Title

Coastal hypoxia reduces trophic resource coupling and alters niche characteristics of an ecologically dominant omnivore

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Abstract

Energy and biomass move through a variety of trophic pathways in coastal ecosystems, with pelagic and benthic pathways often dominating food web dynamics. Consumers that couple these pathways play important structuring roles in these food webs by integrating spatially disconnected or asynchronous production pathways. The objective of this study was to quantify benthic and pelagic trophic pathway contributions to the diet of the mysid *Neomysis americana* in the Choptank and Patuxent rivers (tributaries of Chesapeake Bay) and determine if the relative contribution of these two trophic pathways differs between ecosystems with different environmental, physical, and watershed features. This mysid species undergoes daily vertical migrations throughout the water column and it was hypothesized that hypoxia (< 2 mg/L) in deep-water habitats of the Patuxent River (but not the Choptank) could reduce the importance of the benthic pathway for mysids in the Patuxent River relative to the Choptank River. Mysids and trophic resources were collected in each river during the summers of 2018 and 2019 and analyzed for carbon and nitrogen stable isotope composition and tissue stoichiometry (C:N). Stable-isotope based estimates of benthic trophic pathway contribution, trophic position, and isotopic niche area (proxy for trophic niche) and C:N composition of *N. americana* were compared within and between tributaries. Overall, mysids in the Patuxent River assimilated less benthic material, realized lower trophic positions, and had lower C:N ratios (proxy for lipid content) than Choptank River mysids. No consistent pattern in trophic niche area was observed although niche area (and pelagic trophic pathway contribution) of Choptank River mysids increased during a high river discharge event. This study provides evidence that hypoxic conditions in the Patuxent reduced the strength of benthic-pelagic coupling by *N. americana* and, further, is associated with lower trophic position and a reduced stoichiometric marker of lipid storage. In addition to their role as omnivorous consumers, *N. americana* are a dominant prey taxon for many predators, underscoring the importance of this species to local food webs and the potential for bottom-up effects on the dynamics of coastal ecosystems in response to changes in their trophic ecology.

Keywords: mysids, trophic connectivity, hypoxia, Chesapeake Bay

1. Introduction

Coastal and estuarine environments are among the most productive ecosystems on Earth (Mann, 2000; Day et al., 2012). This productivity is supported by a diversity of autochthonous and allochthonous sources of organic matter representative of distinct trophic pathways (or 'channels') through which energy can enter coastal food webs. Trophic pathways supported by new production in the water column (pelagic) or by bottom-associated production and detrital recycling (benthic) are ecologically dominant pathways in many coastal food webs (Townsend and Cammen, 1988; Dunton et al., 2006; Bartels et al., 2018) that often differ in terms of timing, magnitude and rates of basal productivity (Rooney et al., 2006; Griffiths et al., 2017). Because of these differences, the ability of mobile consumers to couple these disparate energy pathways is critical to ecological function and can support ecosystem stability while also increasing consumer productivity through the availability of spatially- and temporally-variable resources (Rooney and McCann, 2012; McMeans et al., 2015). Recent studies have shown vertically migrating, opportunistic species play an important role in coupling benthic and pelagic trophic pathways in coastal ecosystems (Woodland and Secor, 2013; Baustian et al., 2014; Kiljunen et al., 2020). Understanding the environmental conditions that modulate trophic coupling by these species is critical for predicting the effects of natural and anthropogenic forcing on the productivity of these species as well as the function of coastal ecosystems as a whole (Rezek et al., 2020).

One important modulator of trophic coupling in coastal ecosystems is the interplay between food availability and other controls on habitat conditions. For example, in ecosystems with abundant autochthonous and allochthonous organic matter that supports productive food webs, high levels of organic matter also fuel oxygen consumption in waters that can be strongly stratified due to persistent freshwater inputs. These conditions can lead to the formation of oxygen-depleted (hypoxic) waters below the better-oxygenated surface layers. Globally, the extent of hypoxic coastal waters is growing (Diaz and Rosenberg, 2008; Breitburg et al., 2018), raising the specter of increasing pressure on the integrity and productivity of these ecosystems. Coastal hypoxia is a particularly compelling problem because its influence is wide-ranging, including enhanced nutrient recycling (Testa and Kemp, 2011), reduced habitat availability (Roman et al., 2019), altered consumer community dynamics and associated food-web interactions (Decker et al., 2004), and negative effects on the growth, reproduction and survival of biota (Breitburg et al., 2018). While the direct effects of hypoxia on aquatic organisms include mortality and sublethal physiological damage accrued through exposure (Diaz and Rosenberg, 1995; Breitburg, 2002; Gray et al., 2002; Luther et al., 2004), indirect effects of hypoxia can arise from the spatial and temporal displacement of individuals from preferred habitats (e.g., environmental conditions), inhospitable recruitment areas, loss of biogenic habitats (e.g., seagrasses, corals), and reduction in foraging opportunities (Breitburg, 2002; Eby et al., 2005; Burkholder et al., 2007). Together, the direct and indirect effects of hypoxia on consumer populations, habitat conditions, and food web dynamics can alter the structure, productivity, and stability of coastal ecosystems (e.g., Rabalais et al., 2002; Kemp et al., 2005; Cloern and Jassby, 2012; Gammal et al., 2017; Woodland et al., 2022).

A useful case-study organism for examining the interactions between organic matter composition, location, concentration, and associated hypoxia formation are mysid shrimp. Mysids (Malacostraca) are small, often omnivorous, shrimp-like organisms that commonly undergo diel vertical or horizontal migrations and can reach very high abundances in coastal ecosystems (Jumars, 2007). Despite their putative role as ecologically significant prey (Lasiak and McLachlan, 1987; Pasquaud et al., 2008; Woodland et al., 2011) and consumers (Fockedey and Mees, 1999; Winkler et al., 2003), mysids are often understudied in many coastal ecosystems.

In Chesapeake Bay, Buchheister and Latour (2015) compiled a 10-year dataset of the stomach contents of over 25,000 fish across 47 species in Chesapeake Bay and found that mysids were present in more than 25% of the sampled predator fishes, identifying mysids as a vital forage taxon in the Chesapeake Bay food web. Other studies from Chesapeake Bay and proximal coastal areas have reached similar conclusions regarding the importance of mysids as prey (Boynton et al., 1981; Woodland and Secor, 2013; Ihde et al., 2015); however, little to no research is available on the ecology of mysids as consumers within the Bay, nor on how mysid trophic niche characteristics are affected by fluctuations in the environment. For example, data from a long-term zooplankton survey conducted by the Chesapeake Bay Program (CBP; Mesozooplankton Monitoring Survey, 1984-2002) indicate the presence of several mysid species (*Neomysis americana*, *Americamysis bahia*, *A. bigelowi*, and *Mysidopsis furca*) whose abundance varies seasonally and spatially (Fig. 1), but this survey was not optimized for mysid sampling. Understanding the mechanisms that support mysid production in Chesapeake Bay is needed to better understand which factors govern the transfer of energy across multiple trophic levels and the interactions that help maintain ecosystem structure and function (Pozas-Schacre et al., 2021).

The objective of this study was to fill gaps in our understanding of the habitat use and trophic ecology of an abundant mysid species, *N. americana*, within two tributaries of Chesapeake Bay, the Choptank and Patuxent rivers. These tributaries share many similarities (see section *2.2 Study systems*, below) but differ in watershed characteristics and associated seasonal water quality, specifically the recurrent formation of hypoxic bottom waters (defined as having dissolved oxygen concentrations < 2 mg/L) in the Patuxent River during the summer months. We hypothesized, given the potential for bottom-water hypoxia to reduce benthic foraging opportunities, benthic trophic pathways would contribute less to the diets of *N. americana* in the Patuxent River than in the Choptank River. We also predicted trophic niche area of *N. americana* would be smaller in the Patuxent River. In addition to benthic trophic pathway contribution and trophic niche area, we calculated and compared trophic position and tissue C:N stoichiometry of *N. americana* between the two rivers. The goals of this study were to provide insight into the effects of environmental disturbance on Chesapeake Bay food webs and ecosystem health by providing new knowledge about how local conditions may influence the trophic ecology (and ultimately the productivity) of an ecologically important consumer.

1. Materials and Methods

1.1. Study organism - Neomysis americana

Neomysis americana (S.I. Smith, 1873) is a medium-sized mysid species (maximum body length ~ 14 mm) that inhabits coastal waters ranging in depth from 1–250 m, and is distributed along the North American east coast from Quebec, Canada, to northern Florida and along the east coast of South America (Williams et al., 1974; Bouchard and Winkler, 2018). This species of mysid is typically found in coastal areas across a range of salinities (oligohaline to fully marine) and temperatures (0–25˚C) and often dominates mysid assemblages in Middle Atlantic Bight coastal waters (Cowles, 1930; Mayor et al., 2017; Mayor and Chigbu, 2018). The diet of *N. americana* is diverse and includes bacteria, phyto- and zooplankton, benthic meiofauna, protists (e.g., tintinnids), detritus, and other suspended particulate organic matter (SPOM) (Zagursky and Feller, 1985; Winkler et al., 2003). *N. americana* plays a critical role in many coastal and estuarine food webs, providing an essential link between the lowest trophic levels and higher predators (Schiariti et al., 2006; Woodland and Secor, 2013; Buchheister and Latour, 2015). Typically, *N. americana* are closely associated with bottom habitats during daylight hours but begin to move upwards in the water column at dusk, often concentrating near or above the pycnocline at night (Jumars, 2007). Through its diel vertical migration – migrating between, feeding within, and serving as prey in both benthic and pelagic habitats – this species can facilitate the coupling of benthic and pelagic trophic pathways (Woodland and Secor, 2013). While this flexible foraging behavior may explain the abundance of *N. americana* in many coastal marine habitats, it also suggests the functional role of mysids in these systems may differ significantly under dissimilar environmental or ecological conditions.

2.2 Study systems

The Choptank and Patuxent rivers (Fig. 1), located in the Mid-Atlantic Coastal Plain, are mid-sized riverine-estuarine tributaries of Chesapeake Bay (Fisher et al., 2006a). These rivers and their basins share a comparable latitude and climate and are part of the same estuarine complex, but have important differences, providing ideal comparative study systems on the ecology of *N. americana* in this region. The Choptank River (1,756 km^2) lies on the eastern side of the Bay with an average depth of 3.6 m. This basin is heavily rural, dominated primarily by agricultural (62%) land cover (Fisher et al., 2006b). Additionally, the Choptank River receives large volumes (>1,000 kg N km^{-2} yr^{-1}) of fertilizer input from the surrounding watershed. The Patuxent River (2,260 km^2) is located on the western side of the Bay and has a mean depth of 4.7 m. This basin is primarily forested (64%) and experiences much lower fertilizer input (<200 kg N km^{-2} yr^{-1}) from the surrounding watershed than the Choptank River. Moreover, the Patuxent basin has a land to water ratio that is three times that of the Choptank basin. Because of this disparity, in addition to its deeper depths, the Patuxent River is more susceptible to bottom-water hypoxia (Fisher et al., 2006a; Testa et al., 2008). However, despite their spatial and bathymetric differences, these tributaries have similar salinity ranges, phytoplankton concentrations, and biotic community composition (Fisher et al., 2006a).

2.3 Field and Lab Methods

Sampling was conducted at $n = 3$ stations in each river (Fig. 1) during the months of June, August, and September in 2018, and the months of June and July in 2019. Stations were chosen to span similar salinity ranges in each river while staying within the salinity tolerance of *N. americana* (i.e., salinity > 1 [PSS]; Chapina et al., 2020). Vertical profiles of dissolved oxygen concentration (DO; mg/L) were collected mid-channel at each station prior to sampling at approximately 1-m depth intervals with a Manta sub3.5 multiprobe sonde (Eureka Water Probes, Austin, TX).

Samples for this study were taken at night (during the interval spanning 1hr after sunset to 1hr before sunrise) using multiple gears to collect different food web components. Mysids were sampled at Patuxent (PAX) station P3 and at Choptank (CHOP) station C3 (Fig. 1) using a 0.6-m diameter circular zooplankton net (0.8 m^2 sampling area, 3-m net length) with 300-μm mesh and a windowed cod-end. The net was deployed twice and towed for 3 minutes, once at the surface and once at approximately mid-depth. Upon the completion of each tow, the zooplankton net was retrieved and all contents were rinsed into the cod-end from the sides of the net. Contents in the cod-end were then rinsed into a 330-μm sieve, transferred to a sample container and preserved with calcium carbonate-buffered 95% ethanol at a 1:1 sample:preservative ratio.

Sampling for trophic resources was conducted at each site in 2018 in each tributary from June through September. More limited sampling at P3 and C3 (Fig. 1) was conducted in 2019 during June and July. Larger size-classes of plankton were collected via vertical deployments of a 40-μm mesh plankton net through the water column. Size-fractionation was used to separate different components of the planktonic community for stable isotope analysis (Woodland et al. 2013). Contents in the cod-end were rinsed from the net and sieved using a series of 250, 150, 80 and 40-μm sieves. For this study, we present only the combined data from the > 250 μm and 150–250 μm size classes to target zooplankton prey. Size-fractionated plankton from each sieve were preserved with calcium carbonate-buffered 95% ethanol at a 1:1 sample:preservative ratio. Ambient water was collected just below the surface and poured through a 40-μm mesh sieve. The filtrate was then filtered onto a pre-ashed (500 ˚C for 4 hrs) Whatman™ glass fiber filter (0.7-μm nominal pore-size). This approach provided size-integrated sampling of two pelagic prey resources, zooplankton > 150 μm (ZOOP) and suspended particulate organic matter (SPOM) 40–0.7 μm. Benthic organic matter (BOM) was sampled using a small benthic grab

(Petite Ponar, sample area = 231 cm^2; Wildco). The grab was deployed over the side of the vessel then retrieved and examined. A subsample from the upper 5 mm of sediment was collected using a clean spatula if the sediment appeared undisturbed. The grab was redeployed if there was evidence of disturbance of the surface sediment. The sample was then transferred to a storage container and maintained on ice. BOM samples were collected from the main channel at all stations and from shallow (< 3 m) depths at a subset of stations.

2.4 Stable isotope methods and trophic niche indices

Mysids and potential prey groups were analyzed using standard stable isotope analysis methods. *Neomysis americana* specimens from ethanol-preserved samples were randomly selected for carbon (C) and nitrogen (N) stable isotope analysis (δ^{13}C, δ^{15}N). Studies on the effects of ethanol preservation on tissue stable isotope composition are equivocal with some studies noting an effect of preservation (e.g., Kelly et al., 2006; Le Bourg et al., 2019) while other have failed to find an effect (e.g., Hobson et al., 1997; Gloutney and Hobson 1998; Barrow et al., 2008). Specific to mysids, Gorokhova and Lehtiniemi (2007) found no effect of ethanol preservation on δ^{13}C or δ^{15}N composition of *Mysis relicta* after a 3-week preservation period. A sample size ranging from 10 to 30 individuals was analyzed from each combination of station and sampling trip, yielding a total of 86 mysids in 2018 and 62 mysids in 2019 (Table 1). Both juvenile and adult *N. americana* individuals were analyzed to allow for identification (if present) of consistent isotopic differences between size-classes. Individual mysids were dried in an oven at 60˚C and packed in tin capsules for stable isotope analysis. Size-fractionated ZOOP samples (> 150 μm) were dried at 60˚C, then homogenized and packed in tin capsules. Benthic grab samples were tested for inorganic carbonates by direct application of 1 N HCl to a small subsample of the sediment. Where present, inorganic carbonates were removed by acidifying subsamples of sediment in 50 ml centrifuge tubes with 1 N HCl for 1 hr before decanting and rinsing residual acid via serial centrifugation at 3000 rpm (Waldron et al., 2001). If acidification was required for δ^{13}C analysis of sediment organic matter, a separate unacidified sample was analyzed for δ^{15}N. All stable isotope measurements were conducted using a continuous-flow isotope ratio mass spectrometer paired with an elemental analyzer (EA-IRMS) at Chesapeake Biological Laboratory.

Mysid trophic niche was investigated using stable isotope-based estimates of resource contribution (benthic vs. pelagic), trophic niche area, and trophic position. Prior to all stable isotope analysis, mysid δ^{13}C values were mathematically corrected for lipid content. No specific correction equation is available for *N. americana*; therefore, we calculated lipid-corrected values from a mysid-specific model (Leggett, 1998) and a general aquatic fauna model (Post et al., 2007), then took the average of the two results. Estimated lipid-corrections from both models were strongly correlated (Pearson product-moment correlation, $r_P > 0.99$, $df = 145$, $p < 0.001$) and differed by an average of 0.17 ± 0.05 ‰.

The MixSIAR package for R was used to estimate the contribution of different prey resources to mysids in each month for each tributary in 2018 and 2019. MixSIAR implements multi-endmember mixing models using a Bayesian framework (Stock et al., 2018). Resource (SPOM, ZOOP, BOM) contributions to the diet of *N. americana* were modeled separately with BOM considered the only benthic resource, while SPOM and ZOOP were both considered pelagic resources. For each prey resource, stable isotope values were aggregated across stations and sample months in each tributary to provide a representative average of stable isotope conditions at the base of each food web. Trophic enrichment factors (TEFs) of 0.40 ± 0.14 ‰

for δ^{13}C and either 2.69 ± 0.54 ‰ (SPOM, BOM) or 3.55 ± 1.19 ‰ (ZOOP) for δ^{15}N were specified based on previously published research on diet-based differences in trophic enrichment of δ^{15}N for related taxa and from a meta-analysis (Toda and Wada, 1990; Gorokhova and Hansson, 1999; McCutchan et al., 2003). Tributary (2 levels) and a unique identifier for each Year-Month combination (5 levels, e.g., 2018-June, 2018-August) were included as class variables in the mixing model. Mysid size (dry weight) was initially considered as a potential covariate in the mixing model but was discarded after model diagnostics (Gelman-Rubin diagnostics > 1.1; Gelman et al., 2013) indicated poor model performance. The final mixing model was fitted using a 'residual only' error structure (Stock and Semmens, 2016).

Standard ellipse areas (SEA, ‰^{2}) derived from δ^{13}C and δ^{15}N stable isotope data were used as a proxy for trophic niche area of sampled mysids and prey sources (Jackson et al., 2011). Bayesian $\mathrm{SEA_{mysid}}$ estimates were generated by fitting bivariate normal distributions with vague normal priors to mysids from each combination of year, month and tributary. As initially estimated, $\mathrm{SEA_{mysid}}$ indices did not account for potential spatial or temporal differences in isotopic niche area at the base of local food webs; therefore, $\mathrm{SEA_{mysid}}$ values were standardized using SEA calculated for resource isotope data from each combination of year and tributary following the approach of Warry et al. (2016). This yields a final trophic niche area index ($\mathrm{Niche_{Area}}$) that represents the proportion of the available pelagic and benthic isotopic niche space occupied by mysids ($\mathrm{Niche_{Area}} = \mathrm{SEA_{mysid}}$ ‰^{2} / $\mathrm{SEA_{resources}}$ ‰^{2}).

Mysid trophic position (TP) was calculated using a three-source model: $\mathrm{TP} = \lambda_{(1\text{-}3)} + (\delta^{15}\mathrm{N_{Mysid}} - (\alpha_1 \times \delta^{15}\mathrm{N_{BOM}} + \alpha_2 \times \delta^{15}\mathrm{N_{SPOM}} + \alpha_3 \times \delta^{15}\mathrm{N_{ZOOP}})) / \Delta_{\mathrm{N}(1\text{-}3)}$, where α_1, α_2, and α_3 are the proportional contributions of BOM, SPOM, and ZOOP (respectively) for mysids from a given combination of tributary-year-month as estimated from the MixSIAR mixing model. The prey-weighted trophic position of the resources, $\lambda_{(1\text{-}3)}$, is calculated by multiplying prey-specific α-values by assigned trophic positions of 1.5 for BOM and SPOM (~1:1 mixture of basal resource and primary consumer biomass), and 2.0 for ZOOP (primary consumer). The serial enrichment of δ^{15}N across trophic transfers, $\Delta_{N(1\text{-}3)}$, is calculated by multiplying prey TEF-values (identified above) by prey-specific α-values.

2.5 Data Analysis

Summary estimates (means ± SD) of resource contribution and niche area from Bayesian posterior probability distributions were compared between tributaries using *t*-tests and ANOVA to determine if spatial differences in basal resource pathways or occupied niche area of mysids were present and if those differences changed over time. Tissue C:N ratios of mysids were also compared between tributaries using *t*-tests. Trophic position estimates for individual mysids were analyzed with a general linear model for both the Choptank and Patuxent rivers (GLM). Predictor variables included body size (dry weight, mg), C:N ratios, niche area, and benthic resource contribution. Parametric assumptions of residual normality and homoscedasticity were satisfied. Statistical significance was set to $\alpha = 0.05$ for all tests.

2.6 Environmental Setting

We assessed the potential effects of local physical conditions during the sampling period by examining patterns of riverine discharge measured for the Choptank and Patuxent rivers. For each river, we downloaded the daily river discharge from 1985 to 2020 as measured at respective United States Geological Survey stations (01491000, Choptank River near Greensboro, MD;

01594440, Patuxent River near Bowie, MD). We then computed daily long-term (1985-2020) median discharge and the 10th and 90th percentiles for each station and compared these to daily annual discharge for the years of sampling (2018, 2019). For the years 1998-2020, we also extracted available monthly surface water concentrations of particulate nitrogen (PN) and carbon (PC) measured at stations included in the Chesapeake Bay Program long-term monitoring program. For each river, the station nearest sampling station 3 was included (station ET5.1 in the Choptank, 38.80645 N, -75.90971 W; station RET1.1 in the Patuxent, 38.49090 N, -76.66429 W). To examine the effects of riverine inflows on the ratio of PN to PC in the water-column on a monthly time-scale, we performed linear regressions of the monthly riverine discharge and water-column PN:PC ratio (by weight) for each river.

3. Results

3.1 Habitat conditions

The annual hydrologic cycle in the Choptank and Patuxent watersheds was different for each year of sampling (Fig. 2). A large precipitation event in July and August of 2018 and subsequent high precipitation rates led to extremely high river discharge in both the Choptank and Patuxent rivers, beginning in late July of 2018. For each river, discharge was at or above the 90th percentile of previously-measured discharge frequently over the course of the latter half of 2018 (Fig. 2A, D). These high-discharge conditions persisted into the winter-spring of both rivers in 2019, but discharge returned to long-term mean conditions by mid-summer of 2019. The effect of riverine discharge on the ratio of particulate carbon and nitrogen was different for the two stations analyzed. For the Patuxent River station RET1.1, there was no significant relationship between discharge and the PN:PC ratio at a monthly scale (linear regression; $p=0.1$), but at the

Choptank River station ET5.1, there was a significant negative relationship (r=0.44, p<0.001) between monthly discharge and the PN:PC ratio (Fig. 2B, E). During the highest discharge months in the record (>15 m^3/s; n=6), however, PN:PC was elevated (Fig. 2E). Average DO concentrations were lower at depth at the sampling stations in the Patuxent River than the Choptank River (Fig. 2C, F). Average DO concentrations declined to or below 2 mg/L at the Patuxent stations P3 and P5 in both 2018 and 2019. At depths ≥ 5m, average annual DO was lower (two-sample *t*-test, $df = 18$, $t = 3.25$, $p = 0.004$) at Patuxent stations P3 and P5 (3.54 ± 2.29 mg/L) than the corresponding stations C3 and C5 in the Choptank River (6.17 ± 1.15 mg/L). Previous studies suggest that mysid distribution (and, therefore, foraging location) is most heavily affected by salinity gradient; however, salinity ranges in this study were comparable between sample collection sites (Patuxent P3 = 6.2 ± 2.5 [mean ± SD], Choptank C3 = 2.6 ± 1.4) and in both cases were within the tolerance range of *N. americana* (Pezzack and Corey, 1982; Schiariti et al., 2006; Chapina et al., 2020).

3.2 Prey Resources and Benthic-Pelagic coupling

Mixing model estimates showed spatial and temporal variability in prey contribution to mysid diet in each tributary (Fig. 3). Among the two pelagic prey types, SPOM typically contributed the least to mysid diet, with estimates of mysid diet proportion ranging from 0.04 to 0.27 in the Choptank River and 0.03 to 0.48 in the Patuxent River. SPOM contributions to mysid diet were highest during August 2018 in both rivers (Choptank = 0.27 ± 0.09 [SD]; Patuxent = 0.48 ± 0.08), coincident with high flow events during that period (Fig. 2). With the exception of June 2018 in the Patuxent River, ZOOP was the dominant pelagic prey in both rivers, ranging from 0.66 to 0.78 and from 0.45 to 0.94 in the Choptank and Patuxent rivers, respectively. In 2018, contributions of ZOOP to mysid diet were similar between tributaries in June and September 2018 (two-sample *t*-tests, $df \geq 22$, $t \geq 2.03$, $p > 0.05$) but ZOOP contribution in August was higher in Choptank River (0.71 ± 0.09) than Patuxent River mysids (0.45 ± 0.06; two-sample *t*-test, $df = 23$, $t = 8.03$, $p < 0.001$). In 2019, ZOOP contributed more to mysid diet in the Patuxent River (annual mean ± SD = 0.87 ± 0.10) than the Choptank River (0.72 ± 0.08).

Benthic (BOM) contributions to mysid diet were higher in the Choptank River than the Patuxent River (ANOVA, $F_{df = 1, 8} = 5.79$, $p = 0.04$) except in August 2018 (Fig. 3). In August 2018, Choptank River mysid BOM contribution declined sharply to 0.02 ± 0.01 (from 0.26 ± 0.08 in June), the lowest observed value in the study for that tributary. Otherwise, BOM contributions ranged from 0.19 to 0.34 in the Choptank River. Conversely, BOM contribution to Patuxent River mysid diet ranged from 0.03 to 0.13 with some evidence of a decreasing trend in contribution from early to late summer in both years for that tributary. With the exception of August 2018, BOM consistently contributed more to Choptank River mysids each month than Patuxent River mysids (two-sample *t*-tests, $df \geq 22$, $t \geq 4.85$, $p < 0.001$).

3.3 Niche Area Estimates

Trophic niche area of mysids differed between the Choptank and Patuxent rivers (Table 1). Average niche area of mysids across all sample months was 5.5% (range = 1.4-15.9%) in the Choptank River and 7.3% (range = 4.4-10.2%) in the Patuxent River. Maximum niche areas for both tributaries were observed in 2018, occurring in August for the Choptank (15.9 ± 10.45%) and equivalent values were observed in June and September for the Patuxent (10.1 ± 6.5% and 10.2 ± 7.5%, respectively). Niche areas were more consistent in 2019 (Table 1) and ranged from

1.5 ± 1.1% to 6.0 ± 4.8% in the Choptank River and 4.4 ± 3.2% to 5.8 ± 4.2% in the Patuxent River. The difference in mysid niche area between the Choptank and Patuxent rivers was the greatest in August 2018 (two-sample *t*-test, $df = 23$, $t = 2.88$, $p = 0.009$) as a result of a sharp increase in the niche area of Choptank River mysids. With the exceptions of August 2018 and June 2019 (two-sample *t*-test, $df = 28$, $t = 1.65$, $p = 0.28$), mysid niche areas were greater in the Patuxent River than in the Choptank River (Table 1).

3.4 Trophic Position

Trophic position of mysids differed between the Choptank and Patuxent rivers for all months and years measured in the study (Table 1). Average trophic position across all sample months and years was 3.48 ± 0.49 (range = 1.49-4.40) in the Choptank River and 2.89 ± 0.27 (range = 2.35-3.60) in the Patuxent River. Mysid trophic position was highest in the Choptank River in September 2018 (4.06 ± 0.18) and lowest in August 2018 (2.90 ± 0.50). Meanwhile, the highest mean trophic position for mysids in the Patuxent River occurred in August 2018 (3.24 ± 0.23) and the lowest occurred in June 2019 (2.62 ± 0.17). Mysid trophic position was consistently higher in the Choptank River throughout the study period except in August 2018 (two-sample *t*-test, $df = 21$, $t = -2.34$, $p = 0.029$) (Table 1).

Fitted GLMs for mysid trophic position in both the Choptank and Patuxent rivers identified significant relationships with the following predictor variables: body size (dry weight, mg), C:N tissue stoichiometry, niche area, and benthic resource contribution (Choptank GLM adjusted-$R^2 = 0.79$, *F*-statistic = 68.02, $p < 0.0001$; Patuxent GLM adjusted-$R^2 = 0.51$, *F*-statistic = 17.15, $p < 0.0001$; Table 2). For the Choptank River, both body size and benthic resource contribution were positively correlated with mysid trophic position ($p = 0.002$ and $p =$

0.0004), while both niche area and C:N ratio were significantly negatively correlated with mysid trophic position ($p < 0.0001$). For the Patuxent River, body size was not significantly correlated with mysid trophic position ($p = 0.08$). Unlike the Choptank River GLM, benthic resource contribution was negatively correlated with mysid trophic position ($p < 0.0001$) in the Patuxent River, and niche area was positively correlated with mysid trophic position ($p = 0.02$). Mysid C:N ratios were also negatively correlated with mysid trophic position in the Patuxent River ($p < 0.0001$).

4. **Discussion**

Patterns in the trophic indices we examined in this study suggest *N. americana* is an ecologically flexible consumer capable of exploiting spatially- and temporally-variable resources. Despite this flexibility, we show evidence that local environmental conditions can constrain feeding by *N. americana*, leading to changes in resource coupling, trophic position, and realized niche dimensions. Given the central role of mysids in coastal food webs as consumers that couple disparate energy channels and as prey that serve to distribute energy to predators occupying multiple habitats, environmental disruption of the ecological niche of mysids could have consequences for nutrient cycling and ecosystem productivity.

Analysis of stable isotope data revealed *N. americana* utilized both benthic and pelagic trophic pathways, confirming the putative trophic role of this mysid species as a coupler of distinct energy pathways. Overall, the pelagic trophic pathway contributed an average of 85 % to the assimilated diet of *N. americana*, whereas the average contribution of the benthic trophic pathway was only 15 %. The contribution of the benthic vs. pelagic trophic pathways was also correlated with trophic position in this study, with higher trophic positions generally observed in mysids utilizing and assimilating more of the benthic trophic pathway and lower trophic positions observed in mysids utilizing and assimilating more of the pelagic trophic pathway, (e.g., zooplankton prey). The importance of the pelagic trophic pathway, and the dominance of zooplankton in particular, to the diet of *N. americana* was observed in both tributaries and is consistent with previous studies investigating feeding by this and other species of mysids (Fulton, 1982; Fockedey and Mees, 1999; Winkler et al., 2003; Sierszen et al., 2011; O'Malley and Stockwell, 2019). Using diet choice experiments supported by field-based stable isotope observations in the St. Lawrence River, Winkler et al. (2007) showed *N. americana* preferred small zooplankton prey such as veliger-stage bivalve larvae, rotifers, and copepod nauplii in the presence of a mixed prey assemblage that included large and small zooplankton taxa. In the Winkler et al. (2007) study, limited predation on the benthic copepod *Halectinosoma curticorne* (Harpacticoida) was observed, but the experiments were not designed to test for consumption of detrital organic matter (suspended or settled) or phytoplankton. Still, other work has demonstrated mysids in the *Neomysis* genus (including *N. americana*) are opportunistic omnivores that exploit a wide range of non-zooplankton prey resources, including pollen, colonial and unicellular phytoplankton, macrophyte detritus, nematodes, gastropod larvae, suspended sediment floc material, and benthic organic matter (Zagursky and Feller, 1985; Fockedey and Mees, 1999; Winkler et al., 2003; Vilas et al., 2008), an expansive potential forage base that supports the variability we observed in mysid trophic energy pathway utilization patterns and trophic position estimates.

The propensity of *N. americana* to use such a broad range of prey suggests this species can exploit resources where and when they become available and serves as a dynamic structuring component of coastal food webs. To put this in context, two previously published food webs identified trophic position (TP) and proportional benthic pathway contributions (BP coupling) from Chesapeake Bay (Rooney et al., 2006) and Maryland's shallow coastal ocean (< 20 m, Woodland and Secor, 2013). In those studies, TP ranged 1.9–3.9 (Chesapeake Bay) and 2.0–4.0; (MD coastal ocean), while BP coupling ranged 0–1 in both ecosystems (Fig. 4). In the Choptank and Patuxent tributaries alone, *N. americana* spans ~75 % of the community-level range in TP (i.e., 2.6–4.1) and ~30% of the community-level range in BP coupling (i.e., 0.02–0.34). While not exhaustive, the range of values presented in the previous studies underscore the relative flexibility of *N. americana*'s trophic ecology and its ability to realize a diverse range of trophic niches. It is interesting to note that the proposed mechanism underlying the modal TP-BP coupling relationship at the food web scale, where large mobile predators (high TP) are better able to exploit spatially and (or) temporally disjunct trophic pathways, is not likely to apply to the *N. americana* in this study because of the intraspecific nature of these data. While the specific mechanism supporting this relationship for mysids is not clear, it is possible greater access to benthic food webs allows mysids to selectively increase the proportion of animal prey in their diet.

The reduced dependence on benthic prey sources by mysids in the Patuxent River corresponded with lower oxygen concentrations in bottom-waters of that tributary during the months of the study. The ecological effects of hypoxia on benthic habitats are pervasive, including reduced benthic biodiversity and secondary productivity arising from ecotoxicological effects (lethal and sublethal) on benthic fauna and altered food web dynamics as a consequence of lost foraging opportunities and habitat availability for pelagic and bentho-pelagic predators (Officer et al., 1984; Diaz and Rosenberg, 1995; Breitburg, 2002; Gray et al., 2002; Rabalais et al., 2002; but see Pihl et al., 1992 for an example of hypoxia favoring some mobile predators;

Luther et al., 2004). The net effect of hypoxia is often a weakening of benthic-pelagic coupling within coastal food webs, and the prevalence of hypoxia in the Patuxent River during the study period provides a likely explanation for the reduced contribution of benthic trophic pathways to mysids in that system. With the annual recurrence of seasonal hypoxia affecting much of the near-bottom habitat in the mid-to-lower reaches of the Patuxent River estuary (< 2 mg/L, Testa et al., 2008) and the pulsed nature of seasonal pelagic production in the region (Stross and Stottlemyer, 1965; Sin et al., 1999; Werdell et al., 2009), the loss of benthic trophic connectivity could have negative consequences for mysid growth and population dynamics.

Mysids are just one component of a community of mobile predators in the Chesapeake Bay ecosystem that integrate both benthic and pelagic pathways. A previous analysis of fish community biomass size spectra concluded pelagic production alone was insufficient to support field observations, and benthic trophic subsidies were required to explain predatory fish biomass distributions in the upper and lower Chesapeake Bay mainstem but not the hypoxia-prone mid-Bay region (Jung and Houde, 2005). Similar spatially-dependent benthic-pelagic decoupling has been observed in the Baltic Sea, where hypoxia linked to eutrophication has dramatically increased in many sub-pycnocline areas of the basin (Carstensen et al., 2014), leading to reductions in benthic productivity (Elmgren, 1989; Karlson et al., 2002) that have been linked to lower benthic-pelagic coupling (Kiljunen et al., 2020) and reduced physiological condition among higher trophic levels (Karlson et al., 2020). In contrast to conditions in the Patuxent River, bottom-water DO concentrations and benthic food contributions at the Choptank River study sites were considerably higher throughout the study period, consistent with long-term trends for that system (Fisher et al., 2006a) and well above the 2 mg/L threshold for hypoxia.

Interestingly, ratios of carbon to nitrogen (C:N) in sampled mysids were consistently higher in the Choptank River across all sample months (Table 1). C:N ratios can serve as a proxy for lipid content within a population, where an increase in carbon corresponds with an increase in tissue lipid concentration (DeNiro and Epstein, 1977; Post et al., 2007). Paterson et al. (2022) evaluated the lipid contents of *Mysis diluviana* specimens from 19 different lakes in southern Ontario and found significant differences between populations, concluding *M. diluviana* with higher total lipid concentrations were exposed to a higher quality of primary production. Given that *N. americana* in the Choptank River exhibited consistently higher ratios of whole-body C:N across sample months, it can be inferred that conditions within the Choptank River were more favorable than in the Patuxent River for mysid foraging during the study period, potentially as a result of greater access to benthic food webs. Further, forage species with greater lipid content per unit body mass are often considered better quality prey for predators due to the key role lipids play in energy storage and transformation (Spitz et al., 2010; Euclide et al., 2017). This suggests the *N. americana* population in the Choptank River could be energetically superior to the Patuxent population as forage for predatory fish on a per unit mass basis.

The lack of a clear pattern in niche area between tributaries provides further evidence of *N. americana* mysids as ecologically flexible consumers whose feeding responds primarily to the relative availability of, or access to, various prey over time. In Lake Ontario, O'Malley et al. (2017) found that, while *M. diluviana* prey is dominated by pelagic taxa with cyclopoid copepods and bosminids constituting roughly 60 % of their summertime diet, the mysid displays a highly omnivorous and opportunistic diet that incorporates new prey items as they become available. We found evidence of similar trophic flexibility in *N. americana,* reflected in decreased carbon isotope values following sustained high discharge conditions associated with persistent precipitation in the Chesapeake Bay region in August and September 2018 (Fig. 2;

Table 1). In this study, all SPOM ≤ 40 μm was considered the basal component of pelagic trophic pathways; however, suspended particles in this range can include detrital organic matter in addition to fresh phytoplankton biomass. During precipitation events, overland flows and increased river discharge can mobilize and transport detrital material from terrestrial, wetland, and upstream sources to downstream estuaries. In the study area, fringing wetlands are a potentially important source of detrital vegetative matter (and associated biofilms), which can provide trophic subsidies for proximal pelagic food webs (Heinle and Flemer, 1975). Unlike detritus-based benthic pathways that are typified by refractory material with enriched δ^{13}C values, this newly mobilized detritus derived from terrestrial or emergent vegetation often has lighter (more depleted) δ^{13}C composition. A decline in particulate PN:PC with increasing discharge is often associated with increased concentrations of suspended detritus relative to fresh phytoplankton in the water column, a pattern observed in the Choptank River concomitant with a decline in mysid δ^{13}C values and an increase in estimated SPOM diet contribution (Figs. 2 & 4). In addition to terrestrial or wetland sources of detritus, it is possible isotopically lighter planktonic biomass originating in or upstream of the estuarine turbidity maximum (ETM) was advected downstream, contributing to the subsequently lighter δ^{13}C values of mysids in the Choptank River (Fry, 2002).

5. **Conclusions**

Taken together, our results emphasize the importance of *N. americana* as a dynamic, ecologically flexible consumer capable of feeding throughout the water column and exploiting pulsed trophic subsidies when available. The ability to rapidly adapt diet and foraging behavior conveys a powerful ecological advantage, allowing consumer populations to persist in the face of asynchronous trophic resource availability (Rooney et al., 2006). At the community level, coastal food webs are stabilized by mobile consumers, such as mysids, capable of coupling pelagic trophic pathways, with high biomass and fast turnover rates, and benthic trophic pathways that experience slower turnover (Rooney et al., 2006; Rooney et al., 2008; Rooney and McCann, 2012). Our study indicates that, at least for *N. americana*, this ecological function declines as bottom waters become less oxygenated. In this case, we found that benthic trophic pathways contributed more to *N. americana* diet in the Choptank than the Patuxent River. Further, Choptank *N. americana* typically occupied higher trophic positions and possessed C:N stoichiometry suggestive of higher lipid content than the Patuxent population. While our focus here was on population-level responses, recent work suggests that the trophic effects of coastal hypoxia can vary within populations (Steube et al., 2021), and future research on individual-level dynamics could yield further insights about the effects of hypoxia on *N. americana*. In the face of future climate uncertainty and the continued frequency and magnitude of hypoxic events in many coastal ecosystems, food web structures are likely to continue changing, following the trajectories of trophic pathway decoupling and altered productivity (e.g., Kemp et al., 2005; Kiljunen et al., 2020; Woodland et al., 2021).

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Tables

Table 1. Mean ± SD for carbon and nitrogen stable isotope ratios (δ^{13}C, δ^{15}N), trophic position, trophic niche area (Niche area, %), and C:N tissue ratio for *Neomysis americana* by month and year in the Choptank (CHOP) and Patuxent (PAX) river estuaries. Asterisks show significant between-river pairwise differences by month (based on two-sample t-tests, $\alpha = 0.05$). Statistical testing not applied to raw isotope data or summarized annual and total data.

		δ^{13}C		δ^{15}N		Trophic position		Niche area		C:N ratio	
Year	Month	CHOP	PAX	CHOP	PAX	CHOP	PAX	CHOP	PAX	CHOP	PAX
2018	6	-27.0 ± 0.3	-26.3 ± 1.0	17.0 ± 0.4	16.1 ± 0.5	3.7 ± 0.1	2.8 ± 0.2 *	1.4 ± 0.9	10.1 ± 6.5 *	4.0 ± 0.1	4.0 ± 0.1
	8	-30.2 ± 0.7	-26.5 ± 0.5	17.9 ± 1.7	15.4 ± 0.7	2.9 ± 0.5	3.2 ± 0.2	15.9 ± 10.4	5.8 ± 4.3 *	3.5 ± 0.8	3.0 ± 0.1
	9	-26.0 ± 0.4	-26.7 ± 1.0	16.8 ± 0.6	17.0 ± 0.8	4.1 ± 0.2	3.0 ± 0.2 *	2.8 ± 1.9	10.2 ± 7.5 *	3.4 ± 0.5	3.2 ± 0.2
2019	6	-27.2 ± 0.3	-25.4 ± 0.5	17.1 ± 1.3	16.8 ± 0.6	3.3 ± 0.4	2.6 ± 0.2	6.0 ± 4.8	4.4 ± 3.2	4.3 ± 0.2	3.8 ± 0.1 *
	7	-27.4 ± 0.3	-24.9 ± 0.7	18.1 ± 0.3	17.7 ± 0.5	3.4 ± 0.1	2.9 ± 0.2 *	1.5 ± 1.2	5.8 ± 4.2 *	4.1 ± 0.2	3.8 ± 0.1 *
Summary	2018	-27.7 ± 0.7	-26.5 ± 0.9	17.2 ± 0.9	16.2 ± 0.8	3.6 ± 0.5	3.0 ± 0.4	6.7 ± 2.1	8.7 ± 2.5	3.6 ± 0.7	3.4 ± 0.4
	2019	-27.3 ± 0.5	-25.1 ± 0.8	17.6 ± 0.9	17.3 ± 0.7	3.4 ± 0.5	2.8 ± 0.4	3.7 ± 1.7	5.1 ± 1.9	4.2 ± 0.4	3.8 ± 0.3
	Total	-27.6 ± 0.6	-25.9 ± 0.9	17.4 ± 0.9	16.6 ± 0.8	3.5 ± 0.5	2.9 ± 0.4	5.5 ± 2.0	7.3 ± 2.3	3.9 ± 0.6	3.6 ± 0.3

Table 2. General linear model results for trophic position (dependent variable) of *Neomysis americana* from the Choptank and Patuxent river estuaries. Asterisks indicate parameter significance for the intercept (β_0), proportional benthic pathway contribution (BOM), body dry weight (Wt), tissue C:N stoichiometric ratio (CN), and niche area (Area) at $\alpha = 0.05$.

GLM model results		Choptank		Patuxent	
Model performance	adj-R^2	0.78		0.48	
	RSE	0.23		0.19	
	df	4, 72		4, 65	
	F	68.02		17.15	
	p	<0.0001		<0.0001	
Parameter estimates	β_0	4.86 (0.28)	*	4.18 (0.26)	*
	BOM	1.39 (0.37)	*	-1.93 (0.68)	*
	Wt	0.83 (0.26)	*	0.19 (0.11)	
	CN	-0.46 (0.05)	*	-0.38 (0.07)	*
	Area	-3.74 (0.84)	*	2.42 (1.04)	*

Figure Legends

Figure 1. Maps showing the location of the Choptank (CHOP) and Patuxent (PAX) rivers relative to the Chesapeake Bay ecosystem (A). Sampling stations in the PAX (B, P1-P5) and CHOP (C, C1-C5) are indicated by filled circles. Chesapeake Bay Program long-term water quality monitoring station locations and codes are provided in B (PAX: TF1.6 & TF.1.5 [upper-river of inset], RET1.1, LE1.3) and B (CHOP: ET5.1, ET5.2). Mean seasonal densities of *Neomysis americana* at PAX (D) and CHOP (E) Chesapeake Bay Program Mesozooplankton Monitoring Survey stations (1984-2002, https://www.chesapeakebay.net).

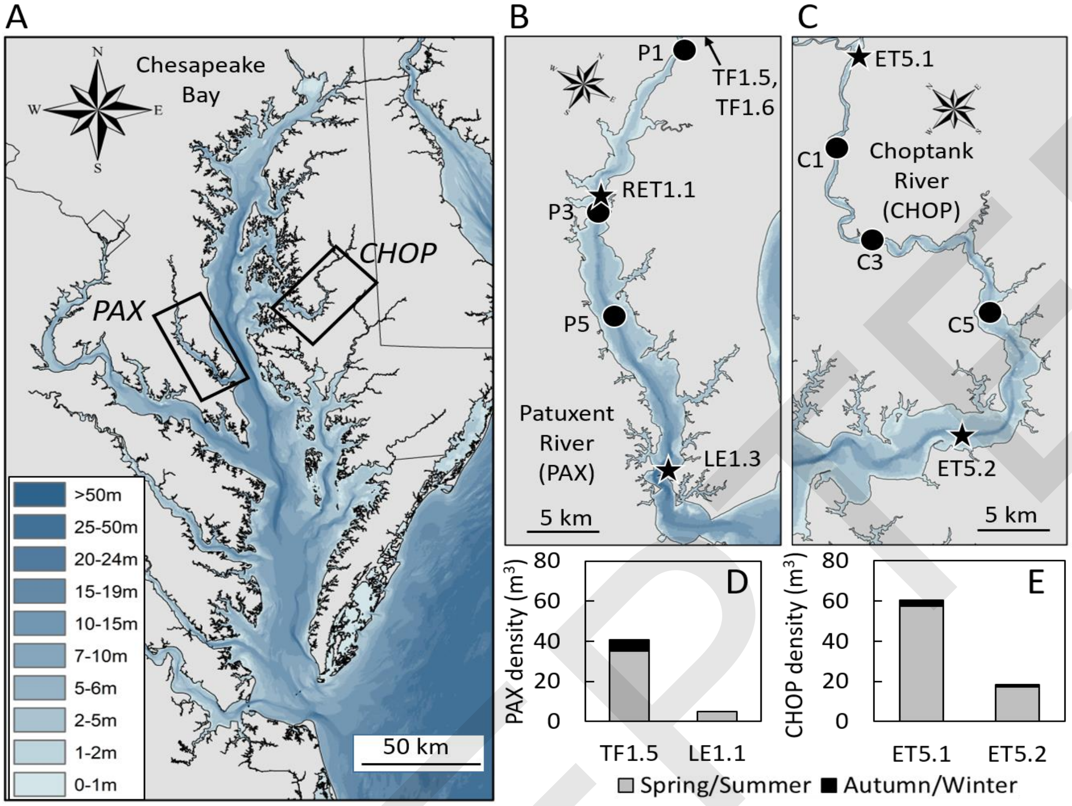
Figure 2. Measured daily discharge over 2018 (green circles) and 2019 (yellow circles) as measured at respective United States Geological Survey stations (01491000, Choptank River near Greensboro, MD; 01594440, Patuxent River near Bowie, MD) as well as long term daily median river discharge from each station (solid black line) with 10th and 90th percentiles (dashed lines) from 1985 to 2020 (A, D). Relationship between mean monthly river discharge and the monthly surface water PN:PC ratio (by weight) measured at a representative station in each river during the 1998–2020 period (B, E). Annual average dissolved oxygen (DO) concentration profiles collected at each representative station in 2018 and 2019 in the Choptank and Patuxent rivers with 2 mg/L hypoxia threshold indicated by vertical dashed line (C, F).

Figure 3. Stable isotope ratio biplot showing the relationship between monthly *Neomysis americana* δ^{13}C and δ^{15}N values and basal resources (Pelagic: SPOM, ZOOP, Benthic: BOM; mean ± SD [error bars]) between the Choptank (black) and Patuxent (red) rivers. Choptank 2018 (upper left), Pax 2018 (upper right), Choptank 2019 (lower left), Pax 2019 (lower right).

Figure 4. Mixing model estimates of monthly proportional diet contributions (± SD) of benthic organic matter (BOM; A), suspended particulate organic matter (SPOM: B), and zooplankton > 150-μm (ZOOP; C) to *Neomysis americana* from the Choptank and Patuxent rivers. Dashed lines indicate grand means for resources in each tributary (black = Choptank, red = Patuxent), asterisks show significant pairwise differences ($\alpha = 0.05$).

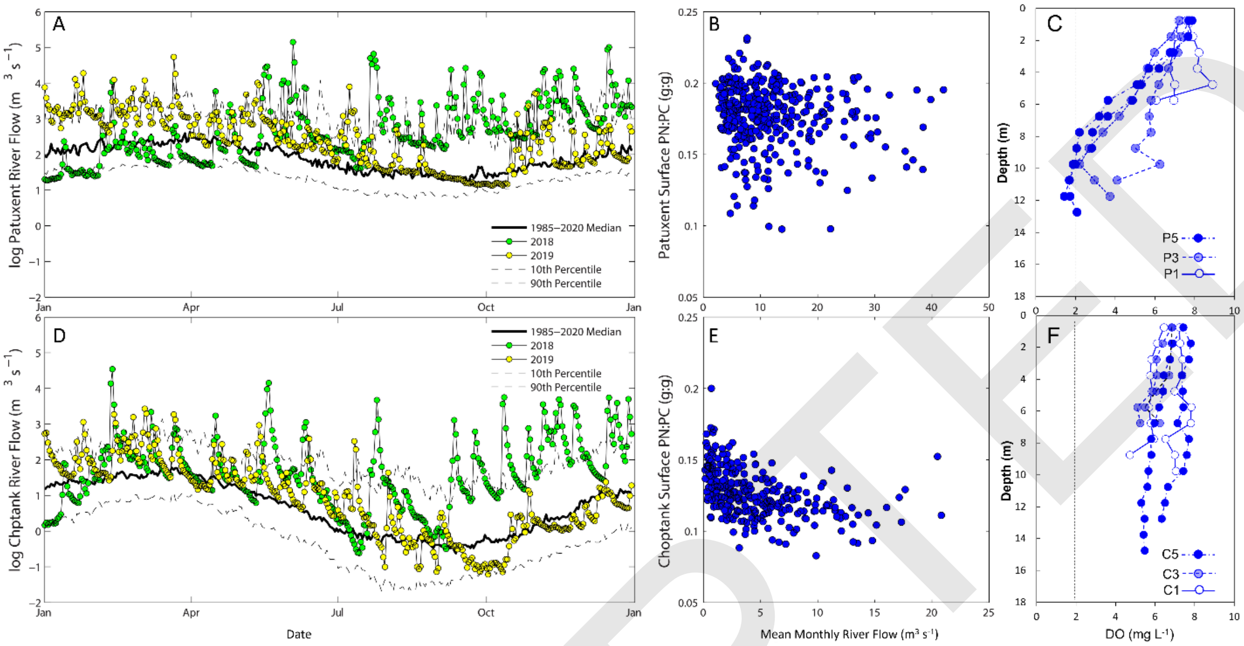
Figure 5. Estimates of trophic position and monthly proportional diet contribution of benthic organic matter (± SD) to *Neomysis americana* from the Choptank (CHOP) and Patuxent (PAX) rivers in this study. Empty symbols show previously published, assemblage-level trophic position and benthic contribution estimates from Chesapeake Bay (orange symbols; Rooney et al., 2006 [interpolated from Supplementary Figure 1a]) and Maryland's coastal ocean (blue symbols; Woodland and Secor, 2013 [interpolated from Figure 2, Table 2]). Dashed lines show least-squares fitted quadratic regressions for previous studies (orange: Chesapeake Bay $R^2 = 0.33$; blue: MD coastal ocean $R^2 = 0.33$).

Figure 1.



A
Chesapeake Bay
N
S
W
E
PAX
CHOP
>50m
25-50m
20-24m
15-19m
10-15m
7-10m
5-6m
2-5m
1-2m
0-1m
50 km
B
P1
TF1.5, TF1.6
RET1.1
P3
P5
Patuxent River (PAX)
LE1.3
5 km
C
ET5.1
C1
Choptank River (CHOP)
C3
C5
ET5.2
5 km
D
PAX density (m³)
80
60
40
20
0
TF1.5
LE1.1
E
CHOP density (m³)
80
60
40
20
0
ET5.1
ET5.2
Spring/Summer
Autumn/Winter

Figure 2.


A
B
C
D
E
F
log Patuxent River Flow (m3 s−1)
log Chptank River Flow (m3 s−1)
1985−2020 Median
2018
2019
10th Percentile
90th Percentile
Jan
Apr
Jul
Oct
Jan
Date
Patuxent Surface PN:PC (g:g)
Choptank Surface PN:PC (g:g)
Mean Monthly River Flow (m³ s⁻¹)
Depth (m)
P5
P3
P1
C5
C3
C1
DO (mg L⁻¹)

850 **Figure 3.**

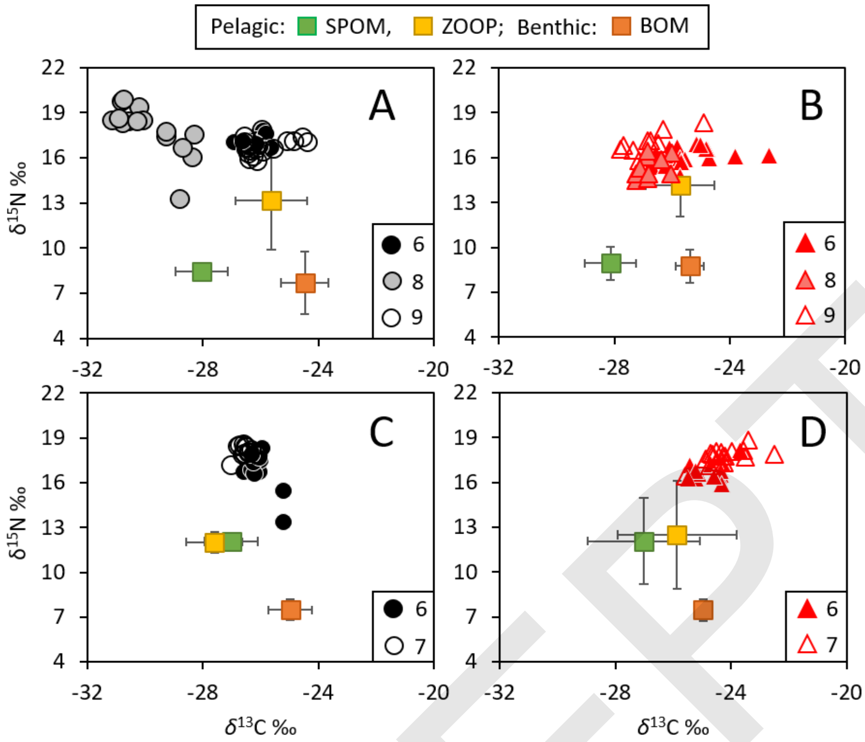


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Figure 4.

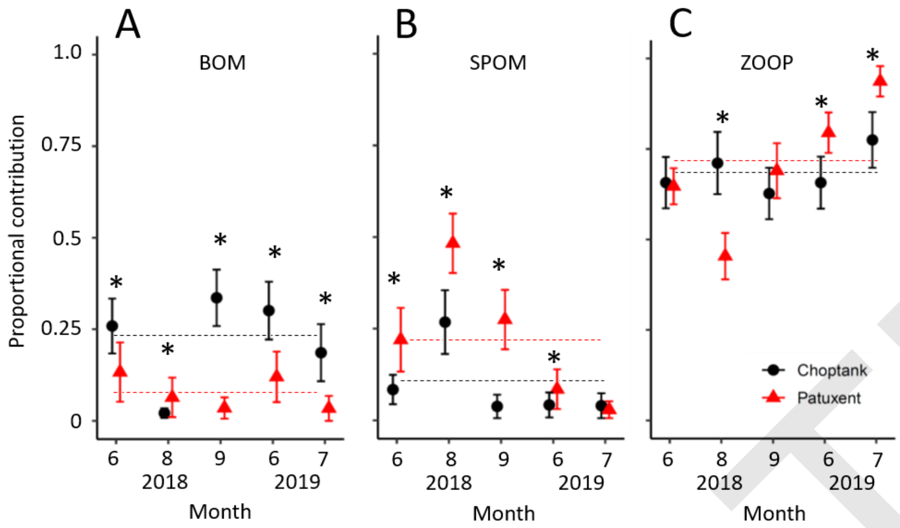
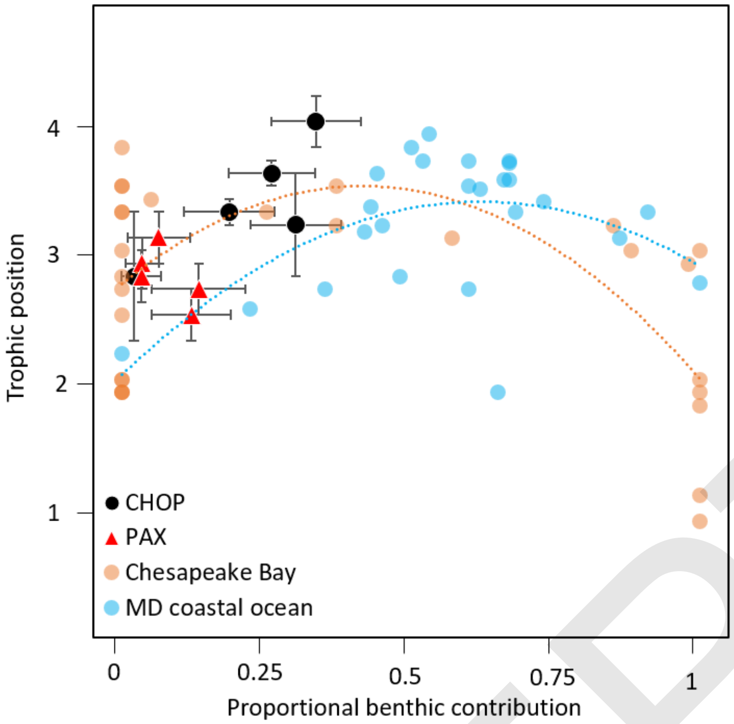

A
B
C
BOM
SPOM
ZOOP
Proportional contribution
1.0
0.75
0.5
0.25
0
*
6
8
9
6
7
2018
2019
Month
Choptank
Patuxent

Figure 5



Trophic position
Proportional benthic contribution
4
3
2
1
0
0.25
0.5
0.75
1
● CHOP
▲ PAX
● Chesapeake Bay
● MD coastal ocean