial and trophic niche overlaps across horseshoe crab age groups in other populations to ascertain a comprehensive perspective of resource use and identify critical habitat requirements for each life history stage.

Isotopic niche breadth size did not differ between horseshoe crabs in Moriches Bay and West Meadow Beach, but variability in $\delta^{15}N$ and $\delta^{13}C$ indicates potential differences in resource use, isotopic baselines, or both. For example, the ranges of $\delta^{15}N$ and the $\delta^{13}C$ were larger and smaller, respectively, in adult horseshoe crabs from West Meadow Beach relative to Moriches Bay. These isotopic range differences between populations are likely a product of spatial variability in nutrient loading and isotopic baselines. Eutrophication primarily derived from anthropogenic nitrogenous wastewater and fertilizers has been present in both waterbodies (Anderson and Taylor 2001) and increased anthropogenic nitrogen loading from wastewater is positively correlated with $\delta^{15}N$ enrichment (Cole et al. 2004). In the central and western Long Island Sound, a variety of estuarine fish, snails, plants (Spartina), and coastal soils have been observed to have enriched $\delta^{15}N$ values relative to estuarine embayments in Long Island's South Shore and is primarily attributed to higher wastewater treatment input into the watersheds in Long Island's North Shore (Watson et al. 2018). This could explain why horseshoe crabs from West Meadow Beach had enriched $\delta^{15}N$ values overall. Disparities in primary producer isotopic baselines may be partially contributing to the geographic disparities in trophic dynamics. For example, Watson et al. (2018) noted that nearshore macrophytes (mainly Spartina spp.) had more deplete $\delta^{13}C$ values, presumably due to increased CO_2 uptake from higher N loading, in the North Shore of Long Island compared to the South Shore estuaries and may partially explain why adult horseshoe crabs in West Meadow had lower $\delta^{13}C$ values. It should be noted that the potential drivers of isotopic niche breadth between populations be inferred with caution, given the lower resolution of basal production sources sampled in Long Island Sound precluded our ability to quantify the dietary contributions of West Meadow Beach crabs and therefore, recommend additional work should focus on the hypotheses presented.

Disparities in movement patterns coupled with differences in primary producer variability could also explain the differences in $\delta^{13}C$ between West Meadow Beach and Moriches Bay populations. For example, in Long Island Sound,

eelgrass (*Zostera marina*) is nearly absent and the loss of *Spartina* spp. was 2-fold greater compared to the South Shore Estuaries between 1974 and 2008 (N.Y.S.D.E.C. 2015). Therefore, fewer primary producer species may be supporting the food web in West Meadow and may explain why horseshoe crab tissues are more deplete in $\delta^{13}C$ value that are reflective of phytoplankton resource pool signatures (Peterson and Howarth 1987) compared to individuals from Moriches Bay. If West Meadow Beach horseshoe crabs heavily rely on SOM like their counterparts in the South Shore, and the SOM may be reflective of phytoplankton $\delta^{13}C$ signatures (−18‰), this could be a potential explanation why most $\delta^{13}C$ signatures in West Meadow Beach were < −16‰ (Fig. 3). However, we are unable to address the dietary contributions to West Meadow Beach horseshoe crabs given the logistical constraints of sampling offshore in Long Island Sound, but mixing polygon results indicated the majority of individuals could reliably have diet contributions quantified from the endmembers and plants sampled in Long Island Sound (Fig. S4). Adult horseshoe crabs in Long Island Sound exhibit at least 2-fold lower exchange rates among adjacent regions relative to adult horseshoe crabs in Long Island's South Shore (Bopp et al. 2019). Therefore, adult horseshoe crabs in West Meadow Beach may experience a reduced ability to assimilate energy from a variety of resource pools. In common snook (*Centropomus undecimalis*), for instance, individuals within downstream habitats within the Shark River Estuary in Florida, USA, exhibited broader trophic niche breadth patterns relative to their less mobile upstream counterparts because they were able to forage across a greater variety of resource pools from freshwater to marine habitats due to their higher dispersal patterns (Rezek et al. 2020).

Although the raw stable isotope values differed between muscle and whole blood tissues, these tissues were positively correlated (Fig. 4), indicating little temporal variation in resource use dynamics (Jardine et al. 2005) or a lack of differences in tissue turnover rates. For instance, American alligator populations that resided in both of freshwater and estuarine habitats in Florida, USA, were observed to have static foraging behavior through time regardless of habitat type after the examination of tissues with 2-fold differences in turnover rates (Rosenblatt et al. 2015). In other marine arthropods, such as in red rock lobster (*Jasus edwardsii*), turnover rates may not differ significantly between hemolymph and muscle tissues (Suring and Wing 2009). One limitation of this study is the lack of knowledge of horseshoe crab tissue turnover rates since this has not been thoroughly quantified in the literature, and unfortunately, we are unable to discern the temporal window of energy assimilation for each tissue. However, the absolute inter-tissue differences in bulk stable isotopes ratio values are presumably a result of different amino acid (AA) compositions between tissues, which result in different isotope fractionation rates

and subsequently, affect bulk stable isotope fractionation rates overall (Whiteman et al. 2019). Future efforts that utilize compound-specific stable isotope analysis coupled with the quantification of bulk tissue turnover rates in horseshoe crabs, particularly with blood tissues, are recommended to ameliorate the ability to evaluate temporal variation in horseshoe crab diet (VanderZanden et al. 2015; Ishikawa 2018).

Our findings provide novel insight into the trophic ecology of horseshoe crabs, and this is first study to robustly quantify resource use and isotopic niche metrics between juveniles and adults in a population that exhibits partial migration. Results presented here corroborate the notion that American horseshoe crabs are dietary generalists (Botton and Ropes 1989; Carmichael et al. 2004), especially considering their strong dependence on SOM which is a conglomerate of detritus (plant and animal), microorganisms, meiofauna, and bacteria (Marchand et al. 2003). Additionally, we demonstrated that SOM is an important energy source assimilated into horseshoe crabs across all life history stages. SOM is perceived to contain dietary constituents that are important for maintaining the chitinous carapaces in arthropods, such as amino acid sugars (Schimmelmann et al. 1998; Carmichael et al. 2004), that may not be supplemented elsewhere in the food web and may partially explain why horseshoe crabs heavily depend on SOM, especially for juveniles that need to replace chitin annually for molting. Identifying the key constituents of SOM assimilated into horseshoe crabs with contemporary approaches, such as compound-specific stable isotope analysis and fatty-acid analyses, would be beneficial for elucidating the critical resources that sustain their metabolic needs (Aichner et al. 2010). Between age groups, we found evidence for resource partitioning, indicating that intraspecific competition may be minimal, especially when considering disparities in their space use and migration patterns. Finally, we recommend that trophic dynamics should be examined in populations with different climes/ habitat characteristics, such as Maine, Delaware Bay, and Florida, to determine if resource use is consistent across the geographic range of American horseshoe crabs, especially considering we found trophic trends across two populations that were < 50 km apart with different migratory patterns. This is imperative because spatial distribution ultimately underpins the breadth of habitats crabs utilize for foraging (Cherel and Hobson 2007) and the diversity of resources varies across latitudinal gradients (Chaudhary et al. 2016). Overall, findings presented here can be used as a reference to determine how the functional roles of horseshoe crabs in local ecosystems may shift under environmental or anthropogenic change.

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