



Carbon and nitrogen stable isotopes of copepods in a tidal estuarine system in Maryland, USA

Blessing O. Edje, Paulinus Chigbu*

NSF CREST-Center for the Integrated Study of Coastal Ecosystem Processes and Dynamics in the Mid-Atlantic Region, Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21853, USA

NOAA Living Marine Resources Cooperative Science Center, Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21853, USA



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ABSTRACT

The carbon and nitrogen stable isotope ratios of copepods, particularly *Acartia tonsa* and suspended particulate organic matter (SPOM) samples, collected seasonally (2014 to 2017) from 13 sites in the tidal estuaries of Maryland were analyzed. We hypothesized that copepods at sites close to the mouths of lagoon tributaries have more depleted $\delta^{13}\text{C}$ than copepods at sites near the inlets, and that variations in the $\delta^{13}\text{C}$ values of copepods mirror that of the SPOM. Copepod $\delta^{13}\text{C}$ values ranged from -27.8‰ to -19.4‰ , and that of SPOM from -26.8 to -20.3‰ . Mean copepod $\delta^{13}\text{C}$ value ($-21.8\text{‰}\pm 0.4$) at a site close to the ocean was the highest, whereas the lowest value ($-24.4\text{‰}\pm 0.5$) occurred at a site located at the mouth of St. Martin River. $\delta^{15}\text{N}$ copepod values varied from 2.9‰ to 13.0‰ (mean= $9.2\text{‰}\pm 0.1$); that of SPOM was -0.58 to 10.51‰ (mean= $5.7\text{‰}\pm 0.2$). Reduction in the $\delta^{15}\text{N}$ mean values from a site near the Ocean City inlet ($10.2\pm 0.4\text{‰}$) to a site close to the tributaries ($8.5\pm 0.6\text{‰}$) suggests that copepods at the site near Ocean City inlet likely fed on food items that contained more marine phytoplankton with enriched $\delta^{15}\text{N}$ and heterotrophic protists, than copepods at a site near the river mouth. The highest ($-21.9\text{‰}\pm 0.4$) and lowest ($-25.8\text{‰}\pm 0.3$) mean copepod $\delta^{13}\text{C}$ values were observed in summer 2015 and winter 2017, whereas the highest ($10.8\text{‰}\pm 0.2$) and lowest ($7.1\text{‰}\pm 0.7$) mean copepod $\delta^{15}\text{N}$ values were recorded in winter and fall 2016, respectively. $\delta^{15}\text{N}$ enrichment between copepods and SPOM ranged from 1.0‰ to 8.9‰ (mean: 3.4‰). The highest enrichment observed in February 2016 stemmed from a reduction in SPOM $\delta^{15}\text{N}$ values, after a major storm event, which was unaccompanied by a decrease in *Acartia* $\delta^{15}\text{N}$. Copepod and SPOM $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, displayed similar spatial patterns and were positively correlated; enrichment at sites near the ocean and depletion near the river mouth indicating that SPOM composition influenced copepods diets in the lagoons.

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1. Introduction

Copepods, the most abundant group of mesozooplankton in estuarine and marine environments, perform important trophic functions, including the transfer of phytoplankton production to fish production (Kleppel, 1993; Chauvelon et al., 2014). Invertebrates such as mysids, ctenophores, and jellyfish prey upon copepods (Fulton, 1983; Purcell and Decker, 2005; Kimmel et al., 2012). The larvae, post-larvae, and juvenile stages of most species of fish, and adults of planktivorous fishes such as bay anchovy (*Anchoa mitchelli*) and European anchovy (*Engraulis encrasicolus*)

also consume copepods (Turner, 1984; Hartman et al., 2004; Chauvelon et al., 2014; Baeta et al., 2017). Copepods feed on diverse food items that include diatoms, dinoflagellates, protozoans and detritus (Roman, 1977, 1984; Kleppel and Pieper, 1984; Kleppel et al., 1988; Kleppel, 1992, 1993; Stoecker and Capuzzo, 1990). They are able to switch their feeding behavior from herbivory to omnivory or carnivory, and vice versa as plankton composition in the environment changes (Gifford and Dagg, 1988; Kiorboe et al., 1996; Gentsch et al., 2009; Goncalves et al., 2012; Chen et al., 2017). The quantity and nutritional quality of food items in the environment therefore, influence copepod diet composition (Kleppel, 1993; Derisio et al., 2014), production (Stottrup and Jensen, 1990; Kleppel et al., 1991; Fileman et al., 2010) and nutritional value to the upper level consumers.

Several studies have used stable isotopes of carbon and nitrogen to examine the trophic ecology and feeding relationships

* Correspondence to: NOAA Living Marine Resources Cooperative Science Center, Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21853, USA.

E-mail addresses: blessingedje@gmail.com (B.O. Edje), pchigbu@umes.edu (P. Chigbu).

of zooplankton in various aquatic systems. For example, [Montoya et al. \(1990\)](#) reported that variations of $\delta^{15}\text{N}$ of *Acartia tonsa* mirrored changes in the $\delta^{15}\text{N}$ of particulate nitrogen in the Chesapeake Bay. [Mukherjee et al. \(2018\)](#) investigated the influence of freshwater discharge on the diets of copepods in the Godavari Estuary, Bay of Bengal, and observed copepod $\delta^{13}\text{C}$ values to be more depleted during wet season than in dry season. [Schell et al. \(1998\)](#) reported spatial differences in the stable isotopes of carbon and nitrogen content of copepods in the Chukchi/Beaufort Sea regions. Furthermore, using $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, [Lopez-Ibarra et al. \(2018\)](#) investigated the trophic structure of copepod species in the eastern tropical Pacific Ocean and found low niche overlap among them, as well as a northward increase in their $\delta^{15}\text{N}$ values. In Riodela Plata, Argentina-Uruguay, [Derisio et al. \(2014\)](#) found that *A. tonsa* in the turbidity front, where Chl. *a* level was low, fed on detritus and had relatively low egg production rate, whereas seaward from the turbidity front where Chl. *a* level was high, the copepods fed on phytoplankton and had higher egg production rate. These studies indicate that copepod diets vary spatially and temporally in relation to environmental factors, and justify the need for further studies on the feeding ecology of copepods, especially in transitional waters. Investigations into the diets of copepods, based on their nitrogen and carbon isotope signatures, in the eutrophic, polyhaline, tidal lagoons of the northeastern United States such as the Maryland Coastal Bays (MCBs) have received little attention.

In the MCBs, the copepod community composition differs from that of the coastal ocean and river-dominated estuaries, such as the Chesapeake Bay and Delaware Bay, because the assemblage is dominated by *A. tonsa*, followed by *Centropages* spp. and *Eurytemora affinis* ([Oghenekaro et al., 2018](#)). The water quality condition of the bays has changed in the past two decades due to human activities in the watershed, and nutrient recycling within the bays, which has affected phytoplankton composition and biomass ([Wazniak et al., 2007](#); [Oseji et al., 2018](#)). Elevated concentrations of nutrients, especially NH_4^+ have caused phytoplankton biomass and the proportions of picoplankton and nanoplankton to increase ([Glibert et al., 2014](#); [Oseji et al., 2018, 2019](#)). A major peak in phytoplankton biomass occurs in the summer, followed sometimes by a minor peak in the winter ([Boynnton et al., 1996](#); [Oseji et al., 2018](#)). Spatially, phytoplankton biomass is higher in the areas of MCBs that are closer to the mouths of tributaries, especially in the northern bays and Newport Bay, than at sites closer to the inlets ([Oseji et al., 2018](#)).

[Duan et al. \(2015\)](#) reported higher dissolved organic carbon (DOC) levels in areas near the mouths of tributaries than at the inlets, and concluded that MCBs may be a major source of DOC to the coastal ocean. Much of the freshwater inflow into the bays is from groundwater, with comparatively less amounts entering directly into the bays from the tributaries. Nevertheless, direct freshwater discharge into the bays is higher from fall through spring than in the summer ([Oseji et al., 2019](#)), which influences the amounts and distribution of terrestrially derived and riverine particulate organic matter as well as salinity in the bays. It is unknown the extent to which spatial and temporal variations in the composition and amounts of suspended particulate organic matter in the bays influence the diet of copepods.

Stable isotopes of carbon are useful for inferring sources of organic matter, whereas nitrogen stable isotopes can be used to determine the trophic level of consumers ([De Niro and Epstein, 1978](#); [Minagawa and Wada, 1984](#); [Post, 2002](#)). The objectives of this study were to: (1) Determine spatial variations in the stable C and N isotope ratios of copepods, (2) Assess temporal variation in the stable C and N isotope ratios of copepods, and (3) Evaluate whether spatial and temporal variations in the stable C and N isotope ratios of copepods are related to variations in the isotopic

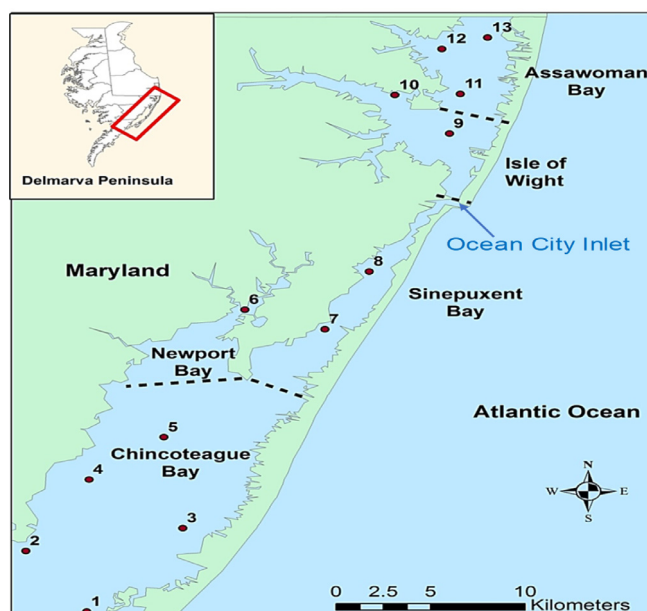


Fig. 1. Map of the Maryland Coastal Bays showing sampling sites.

ratios of SPOM. We hypothesized that copepods inhabiting MCBs sites close to the mouths of tributaries have more depleted C than those at sites close to the ocean, and that seasonal and inter-annual variations in the stable C and N isotope ratios of copepods are related to the composition of the SPOM.

2. Materials and methods

2.1. Study area

The Maryland Coastal Bays (Fig. 1) are well-mixed, shallow (mean depth of about 2 m) estuaries located in the eastern region of United States ([Duan et al., 2015](#)). The Bays are divided into two main regions (north and south) by the Ocean City inlet. The northern region of the Bays includes St. Martin River, Isle of Wight Bay, and Assawoman Bay, whereas the southern region of the Bays includes Sinepuxent, Newport, and Chincoteague Bays. The Ocean City and Chincoteague inlets connect the Bays directly to the Atlantic Ocean. The salinity in the open water portions of the Bays is similar to that of the coastal ocean while the upstream portion is freshwater ([Wazniak and Hall, 2005](#)). In the northern part of the Bays, land use is mostly urban while in the south, land use is mainly forest and agriculture ([Wazniak et al., 2007](#); [Duan et al., 2015](#)).

2.2. Sample collection and preparation

Samples for suspended particulate organic matter (SPOM) and copepods were collected at the same time from 13 sites (Fig. 1) at least once every season over multiple years from October 2014 to October 2017; winter (February); spring (April/May); summer (July), and fall (October). Zooplankton samples were collected by horizontally towing a plankton net with 200 μm mesh size net for 2 min. The zooplankton were concentrated and stored in ice until reaching the laboratory, where the samples were stored in -80°C for a period of 1 to 4 weeks until they were analyzed. Based on the relative abundance of the copepods, *A. tonsa* was the only species used in this study except in April 2015, when *C. hamatus* was the only species used, and in February 2016 and 2017, when both species were used.

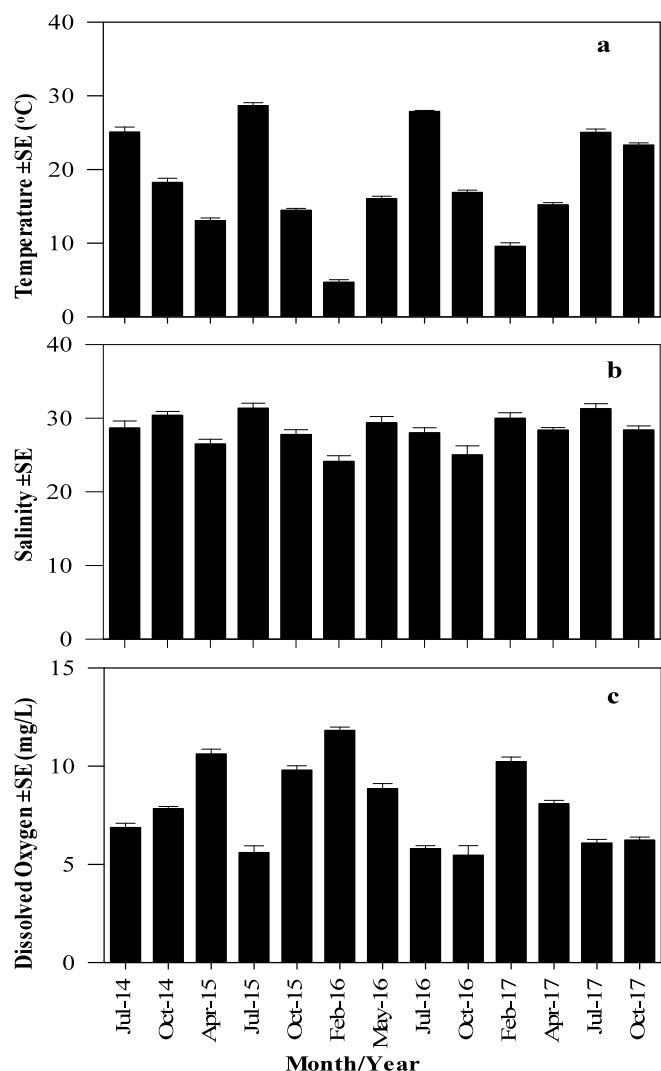


Fig. 2. Temporal variation in temperature (a), salinity (b) and dissolved oxygen (c) in Maryland Coastal Bays (averaged across 13 sites).

Water samples for SPOM were collected at each site from a depth of about 0.5 m using a horizontal Van Dorn water sampler. Samples were transferred into 2 L high-density polyethylene bottles and immediately stored in ice. At each location, environmental variables such as temperature, salinity, and DO were measured using a YSI 6000 QS Sonde. For SPOM analysis, water samples were pre-filtered with a 200-µm sieve, and then filtered onto a pre-combusted Whatman GF/F filter (25-mm diameter; 450 °C for 4 h) and stored at -80 °C before analysis.

2.3. Stable isotopes analyses

All samples for stable isotope analysis were shipped to the University of California Davis for analysis. Filter papers containing SPOM samples were oven dried for 48 h at 60 °C, allowed to cool, and wrapped in a tin capsule before shipping. Copepods for stable isotope analysis were rinsed in distilled water and sorted by species. Bulk samples containing approximately 60–400 individual copepods were dried in the oven at 60 °C for 48 h. Weighed, dried ground copepod samples (0.8–1.2 mg) were wrapped in tin capsule before shipping to University of California Davis for analysis. As SPOM and copepod samples may contain

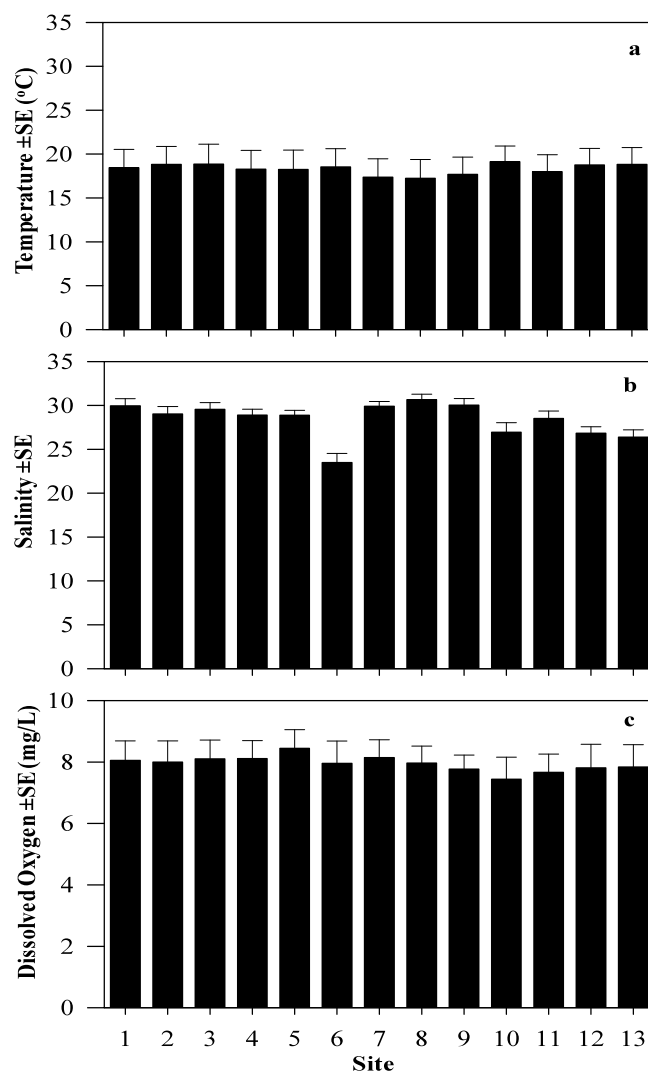


Fig. 3. Spatial variation in temperature (a), salinity (b) and dissolved oxygen (c) in Maryland Coastal Bays averaged across months (2014–2017).

carbonates, an acidification step using the drop-by-drop method was used to remove enriched carbonate in the sample for stable isotope carbon analysis (Guerra et al., 2013).

The University of California Davis uses the Elementar Vario EL Cube/Micro Cube elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) for sample analysis. First, the samples were combusted at 1080 °C in a micro-cube elemental analyzer, then Europa 20–20 IRMS was used to determine the stable isotope ratios. The results are expressed in δ notation and are reported based on air δ¹⁵N and Pee Dee Belemnite for δ¹³C. Negative (–) sign indicates depletion and positive (+) sign indicates enrichment of the heavy (¹³C and ¹⁵N) relative to the lighter (¹²C and ¹⁴N) isotopes when compared with the standard materials using the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

Where X = ¹³C or ¹⁵N and R represents the corresponding ratio of ¹³C/¹²C or ¹⁵N/¹⁴N.

Data analyses

A Kruskal–Wallis test was used to compare spatial and temporal changes in stable isotopic (SI) carbon and nitrogen levels of copepod data, followed by post-hoc tests to compare variables between sites, seasons and years. Linear regression analyses

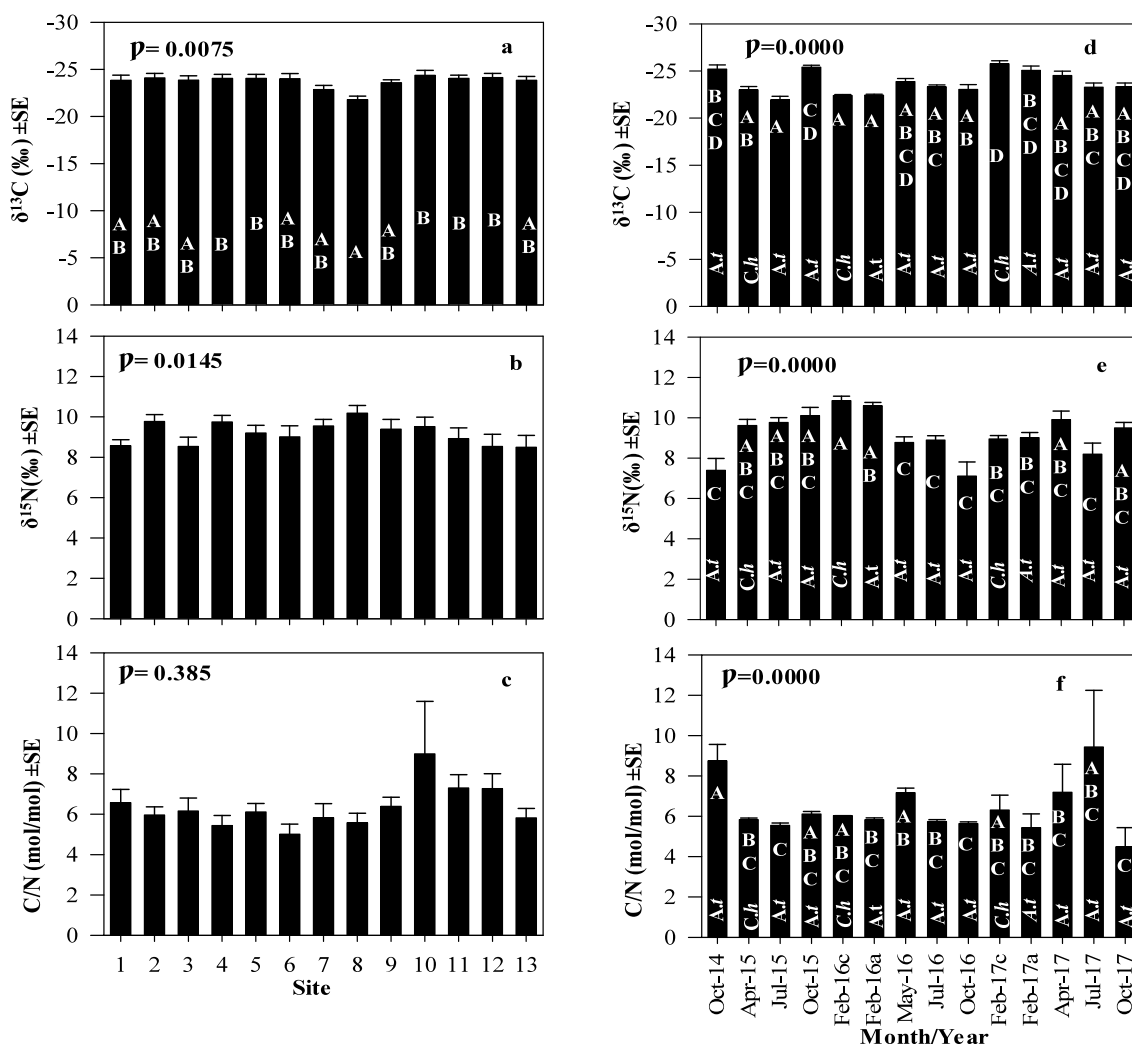


Fig. 4. Spatial variations in $\delta^{13}C$ (a), $\delta^{15}N$ (b) and C/N (c) and temporal variations in $\delta^{13}C$ (d), $\delta^{15}N$ (e) and C/N (f) of copepods in MCBs. *Acartia tonsa* (A.t) and *Centropages hamatus* (C.h).

were performed to evaluate spatial and temporal relationships between SPOM and copepod SI carbon and nitrogen data.

3. Results

3.1. Environmental factors

Water temperature ($^{\circ}C$) varied from a minimum value of $4.7 \pm 0.3SE$ in February 2016 to a maximum of $28.7 \pm 0.4SE$ in July 2015 (Fig. 2a). The highest salinity was recorded in July 2015 ($31.33 \pm 0.70SE$) and the lowest (Fig. 2b) occurred in February 2016 ($24.12 \pm 0.78SE$). Mean dissolved oxygen level was above 5.47 mg/L each month (Fig. 2c).

The lowest mean temperature, $^{\circ}C$ ($17.4 \pm 2.1SE$) was observed at site 8 closest to the Ocean City inlet, whereas the highest mean temperature ($19.1 \pm 1.8SE$) was recorded at site 10 at the mouth of St. Martin River (Fig. 3a). Salinity varied from the lowest mean value of $23.49 \pm 1.06SE$ at site 6 to the highest value of $30.66 \pm 0.62SE$ at site 8 (Fig. 3b). Mean concentration of dissolved oxygen was above 6 mg/L at all the sites (Fig. 3c).

3.2. Spatial variations in copepod $\delta^{13}C$, $\delta^{15}N$ and C/N

Copepod $\delta^{13}C$ values observed during this study ranged from -27.8‰ to -19.4‰ (mean \pm standard error, $SE = 23.7\text{‰} \pm 0.1$),

and significant spatial variations were observed (Kruskal–Wallis test, $p = 0.0075$). Copepod mean $\delta^{13}C$ value ($\pm SE$) at site 8 (Sinepuxent Bay) located closest to the ocean was the highest ($-21.8\text{‰} \pm 0.4$), and was significantly different from values at sites 4, 5, and 10 to 12 (Fig. 4a). Copepods at site 10 located at the mouth of St. Martin River had the lowest mean $\delta^{13}C$ value ($\pm SE$) of $24.4\text{‰} \pm 0.5$; there was thus an increasing trend in the values from sites 10 through 9 to 8.

$\delta^{15}N$ copepod values ranged from 2.9‰ to 13.0‰ with a mean value ($\pm SE$) of $9.2\text{‰} \pm 0.1$. Spatial variations were observed for $\delta^{15}N$ copepod values (Kruskal–Wallis test, $p = 0.0145$), although pair-wise post-hoc comparisons showed no significant differences ($p > 0.05$), Fig. 4b. There was, however, a decreasing trend in the $\delta^{15}N$ mean values from sites 8 ($10.2 \pm 0.4\text{‰}$) to 13 ($8.5 \pm 0.6\text{‰}$). Copepod mean C:N values ranged from $5.0 \text{ mol/mol} \pm 0.5SE$ (site 6) to $9.0 \text{ mol/mol} \pm 2.6SE$ (site 10). No significant spatial variations (Kruskal Wallis test, $p = 0.385$) were observed in the copepod C:N ratio which varied from 2.0 to 39.5 mol/mol (Fig. 4c).

3.3. Temporal variations in copepod $\delta^{13}C$, $\delta^{15}N$ and C/N

Significant temporal variations were observed in copepod $\delta^{13}C$ values (Kruskal–Wallis test, $p = 0.000$). The highest ($-21.9\text{‰} \pm 0.4SE$) and lowest ($-25.8\text{‰} \pm 0.3SE$) mean copepod $\delta^{13}C$ values were observed in July 2015 and February 2017, respectively

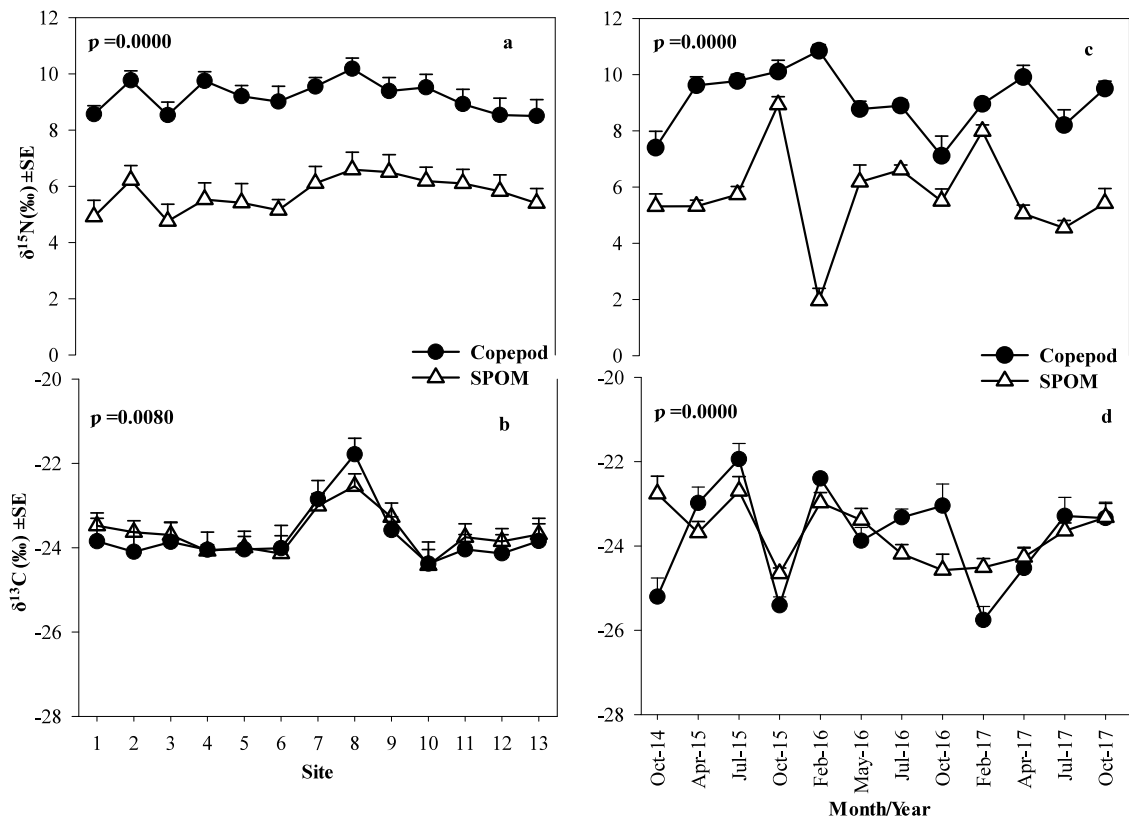


Fig. 5. Spatial variations of copepod $\delta^{15}\text{N}$ relative to SPOM $\delta^{15}\text{N}$ (a) and copepod $\delta^{13}\text{C}$ relative to SPOM $\delta^{13}\text{C}$ (b). Temporal variations of copepod $\delta^{15}\text{N}$ relative to SPOM $\delta^{15}\text{N}$ (c) and copepod $\delta^{13}\text{C}$ relative to SPOM $\delta^{13}\text{C}$ (d).

(Fig. 4d). Significant differences were observed in copepod $\delta^{13}\text{C}$ values among months within the same year (Fig. 4d), in 2015 when October data were significantly more depleted than April and July data, and in 2017 when February *C. hamatus* data were significantly more depleted than July *A. tonsa* data (Fig. 4d), but not in 2016. When compared among years (Fig. 4d), significant differences in copepod $\delta^{13}\text{C}$ values were observed between October 2015 ($-25.4\text{‰} \pm 0.2\text{SE}$) and 2016 ($-23.1\text{‰} \pm 0.5\text{SE}$), and between February 2016 ($-22.4\text{‰} \pm 0.1\text{SE}$) and 2017 ($-25.8\text{‰} \pm 0.3\text{SE}$) for both copepod species.

Copepod $\delta^{15}\text{N}$ values varied temporally (Kruskal–Wallis test, $p=0.000$) with highest and lowest mean values ($\pm\text{SE}$) of $10.8\text{‰} \pm 0.2$ (*A. tonsa*) and $7.1\text{‰} \pm 0.7$ (*A. tonsa*) in February and October 2016, respectively (Fig. 4e). No significant differences in copepod $\delta^{15}\text{N}$ mean values were observed among months within the same year ($p>0.05$), except in 2016 when data obtained in February for *A. tonsa* was significantly higher ($10.8\text{‰} \pm 0.2\text{SE}$) than values obtained in the other months. There was no significant difference between *A. tonsa* and *C. hamatus* $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values observed in February 2016 and 2017 (Fig. 4d and e).

Significant temporal variations (Kruskal–Wallis test, $p = 0.000$) were observed in the copepod C:N values (Fig. 4f). October 2017 had the lowest mean value (4.5 ± 1.0 mol/mol) and July 2017 had the highest value (9.4 ± 2.8 mol/mol) of C:N ratio.

3.4. Relationships between copepod and SPOM $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

There was enrichment of $\delta^{15}\text{N}$ in copepod samples relative to SPOM samples (Fig. 5a–d). Spatially, copepods had mean $\delta^{15}\text{N}$ enrichment value of 3.4‰ over the SPOM value (Fig. 5a), but the spatial patterns of variation of $\delta^{15}\text{N}$ in copepods and SPOM were similar. A similar spatial pattern was observed for the copepod and SPOM $\delta^{13}\text{C}$ values with enrichment at sites close to the Ocean

City Inlet (7, 8 and 9), and depletion at other sites, especially at sites (6 and 10) close to the mouths of tributaries (Fig. 5b). The differences between copepod and SPOM $\delta^{13}\text{C}$ values were, however, very small ranging from -2.4 to 0.8 compared to that of $\delta^{15}\text{N}$, which ranged from 2.7 to 4.2 .

On a temporal scale, there was a wide variation in the enrichment of copepod $\delta^{15}\text{N}$ relative to the SPOM values, with values ranging from 1.0‰ in February 2017 to 8.9‰ in February 2016 (Fig. 5c). Mean values of copepod $\delta^{13}\text{C}$ were more depleted than SPOM values in October 2014 and 2015, May 2016 and February 2017 (Fig. 5d).

Spatially, significant linear relationships (Fig. 6a,c) were observed between mean values of SPOM and copepod $\delta^{13}\text{C}$ ($r^2 = 0.82$, $p = 0.0000$) and $\delta^{15}\text{N}$ ($r^2 = 0.49$, $p = 0.008$), but when examined temporally, no significant relationships ($r^2 = 0.02$ – 0.21 , $p>0.771$) were observed (Fig. 6b,d).

4. Discussion

4.1. General pattern of copepod $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N

Copepod $\delta^{13}\text{C}$ content in this study ranged from -27.8‰ to -19.4‰ (mean = $23.7\text{‰} \pm 0.1$) and is comparable to what was observed in some other aquatic ecosystems. Schell et al. (1998) reported mean copepod $\delta^{13}\text{C}$ values for various species in the Chukchi/Bering/Beaufort Sea regions that ranged from $-21.8\text{‰} \pm 0.12\text{SE}$ (East Chukchi subregion) to $-25.7\text{‰} \pm 0.20\text{SE}$ (East Alaskan Beaufort subregion) with an overall mean of -22.74‰ for all samples. Mean calanoid copepod $\delta^{13}\text{C}$ values in Godavari Estuary (Bay of Bengal) were -29.3 ± 1.2 during the wet season and -23.8 ± 0.5 in the dry season (Mukherjee et al., 2018). Additionally, in Waquoit Bay, Massachusetts, USA, Martinetto et al. (2006) reported *A. tonsa* $\delta^{13}\text{C}$ mean value of -20.3 ± 0.9 ,

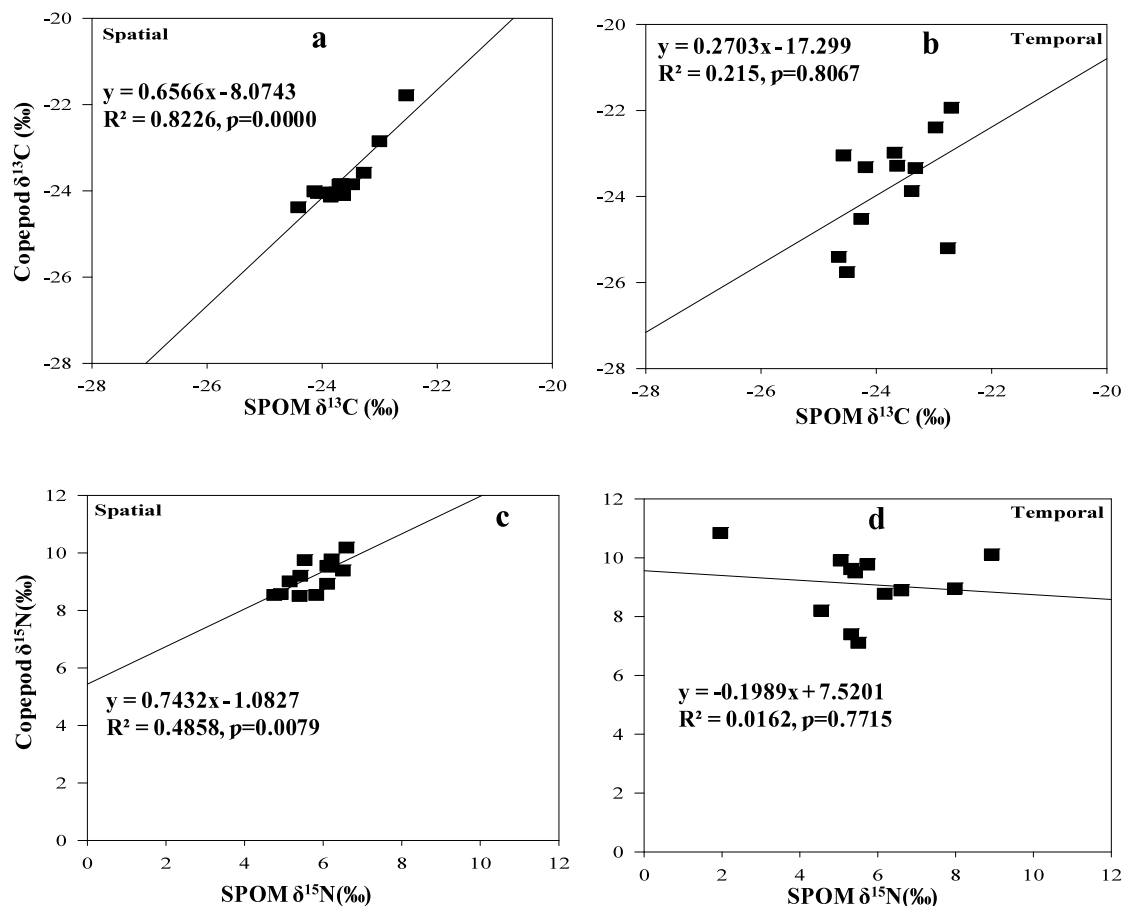


Fig. 6. Spatial (a and c) and temporal (b and d) relationships between copepod and SPOM mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

whereas in the Bay of Marseille (northwest Mediterranean Sea), Banaru et al. (2014) observed mean value (-21.45 ± 0.58) for copepods that are more enriched than the value reported in the MCBs in this study. In general, more negative values ($< -24\text{‰}$) of $\delta^{13}\text{C}$ SPOM indicate a higher amount of terrestrially derived materials whereas higher values ($> -21\text{‰}$) indicate larger contributions of marine organic materials (Fry and Sherr, 1984; Andrews et al., 1998; Harmelin-Vivien et al., 2008). Therefore, the more depleted values within the range reported for copepod $\delta^{13}\text{C}$ in this study suggest that terrestrially derived organic materials sometimes formed part of the copepods diets.

The $\delta^{15}\text{N}$ values recorded for copepod samples in this study (2.9 to 13.0‰) were more enriched than values reported for SPOM (-0.6 to 10.5‰) which, as expected, is an indication of higher trophic level of the copepods compared to the SPOM. In Chesapeake Bay, the $\delta^{15}\text{N}$ of *A. tonsa* ranged between 10.1 and 18.7‰ in spring and between 10.1 and 13.6‰ in the fall (Montoya et al., 1990). In Chukchi/Bering/Beaufort Sea regions, Schell et al. (1998) reported mean copepod $\delta^{15}\text{N}$ values of $5.8\text{‰} \pm 0.21\text{SE}$ in the South Bering Sea and $11.6\text{‰} \pm 0.44$ in the West Alaskan Beaufort Sea, whereas in Waquoit Bay, Massachusetts, the mean value for *A. tonsa* adults was $7.08\text{‰} \pm 2.45$ (Martinetto et al., 2006). C:N values recorded for copepod samples ranged from 2.0 to 39.5 mol/mol in this study, and are higher than the values (1.7 to 11.4 mol/mol) recorded for SPOM.

4.2. Spatial variations of copepod $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N

Spatial variations were observed in copepod $\delta^{13}\text{C}$ such that mean values were more enriched at sites 7, 8 and 9 than at the other sites, with the highest mean value recorded at site 8

($-21.8\text{‰} \pm 0.4\text{SE}$). Copepods in these sites, perhaps, consumed a higher proportion of plankton of estuarine/marine organic matter origin, and less of terrestrially derived organic matter than copepods at site 10. Site 8 is located closest to the Ocean City inlet through which water enters the bays from the adjacent ocean. Site 10, directly influenced by freshwater discharge from St. Martin River, had mean SPOM $\delta^{13}\text{C}$ value ($-24.4\text{‰} \pm 0.4\text{SE}$) similar to the values recorded in sites 1 to 7 and sites 9 to 11, indicating the contributions of terrestrial/riverine organic matter to the SPOM. Copepod $\delta^{13}\text{C}$ collected at site 10 ($-24.4\text{‰} \pm 0.5\text{SE}$) reflected the low stable carbon isotopic value, an indication of terrestrial/riverine organic matter influence on the diets of the copepods collected at the site. Other investigators have reported spatial differences in the signatures of copepod $\delta^{13}\text{C}$ in various estuarine and marine systems, which they related to the isotopic signatures in the SPOM. At sites close to the mouths of rivers such as the Mackenzie River in the Bering, Chukchi and Beaufort Seas, copepod $\delta^{13}\text{C}$ values were highly depleted (mean = -26.1‰) which Schell et al. (1998) attributed to the inputs of nutrients and carbon from terrestrial sources that had low $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Vizzini et al. (2005) observed that in Manguio lagoon, southern France, $\delta^{13}\text{C}$ of particulate organic matter was 1.5–2‰ higher at a site with marine influence than at a site influenced by freshwater discharge. The isotopic signatures of invertebrates and fish at upper trophic levels in the lagoon reflected the pattern they found in the SPOM. Suzuki et al. (2014) reported that two copepod species (*Sinocalanus sinensis* and *Pseudodiaptomus inopinatus*) in the estuarine turbidity maximum (ETM) of Chikugo River estuary, Japan had depleted $\delta^{13}\text{C}$ ($< -24\text{‰}$) whereas copepods (*P. inopinatus*) inhabiting downstream of the ETM had more enriched $\delta^{13}\text{C}$

values ($> -24\text{‰}$). Furthermore, they observed that terrestrial-plant detritus $\delta^{13}\text{C}$ signature in the aquatic system was -24‰ , and concluded that spatio-temporal variations in copepod $\delta^{13}\text{C}$ were due to their feeding on detritus and/or phytoplankton. Nevertheless, other factors might have contributed to the observed spatial distribution of SPOM and copepods $\delta^{13}\text{C}$ in MCBs. The uptake of HCO_3^- instead of CO_2 under conditions of CO_2 limitation in eutrophic systems results in less discrimination of stable isotope causing phytoplankton to become enriched with ^{13}C (Ogawa and Ogura, 1997; Ke et al., 2017). Additionally, depleted $\delta^{13}\text{C}$ of SPOM may arise from phytoplankton uptake of isotopically light dissolved inorganic carbon from bacterial decomposition of terrestrial organic matter (Coffin and Cifuentes, 1999) which is then, transferred to the copepods that feed on them. It is unknown the extent, if any, to which these factors contributed to the enrichment observed in samples collected close to the ocean, and depletion of samples collected close to the mouth of tributaries.

Aside from site 8 with most enriched copepod $\delta^{13}\text{C}$ values, sites in the northern and southern parts of the MCBs had similar copepod $\delta^{13}\text{C}$ values. Perhaps, this is due to the hydrodynamic mixing of marine and terrestrially derived organic matter by winds and tidal action (Kang et al., 2017). Sites near the mouths of tributaries with depleted $\delta^{13}\text{C}$ values also had higher phytoplankton biomass than sites near the inlets (Oseji et al., 2018). It is likely that the high $\delta^{13}\text{C}$ signature of the phytoplankton component of the SPOM dampened the terrestrial $\delta^{13}\text{C}$ signal at the nearshore sites. Similar to the pattern observed for copepod $\delta^{13}\text{C}$ value, $\delta^{15}\text{N}$ values were more enriched at site 8 than at the other sites (9 – 13) in the northern bays. In fact, copepod $\delta^{15}\text{N}$ values decreased from site 8 to site 13 similar to what was observed for SPOM $\delta^{15}\text{N}$. It is likely that copepods in site 8 near the Ocean City inlet, with lower phytoplankton biomass, exhibited higher level of carnivory/omnivory by feeding selectively on heterotrophic protists, and phytoplankton, especially diatoms transported into the bays from the ocean, and less on terrestrially derived materials than copepods at sites 9 to 13.

4.3. Temporal variation of copepod $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N

Temporal variations were recorded in the values of copepod $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N. Temporal variabilities in the SPOM and zooplankton $\delta^{13}\text{C}$ may be due to variations in the composition and isotopic ratios of phytoplankton species and heterotrophic flagellates, input of terrestrial materials and detritus, and physico-chemical conditions (Needoba et al., 2003; Aberle and Malzahn, 2007; Banaru et al., 2014). A more enriched $\delta^{13}\text{C}$ value occurred in summer (July 2015), whereas a more depleted value was found in winter (February 2017). Freshwater discharge into the MCBs is higher from fall through spring than in the summer, which could have transported more riverine/terrestrial organic materials into the bays. It is likely, therefore that copepods ingested a higher proportion of terrestrial/riverine organic materials in February 2017 than in July 2015. Mukherjee et al. (2018) found that zooplankton diet comprised detrital organic matter during wet season whereas during dry season, phytoplankton and detrital organic matter were the major components in Godavari estuary, Bay of Bengal, Indian Ocean. During wet season, zooplankton $\delta^{13}\text{C}$ value averaged -29.1 ± 0.1 whereas in dry season, it was less depleted (-23.3 ± 0.8) in Godavari estuary. In the Bay of Marseille, Mediterranean Sea, Banaru et al. (2014) observed seasonal variations of the C and N isotopic composition of zooplankton that reflected those of the POM sources implying that the POM was integrated in the food web of the zooplankton.

Seasonal variation in the abundance of small planktonic cells (e.g. flagellates and dinoflagellates) and nanoplankton and picoplankton may influence the $\delta^{13}\text{C}$ of SPOM since these small

cells have more depleted $\delta^{13}\text{C}$ than larger microphytoplankton such as diatoms (Rau et al., 1990; Fry and Wainwright, 1991). Phytoplankton composition varies seasonally in MCBs. Diatoms contributed $>40\%$ of the community in winter which decreased in spring ($<40\%$) at which time microphytoflagellates (MPF) were relatively abundant, and by July were among the dominant groups in the assemblage (Oseji et al., 2019). From August to October, diatoms dominated again, whereas the relative density of dinoflagellates was highest in winter and early spring. The seasonal variation in the relative abundance of MPF and dinoflagellates, however, does not seem to be responsible for the observed differences in the signatures of SPOM and copepods $\delta^{13}\text{C}$. This is because, in 2015, the most depleted copepod $\delta^{13}\text{C}$ occurred in October when diatoms dominated the phytoplankton assemblage, and the least depleted $\delta^{13}\text{C}$ occurred in July when MPF were relatively abundant (Oseji et al., 2019). Temperature influences $\delta^{13}\text{C}$ such that as temperature increases, $\delta^{13}\text{C}$ increases (Goerick and Fry, 1994). Hence, higher temperatures in the summer might cause more enriched $\delta^{13}\text{C}$. Nevertheless, we observed in this study that $\delta^{13}\text{C}$ values in summer (July 2016), when temperature was at its peak, were comparable to values in winter (February 2016) when temperature was much lower.

Copepod $\delta^{13}\text{C}$ was significantly more depleted in October 2015 than in October 2016, and more depleted in February 2017 than in February 2016. These differences are unlikely due to variations in freshwater discharge as higher river flow occurred in October 2016/February 2016 than October 2015/February 2015. Instead, it might have been due to variations in copepod selective feeding and isotopic fractionation. In MCBs, mean copepod $\delta^{15}\text{N}$ was highest in February 2016 and lowest in October 2016, perhaps due to the fact that the two months differed with regard to the amounts of freshwater discharge that occurred. It might also have been contributed by the differences in the amount of heterotrophic organisms in the SPOM ingested by the copepods since higher SPOM $\delta^{15}\text{N}$ may result from higher contributions of heterotrophic organisms (Aberle et al., 2010).

Montoya et al. (1990, 1991) observed that $\delta^{15}\text{N}$ of SPOM and *A. tonsa* can change rapidly within a period of days in Chesapeake Bay. An increase in the amounts of NH_4^+ in surface waters due to storm-induced mixing of the water column caused major alterations in the $\delta^{15}\text{N}$ of dissolved and particulate nitrogen due to isotopic fractionation associated with NH_4^+ uptake by phytoplankton, which was reflected in the *A. tonsa* $\delta^{15}\text{N}$ values (Montoya et al., 1991). Enrichment of $\delta^{15}\text{N}$ between *A. tonsa* and particulate nitrogen was not much different before (4.1‰) and after (3.6‰) the mixing of the water column due to the storm event.

A comparison between *A. tonsa* and *C. hamatus* $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, based on samples collected in February 2016 and 2017, showed no significant differences, suggesting that both species fed on similar food items. This is consistent with observations by other investigators who conducted stable carbon and nitrogen isotopic studies on the species (Aberle et al., 2010; Schoo et al., 2018).

4.4. Relationships between copepod and SPOM $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

A similar spatial pattern was observed for copepod and SPOM $\delta^{13}\text{C}$ values in this study with little or no enrichment (-2.4 to 0.8) observed between them. An enrichment in $\delta^{13}\text{C}$ of 0.5 to 1‰ from one trophic level to another has been reported (Minagawa and Wada, 1984); Post (2002) calculated the mean value to be $0.39\text{‰} \pm 1.3\text{SD}$. The results from the MCBs study agree with findings from other studies, which concluded that $\delta^{13}\text{C}$ could be used to infer carbon source in a food web rather than the trophic level (Van Zanden and Rasmussen, 2001). In October 2014 and 2015, and February 2017, copepod $\delta^{13}\text{C}$ was depleted relative

to SPOM $\delta^{13}\text{C}$. This was probably because of low phytoplankton biomass during those periods, and copepod selective consumption of a portion of the SPOM, likely terrestrial/river derived organic matter with lighter isotopic carbon.

The significant positive relationships observed between copepod $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and SPOM $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ when examined spatially; indicate that the composition of SPOM in the various sites influenced the diets of the copepods. When examined temporally, however, no significant relationships were observed, perhaps due to substantial seasonal variability in the composition of the SPOM, isotopic fractionation, and selective feeding by copepods. The isotopic signature of the diet and trophic fractionation influence copepod $\delta^{15}\text{N}$ content (Minagawa and Wada, 1984; El-Sabaawi et al., 2009; McCutchan et al., 2003). Spatially and temporally, enrichment of $\delta^{15}\text{N}$ was observed between SPOM and copepod, which averaged 3.4‰. This value is similar to the enrichment value, $3.4 \pm 1.1\text{‰}$ (range: 1.3 to 5.3) expected from one trophic level to a higher level (Minagawa and Wada, 1984; Post, 2002). An enrichment value of 4.3 ± 0.1 SE was observed between the SPOM $\delta^{15}\text{N}$ and *Acartia* $\delta^{15}\text{N}$ in April 2015. This value is similar to $4.2 \pm 2.3\text{‰}$ (mean \pm SD) obtained between *A. tonsa* and particulate nitrogen in spring in the Chesapeake Bay, but higher than $3.3 \pm 1.0\text{‰}$ recorded in fall (Montoya et al., 1990). The enrichments between SPOM and copepods in some months during this study were greater than the mean value of 3.4‰. $\delta^{15}\text{N}$ enrichment between SPOM and copepod was 1.0‰ in February 2017 and 8.9‰ in February 2016. The low and high enrichment values could have been due to selective feeding by copepods on items in the SPOM that contained depleted or more enriched $\delta^{15}\text{N}$, and/or due to differences in the fractionation of $\delta^{15}\text{N}$ content in their diets (Needoba et al., 2003; Aberle and Malzahn, 2007). In the fall, it was observed that $\delta^{15}\text{N}$ of *Acartia* decreased concurrently with a reduction in $\delta^{15}\text{N}$ of particulate nitrogen after a storm event (Montoya et al., 1990). This is incongruent with the results of our study in MCBs, perhaps due to the fact that the storm events had different effects on the two ecosystems. The very high enrichment between SPOM and copepods $\delta^{15}\text{N}$ observed in February 2016 during this study stemmed from the dramatic reduction in the $\delta^{15}\text{N}$ content of the SPOM following a major storm event that likely transported untreated sewage into the bays. This decrease in SPOM $\delta^{15}\text{N}$ signature was unaccompanied by a reduction in *Acartia* $\delta^{15}\text{N}$. Therefore, using SPOM $\delta^{15}\text{N}$ values obtained soon after major storm events to calculate trophic levels of copepods in shallow urbanized lagoons such as the MCBs could result in unreliable estimates.

5. Conclusions

This study suggests that spatial and temporal variability in the composition of SPOM affects the feeding habits of copepods in MCBs. The more depleted values within the range of copepod $\delta^{13}\text{C}$ content (-27.8‰ to -19.4‰) suggest that terrestrially derived organic materials were consumed by copepods. SPOM $\delta^{13}\text{C}$ values were depleted at the river mouth, indicating the contributions of terrestrial organic matter to the SPOM, and enriched near the ocean inlet suggesting minimal contribution of terrestrial organic matter at the site. The significant positive relationships observed between copepod $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and SPOM $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ when examined spatially indicate that the composition of SPOM in the various sites influenced the diets of the copepods.

A more enriched $\delta^{13}\text{C}$ value occurred in summer (July 2015), whereas a more depleted value was found in winter (February 2017). This perhaps occurred because freshwater discharge into the MCBs was higher from fall through spring than in the summer, which could have transported more terrestrial organic materials into the bays. It is likely, therefore that copepods ingested

a higher proportion of terrestrial/riverine organic materials in February 2017 than in July 2015. In October 2014 and 2015, and February 2017, copepod $\delta^{13}\text{C}$ was depleted relative to SPOM $\delta^{13}\text{C}$. This was probably because of low phytoplankton biomass during those months, and copepod selective consumption of a portion of the SPOM with lighter isotopic carbon.

$\delta^{15}\text{N}$ enrichment between SPOM and copepod was 1.0‰ in February 2017 and 8.9‰ in February 2016. The very high enrichment between SPOM and copepods $\delta^{15}\text{N}$ observed in February 2016 stemmed from the dramatic reduction in the $\delta^{15}\text{N}$ content of SPOM following a major storm event that likely transported untreated sewage into the bays. This decrease in SPOM $\delta^{15}\text{N}$ signature was unaccompanied by a reduction in *Acartia* $\delta^{15}\text{N}$.

CRedit authorship contribution statement

Blessing O. Edje: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing.
Paulinus Chigbu: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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