Assessing $\delta^{15}N$ values in the carbonate-bound organic matrix and periostracum of bivalve shells as environmental archives

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ABSTRACT

Though previous studies demonstrate the utility of nitrogen and carbon stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$, respectively) in bivalve soft tissues as biogeochemical proxies, it is necessary to develop alternative proxies for environmental reconstructions when soft tissues are unavailable, such as with fossils or in museum-archived specimens. This study assesses the reliability of the $\delta^{15}N$ values of carbonate-bound organic matrix ($\delta^{15}N_{CBOM}$) and periostracum ($\delta^{15}N_{periostracum}$) in bivalve shells as recorders of the $\delta^{15}N$ values of particulate nitrogen ($\delta^{15}N_{PN}$) by comparing the $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values of live-collected freshwater mussels (*Elliptio complanata*) and estuarine clams (*Rangia cuneata*) to the $\delta^{15}N$ values of particulate nitrogen ($\delta^{15}N_{PN}$) in the water column. The $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in both species were within the range of the $\delta^{15}N_{PN}$ values that have been corrected for trophic-level enrichment. Thus, our findings illustrate that $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values reliably record $\delta^{15}N_{PN}$ values in rivers and estuaries. The significant positive correlation between $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in both species indicates that they may be used in a similar manner to record $\delta^{15}N_{PN}$ values. The $\delta^{15}N$ values in *E. complanata* muscle, mantle, and gill tissues were enriched by about +3.4‰ compared to $\delta^{15}N_{PN}$ from the water column, which suggests that they are primary consumers that reflect baseline trophic levels. On the other hand, $\delta^{15}N$ values in the soft tissues of *R. cuneata* have trophic-level enrichment consistent with both primary and secondary consumption. Therefore, variations in the $\delta^{15}N$ values of tissues in *R. cuneata* may be related to trophic-level shifts and/or changes in N sources. Differences between the $\delta^{15}N$ values of soft tissue, CBOM, and periostracum in *E. complanata* and *R. cuneata* can be attributed to asynchronous growth, metabolic rate, and organic molecule composition. The $\delta^{15}N_{CBOM}$ values vary along a freshwater-estuarine gradient because of land-use change and differences in the trophic level of the compared species. The $\delta^{15}N_{CBOM}$ values between neighboring sites reflect influences from biosolid application and treated wastewater discharge. While $\delta^{15}N_{CBOM}$ values did not differentiate between sites dominated by urban and forested land-cover, $\delta^{15}N_{CBOM}$ values were highest at the site with the highest agricultural land-use. These results demonstrate the potential of $\delta^{15}N_{CBOM}$ values in bivalve shells to record long-term changes in watershed land use.

1. Introduction

Nitrogen loading to estuaries from coastal watersheds has well-documented detrimental impacts on ecosystem health (e.g., Nixon et al., 1996; Howarth, 2008; Diaz and Rosenberg, 2008). This nitrogen may be derived from sources such as in-stream processes, atmospheric deposition, animal waste, fertilizer, urban runoff, and wastewater effluent (Nixon et al., 1996; Howarth, 2008). Excess nutrient pollution is a detriment to more than 60% of coastal rivers and estuaries in the United States (Howarth et al., 2002). For example, more than 30 years of chronic nutrient loading to the Neuse River Basin in North Carolina has resulted in seasonal hypoxia and anoxia despite the implementation of nutrient reduction strategies (e.g., Stow et al., 2001; Paerl et al., 2006). As in many watersheds, in the Neuse River Basin, excess nutrients mainly originate from non-point sources, which are difficult to trace and to regulate. Understanding the transfer of nitrogen from land to rivers to

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coastal environments is crucial to maintaining ecosystem health but is exceedingly complex. While current environmental monitoring allows for understanding modern biogeochemical processes, less is known about changes in biogeochemical processing prior to the instrumental record. The latter is vital for understanding how land-use and environmental change will continue to impact coastal water quality in the future.

Nitrogen stable isotope values ($\delta^{15}N$) are a useful tool for tracing N sources to aquatic ecosystems based on predictable fractionations that result in distinct $\delta^{15}N$ values between N sources. For example, the $\delta^{15}N$ values in organic matter, such as bivalve soft tissues, have been used to trace nutrient sources and identify pollutants in modern freshwater (e.g., Gustafson et al., 2007; Bucci et al., 2011; Lee et al., 2018) and estuarine (e.g., O'Donnell et al., 2003; Piola et al., 2006; Kovacs et al., 2010; Carmichael et al., 2012) environments. These $\delta^{15}N$ values are used to understand how excess nutrient loading and land-use change affect aquatic biogeochemistry (Vander Zanden et al., 2005; Anderson and Cabana, 2005, 2006; Vandermyde and Whitledge, 2008; Gustafson et al., 2007; Bucci et al., 2011; Thibault et al., 2020). For example, Vander Zanden et al. (2005) established that tissue $\delta^{15}N$ values from a variety of primary consumers including bivalves, zooplankton, and small fish, reflect riparian zone and watershed land use in 27 Danish lakes that represent various trophic states and land-use types.

Likewise, dual $\delta^{15}N$ and stable carbon isotope values ($\delta^{13}C$) in organic matter provide useful information for reconstructing modern and ancient food web dynamics (e.g., Peterson and Fry, 1987; Fry and Sherr, 1989). For aquatic food web reconstructions, it is necessary to establish the $\delta^{15}N$ and $\delta^{13}C$ values at the base of an ecosystem using primary consumers before inferring the trophic levels of higher organisms (e.g., Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1999; Raikow and Hamilton, 2001; Post, 2002; Howard et al., 2005; Gustafson et al., 2007). Generally, primary consumers are preferred over primary producers to establish this baseline because they integrate $\delta^{15}N$ and $\delta^{13}C$ values throughout their lifetime and are less sensitive than primary producers to very rapid variations in environmental conditions (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1999; Post, 2002; Gustafson et al., 2007; McMahon et al., 2013). For this reason, $\delta^{15}N$ and $\delta^{13}C$ values of bivalve tissue are commonly used to estimate the base of aquatic food webs (e.g., Post, 2002; Gustafson et al., 2007; Nerot et al., 2012).

Although the stable isotope ratios of bivalve soft tissues have served as useful tools in modern studies, extending these records beyond the instrumental record is complex because this substrate is not preserved in the historical and fossil record. Even considering the availability of bivalve soft tissues in some museum collections, wet-preservation in ethanol and/or formalin may render them unsuitable for isotopic studies because of variable effects on the $\delta^{15}N$ and $\delta^{13}C$ values of soft tissues (Versteegh et al., 2011). Fortunately, bivalve shells are often dry-preserved and potentially serve as a suitable substitute for soft tissues (Gillikin et al., 2017; Darrow et al., 2017; Black et al., 2017). For example, previous studies have determined that the outer organic layer of bivalve shells, called the periostracum, is a suitable alternative to soft tissues for paleoenvironmental reconstructions (Delong and Thorp, 2009; Whitney et al., 2019). Often, the periostracum is preserved in historical and archaeological shells, but in the absence of soft tissues and periostracum, previous studies have also demonstrated that the carbonate-bound organic matter (CBOM) in bivalve shells have the potential to extend $\delta^{15}N$ records back through time (e.g., O'Donnell et al., 2003; Carmichael et al., 2008; Versteegh et al., 2011; Dreier et al., 2012; Graniero et al., 2016; Oczkowski et al., 2016; Black et al., 2017; Darrow et al., 2017; Gillikin et al., 2017). For example, Black et al. (2017) used $\delta^{15}N_{CBOM}$ values from archaeological oysters (*Crassostrea virginica*) from shell middens to provide evidence of N-loading to the Chesapeake Bay as early as the 19th century. Similarly, Darrow et al. (2017) demonstrated that $\delta^{15}N_{CBOM}$ values in modern and historical *C. virginica* could be used as indicators of N sources in the northern Gulf of Mexico. The availability of fossil and historical shells in museum collections, such as those at the North Carolina Museum of Natural Sciences, makes extending records of paleoenvironmental change beyond instrumental records possible. Moreover, the abundance of shells from species such as *Rangia cuneata* in archaeological shell middens makes utilizing this species for archaeological studies enticing.

Though previous studies have demonstrated the efficacy of $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in estuarine and marine bivalves as biogeochemical proxies, few have examined the utility of this proxy in freshwater bivalves (Gillikin et al., 2012). Consequently, the goal of this study is to assess whether $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in freshwater mussels and estuarine clams serve as recorders of N fluxes in aquatic settings. For the first time, this study examines $\delta^{15}N_{CBOM}$ values as environmental recorders in the aragonite shells of the bivalves *Elliptio complanata* and *Rangia cuneata*. This study tests the following hypotheses: (1) $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values are indicators of the $\delta^{15}N$ values of suspended particulate nitrogen ($\delta^{15}N_{PN}$) in the water column; (2) $\delta^{15}N$ and $\delta^{13}C$ values in *E. complanata* and *R. cuneata* tissues can be used as baselines for trophic level reconstructions; and (3) variations in $\delta^{15}N_{CBOM}$ values between study sites reflect land-use change.

2. Study site

The Neuse River Basin lies entirely within North Carolina and empties into the Albemarle-Pamlico Sound, the second-largest estuary system in the United States (Fig. 1). The Neuse River estuary is a microtidal estuary, bound by barrier islands, with wind-driven circulation and semi-diurnal seiching (e.g., Luettich et al., 2002; Reynolds-Flemming and Luettich Jr., 2004). For more than 20 years, the Neuse River Basin has been classified as a Nutrient Sensitive Watershed by the North Carolina Department of Environmental Quality (NCDEQ), which designates it as a watershed requiring increased nutrient management strategies due to excessive growth of micro- and macroscopic algae. Chronic nutrient pollution in the watershed results in harmful algal blooms, hypoxia, fish kills, and degrading water quality (e.g., Showers et al., 1990; Paerl et al., 1995; Paerl et al., 1998; Christian and Thomas, 2003; Hounshell and Paerl, 2017).

A total of seven sites were chosen along the Neuse River and its estuary due to their close proximity to existing sites monitored by RiverNet (http://rivernet.ncsu.edu/) and ModMon (http://paerllab.web.unc.edu/projects/modmon/) programs at North Carolina State University and the University of North Carolina at Chapel Hill, respectively. Three sites are located on the main Neuse River channel: upriver at Auburn-Knightdale and Clayton, and mid-river at Seven Springs. The Auburn-Knightdale and Clayton sites are located downstream of the City of Raleigh and are classified by medium- and high-intensity developed land-use (North Carolina Division of Water Quality (NCDWQ), 2009). They are within about 7 km of each other and are separated by a wastewater treatment facility. Sites Seven Springs and Grifton are in the middle of the Neuse River Basin; however, Grifton is situated on a tributary of the main Neuse River channel called Contentnea Creek. The middle of the Neuse River basin is characterized by agricultural land-use (34.4% in the middle Neuse, 44.1% in Contentnea Creek; Lebo et al., 2012) and a high concentration of confined animal operations (NCDWQ, 2009). Three sites were also chosen along the Neuse River Estuary: New Bern in the upper estuary, Flanners Beach in the upper-middle estuary, and Pinecliff in the mid-estuary.

3. Bivalve ecology

3.1. *Elliptio complanata*

Unionids are freshwater mussels that are globally distributed and economically valuable; however, many species are now classified as endangered or threatened. They are infaunal, living in the substrate, but they can occasionally be found on top of the sediment surface and prefer

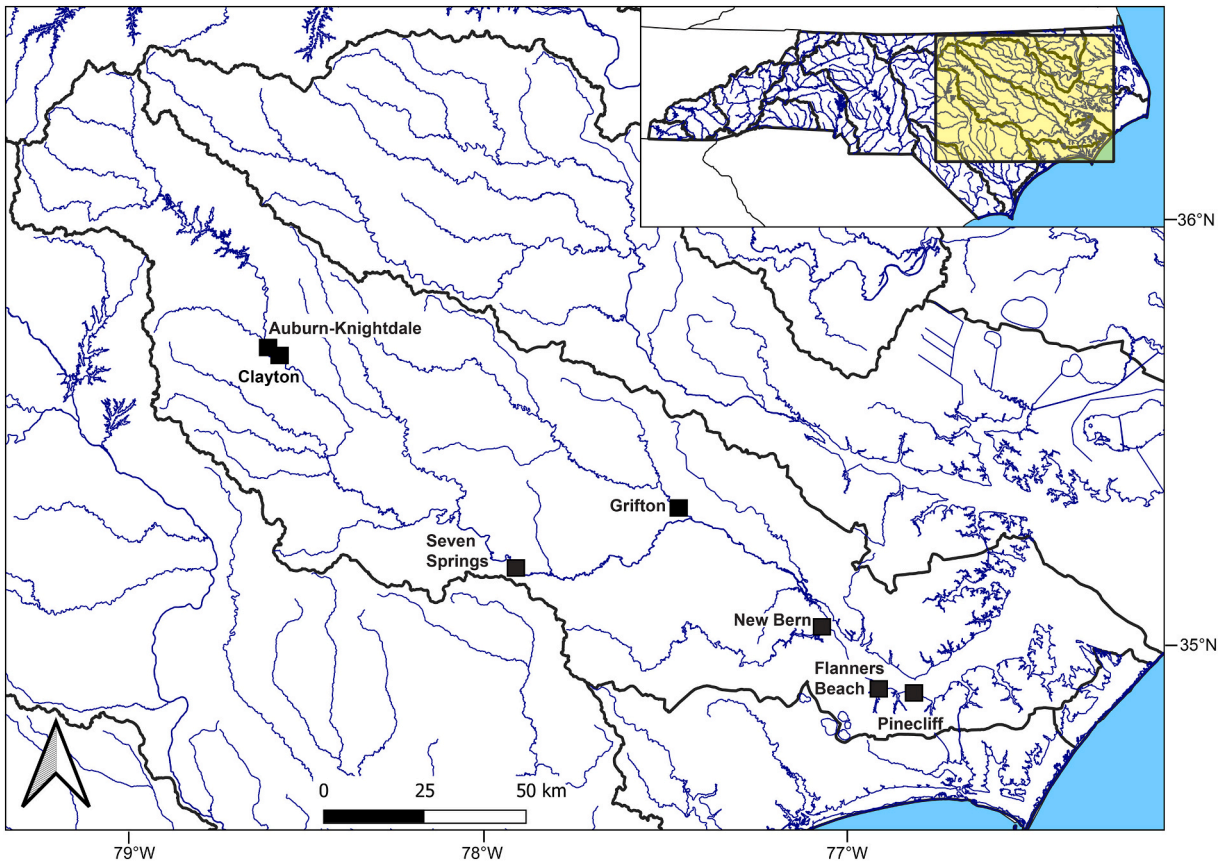

Fig. 1. Map of the Neuse River Basin in North Carolina. Study sites are denoted with squares along the Neuse River and its estuary. Descriptions of study site and sampling information can be found in Table 1.

sandier substrates (Leff et al., 1990). Their critical thermal maximum is around 40 °C (Galbraith et al., 2012), and growth cessation occurs below about 12 °C (Dettman et al., 1999; Goodwin et al., 2019). Growth rates and longevity in *E. complanata* are highly variable due to factors such as habitat heterogeneity, shell length, and growth cessation (Anthony et al., 2001). On average, spatial aggregation and horizontal locomotion in *E. complanata* increase with spawning and day length, but overall horizontal movement is low in this species (Amyot and Downing, 1997; Amyot and Downing, 1998). *Elliptio complanata* is a suspension-feeding bivalve with a diet comprised of phytoplankton, detritus, and bacteria (Raikow and Hamilton, 2001; Post, 2002). One study determined that unionids consume roughly 80% deposited and 20% suspended organic matter, on average, but they may preferentially assimilate ^{15}N-enriched material (deposited or suspended) rather than bulk suspended matter (Raikow and Hamilton, 2001).

3.2. *Rangia cuneata*

Rangia cuneata are estuarine clams that are indigenous to the Atlantic and Gulf Coasts but are considered invasive to parts of Europe (Poirrier and Caputo, 2015; Warzocha et al., 2016). Historical and fossil shells of this species are commonly found in archaeological shell middens along the coast of the Gulf of Mexico (Peregrine and Ember, 2001; Henderson et al., 2002; Perttula, 2004) and in Pliocene and Pleistocene deposits along the eastern coast of North America and the northern coast of South America (LaSalle and de la Cruz, 1985). Modern *R. cuneata* inhabit sandy substrates in the oligohaline and mesohaline zones of estuaries, where salinities range from 0 to 18 (e.g., LaSalle and de la Cruz, 1985; Verween et al., 2006; Wong et al., 2010; Poirrier and Caputo, 2015). They have broad salinity and temperature tolerance but prefer lower salinity environments where competition and predation are less prevalent (Cooper, 1981). *Rangia cuneata* are non-selective filter feeders, consuming primarily seston and detritus (LaSalle and de la Cruz, 1985; Gaston et al., 1997). Seston includes the living and non-living components of suspended matter in the water column. Gaston et al. (1997) studied *R. cuneata* from the Gulf of Mexico and found that greater than 90% of gut contents were composed of sand, silt, and clay particles with smaller contributions from living and dead diatoms and bits of plant matter.

4. Methods

4.1. *Bivalve organic matter*

In the Neuse River, ten *E. complanata* were hand-collected alive upstream at Clayton by snorkeling with aid from the North Carolina Wildlife Resources Commission (Table 1). Ten *R. cuneata* in the Neuse River estuary were hand-collected alive at New Bern by wading into shallow water. To assess the effect of land-use on $\delta^{15}N_{shell}$ values, additional *E. complanata* were collected alive from nearby Auburn-Knightdale, mid-river Seven Springs, and Grifton, located on Contentnea Creek, a tributary of the main Neuse River channel (Table 1). Additional *R. cuneata* were collected alive from sites at New Bern, Flanners Beach, and Pinecliff, in the upper, upper-middle, and middle of the Neuse River Estuary, respectively (Table 1). It was substantially more challenging to find *R. cuneata* at Flanners Beach and Pinecliff than at New Bern. All shells chosen were of similar size and were the smallest collected at each study site. Therefore, all individuals were expected to be at a similar life stage.

All specimens remained on ice until they were returned to the laboratory, where they were immediately frozen. Soft tissues for 10 *E. complanata* from Clayton and 10 *R. cuneata* from New Bern were chosen for the following analyses (5 collected on 12 August 2015 and 5

Table 1
A summary table containing the study site and sample collection information.

Study site	Latitude	Longitude	Sampling date	Sample type	# shells collected
Auburn-Knightdale (AK)	35°43′36.11"N	78°30′48.80"W	28 July 2016	*Elliptio complanata*	2
Clayton (CT)	35°42′9.48"N	78°28′41.82"W	28 July 2016	*Elliptio complanata*	10
Seven Springs (SS)	35°13′45.1"N	77°50′46.6"W	09 September 2016	*Elliptio complanata*	3
Grifton (G)	35°22′11.98"N	77°26′46.21"W	07 August 2017	*Elliptio complanata*	2
New Bern (NB)	35°6′15.156"N	77°2′4.804"W	12 August 2015	*Rangia cuneata*	5
New Bern (NB)	35°6′15.156"N	77°2′4.804"W	05 October 2015	*Rangia cuneata*	5
New Bern (NB)	35°6′15.156"N	77°2′4.804"W	17 July 2017	*Rangia cuneata*	3
Flanners Beach (FB)	34°59′02.3"N	76°56′53.4"W	17 July 2017	*Rangia cuneata*	3
Pinecliff (PC)	34°56′22.0"N	76°49′20.0"W	17 July 2017	*Rangia cuneata*	1

collected on 05 October 2017). First, individuals were thawed and dissected so that the adductor muscle, mantle, gill, and stomach could be separated for $\delta^{15}N$ and $\delta^{13}C$ analyses. In this case, the stomach refers to the combined stomach tissue and stomach contents. All soft tissues were dried for ~6–8 h at 60–65 °C, then ground and homogenized using a mortar and pestle. Powdered samples were packed into tin capsules and analyzed on a Thermo Delta V Advantage isotope ratio mass spectrometer (IRMS) in continuous flow mode connected to a Costech Elemental Analyzer (EA) via ConFlo IV at Union College (Schenectady, NY). Reference standards sucrose [IAEA-CH-6] ($\delta^{13}C = -10.449‰$), acetanilide ($\delta^{13}C = -34.07‰$, $\delta^{15}N = -0.96‰$), ammonium sulfate [IAEA-N-2] ($\delta^{15}N = +20.3‰$), and caffeine [IAEA-600] ($\delta^{13}C = -27.771‰$, $\delta^{15}N = +1.0‰$) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale. Percent C and N were calculated using an additional 2 acetanilide standards per run of varying mass. Corrections were done using a regression method. The combined uncertainty (analytical uncertainty and average correction factor) for $\delta^{13}C$ (VPDB) and $\delta^{15}N$ (air) was ±0.09‰ and ±0.09‰, respectively, based on 8 acetanilide standards over 2 analytical sessions.

After dissection, all shells were scrubbed thoroughly with a toothbrush and dilute soap and water. The outer surface of the shell was lightly sanded to remove surface contaminants. An approximately 1 mm wide line of periostracum was carefully removed from the growth margin using a Brasseler carbide drill bit (catalog #H71–008) and a NSK Volvere Vmax drill. The coeval CBOM beneath the periostracum line was removed using the same hand-drilling technique to obtain ~5 mg of powder for analysis. Care was taken to drill at the shallowest depth possible to get enough powder for analysis. The CBOM and periostracum were packed in tin cups, and the periostracum was combusted in an elemental analyzer following previous studies (Versteegh et al., 2011; Graniero et al., 2016; Black et al., 2017; Darrow et al., 2017; Gillikin et al., 2017; Whitney et al., 2019). Gillikin et al. (2017) previously established that vigorous chemical cleaning of bivalve shell carbonate is not required for CBOM analysis in bivalves. The $\delta^{15}N_{CBOM}$ samples were analyzed at KU Leuven on a Thermo Flash HT with a Costech zero blank autosampler, coupled to a Thermo Delta V Advantage IRMS via a Thermo ConFlo IV with an inline CO_2 trap. Approximate analytical uncertainty was less than ±0.5‰ (see Gillikin et al., 2017).

4.2. $\delta^{15}N_{PN}$ and $\delta^{13}C_{POC}$ values

Suspended particles from the water column were analyzed for $\delta^{15}N_{PN}$ and the $\delta^{13}C$ values of particulate organic carbon ($\delta^{13}C_{POC}$) fortnightly to monthly from August 2015 through November 2016. For $\delta^{15}N_{PN}$ and $\delta^{13}C_{POC}$ analysis, 200 to 1000 mL of water was filtered through pre-combusted (4 h at 500 °C; Bouillon et al., 2012), pre-weighed 47 mm GF/F filters, which were then dried for approximately 12 h at room temperature and placed in a desiccator overnight. The volume of water analyzed was dependent on the concentration of suspended particles in the water column at the time of filtration. One-half of these filters were used for $\delta^{15}N_{PN}$ analyses (and did not undergo any additional treatment), and the remaining half was decarbonated in HCl fumes for 4 h for $\delta^{13}C_{POC}$ analysis (Lorrain et al., 2003) and stored in a desiccator. These filters were then packed into silver capsules and were analyzed in continuous flow mode using a Costech EA connected to a Thermo Delta V Advantage IRMS via ConFlo IV at Union College. The combined uncertainty for $\delta^{13}C$ was ±0.09‰ (VPDB) based on 8 acetanilide standards over 3 analytical sessions. The combined uncertainty for $\delta^{15}N$ was ±0.02‰ (air) based on acetanilide standards over multiple analytical sessions.

5. Results

5.1. $\delta^{15}N_{PN}$ and $\delta^{13}C_{POC}$ values

The $\delta^{15}N_{PN}$ values in this study ranged from −1.4 to +9.1‰, with considerable overlap in $\delta^{15}N_{PN}$ values between river and estuary sites (Fig. 2). The range in values within sites is due to seasonal variations in $\delta^{15}N_{PN}$ values at each site (Appendix I). Measured $\delta^{13}C_{POC}$ values in this study ranged from −35.8 to −24.7‰, with a notable increase in average values from upstream to downstream along the Neuse River and its estuary (Fig. 2).

5.2. Bivalve $\delta^{15}N$ and $\delta^{13}C$ values

There was a wider range in $\delta^{15}N$ values between soft tissues in *E. complanata* than in *R. cuneata*, though the trends were similar. In *E. complanata*, the muscle tissues had higher $\delta^{15}N$ values (+9.5 ± 0.4‰, $n = 9$) compared to the mantle (+8.2 ± 0.5‰, $n = 9$, *t*-test $p < 0.001$) and gill (+7.7 ± 0.5‰, $n = 10$, t-test $p < 0.001$) tissues (Fig. 3). Similarly, the muscle tissues of *R. cuneata* had higher $\delta^{15}N$ values (+12.6 ± 0.6‰, $n = 10$) compared to the mantle (+11.4 ± 0.3‰, $n = 10$, *t*-test $p < 0.001$) and gill (+11.2 ± 0.1‰, $n = 10$, t-test $p < 0.001$) tissues (Fig. 3). The stomach $\delta^{15}N$ values, which contain both assimilated and unassimilated material, were the lowest for both species (*E. complanata* = +5.7 ± 0.8‰, $n = 10$; *R. cuneata* = +10.9 ± 0.7‰, $n = 9$; Fig. 3). There were significant differences between the average $\delta^{15}N_{CBOM}$ values for *E. complanata* and *R. cuneata* (+7.0 ± 0.3‰ ($n = 10$) and + 9.7 ± 0.5‰ ($n = 10$), respectively; *t*-test $p < 0.001$).

Significant positive correlations were found between the $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in *E. complanata* (Pearson's $R^2 = 0.67$, $p = 0.001$) and *R. cuneata* (Pearson's $R^2 = 0.60$, $p = 0.004$; Fig. 4). However, only *E. complanata* showed a significant positive correlation between the $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{mantle}$ (Pearson's $R^2 = 0.41$, $p = 0.047$) and the $\delta^{15}N_{periostracum}$ and $\delta^{15}N_{mantle}$ values (Pearson's $R^2 = 0.46$, $p = 0.030$) (Fig. 4). In *E. complanata*, the relationship between the $\delta^{15}N_{periostracum}$ and $\delta^{15}N_{gill}$ values was a weak, positive correlation (Pearson's $R^2 = 0.27$, $p = 0.057$; Fig. 4). Finally, there is a strong positive correlation between the $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values when including both species (Pearson's $R^2 = 0.91$, $p < 0.001$).

The offsets between $\delta^{15}N_{tissue}$ and $\delta^{15}N_{CBOM}$ values ($\Delta^{15}N_{tissue\text{-}CBOM}$) varied by tissue type. The average offset between $\delta^{15}N_{periostracum}$ and $\delta^{15}N_{CBOM}$ values was smaller for *E. complanata* than for *R. cuneata* (Table 2). In both species, the $\Delta^{15}N_{tissue\text{-}CBOM}$ offset was the largest between for muscle tissue, followed by mantle and then by gill. Stomach $\delta^{15}N$ values were excluded from this comparison because of the

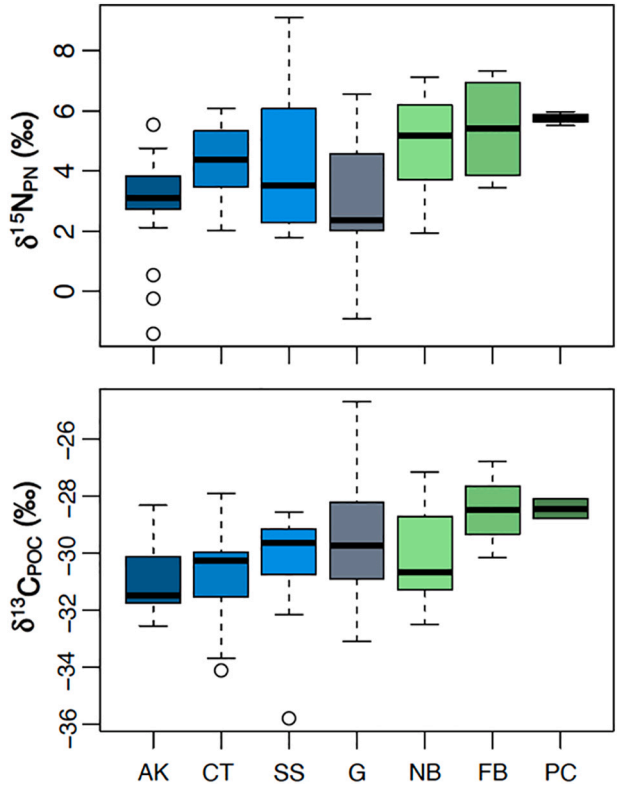

Fig. 2. (Top) Average $\delta^{15}N_{PN}$ values by site. (Bottom) Average $\delta^{13}C_{POC}$ values by site. For river and upper estuary sites, samples were collected approximately fortnightly from August 2015 through August 2016 (November 2016 at Auburn-Knightdale). Sampling at Flanners Beach and Pinecliff occurred from June through November 2016. Site abbreviations are listed in Table 1.

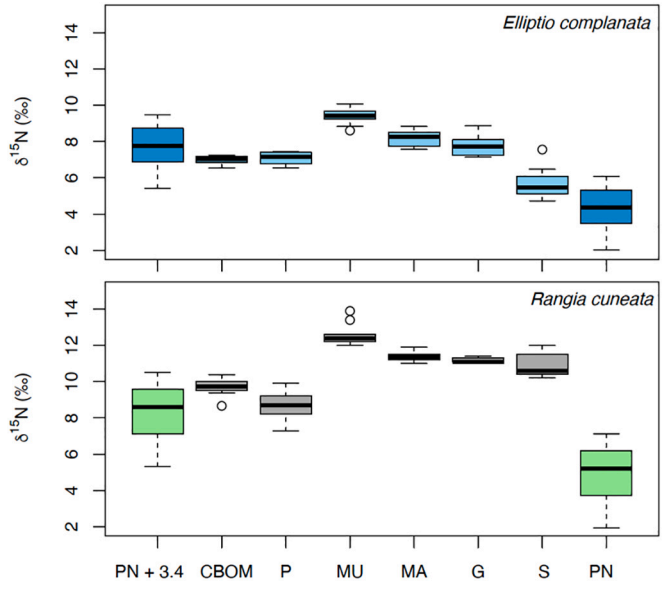

Fig. 3. Summary of $\delta^{15}N$ values for *E. complanata* (top) and *R. cuneata* (bottom) compared to the $\delta^{15}N_{PN}$ values that have been adjusted for an increase in one trophic level, where PN + 3.4 is the trophic-correct $\delta^{15}N_{PN}$ value, p = periostracum, MU = muscle, MA = mantle, G = gill, S = stomach, and PN = $\delta^{15}N_{PN}$ values at Clayton (*E. complanata*) and New Bern (*R. cuneata*).

unassimilated material present in the stomach that we would not expect to be present in the $\delta^{15}N_{CBOM}$ values. The offsets between the average PN +3.4 and $\delta^{15}N_{CBOM}$ values for *E. complanata* and *R. cuneata* are +0.8 and −1.4, respectively. Regardless of tissue type, the $\Delta^{15}N_{tissue\text{-}CBOM}$ values were greater in *R. cuneata* than in *E. complanata* (Table 2).

In general, the average $\delta^{15}N_{CBOM}$ values increased from upriver toward the upper estuary (Fig. 5). In the river, statistically significant differences between average $\delta^{15}N_{CBOM}$ values occurred between Auburn-Knightdale (+5.8 ± 0.2‰, $n = 3$) and Clayton (+7.0 ± 0.3‰, $n = 10$), the sites that are the closest together (t-test $p < 0.05$). The average $\delta^{15}N_{CBOM}$ values from Clayton and Seven Springs (+7.1 ± 0.5‰, $n = 3$) were statistically indistinguishable from each other (t-test $p < 0.05$; Fig. 5). Grifton had slightly higher average $\delta^{15}N_{CBOM}$ values (+7.5 ± 0.3‰, $n = 3$) which were statistically indistinguishable from Seven Springs (t-test $p > 0.05$), but statistically different from those at Clayton (t-test $p < 0.05$). In general, estuarine sites showed higher average $\delta^{15}N_{CBOM}$ values than riverine sites. From New Bern to Flanners Beach, there was a statistically insignificant increase in $\delta^{15}N_{CBOM}$ values from +9.0 ± 0.1‰ ($n = 3$) to +9.5 ± 0.4‰ ($n = 3$), respectively. The one specimen from Pinecliff had a $\delta^{15}N_{CBOM}$ value of +8.5‰.

The relationship between the $\delta^{13}C$ values of tissues differs by species. The aaverage *E. complanata* muscle tissues (−30.4 ± 0.4‰, $n = 9$) are significantly higher than gill (−31.0 ± 0.4‰, $n = 10$, t-test $p < 0.01$), mantle (−32.0 ± 0.3‰, $n = 9$, t-test $p < 0.001$), and stomach (−33.2 ± 0.6‰, $n = 9$, t-test $p < 0.001$) tissues (Fig. 6). This relationship contrasts with results for *R. cuneata*, which show substantial overlap in $\delta^{13}C$ values between tissue types (average all tissues = −30.3 ± 0.4‰, $n = 38$; Fig. 6). However, in both species, stomach tissues had the lowest average $\delta^{13}C$ values.

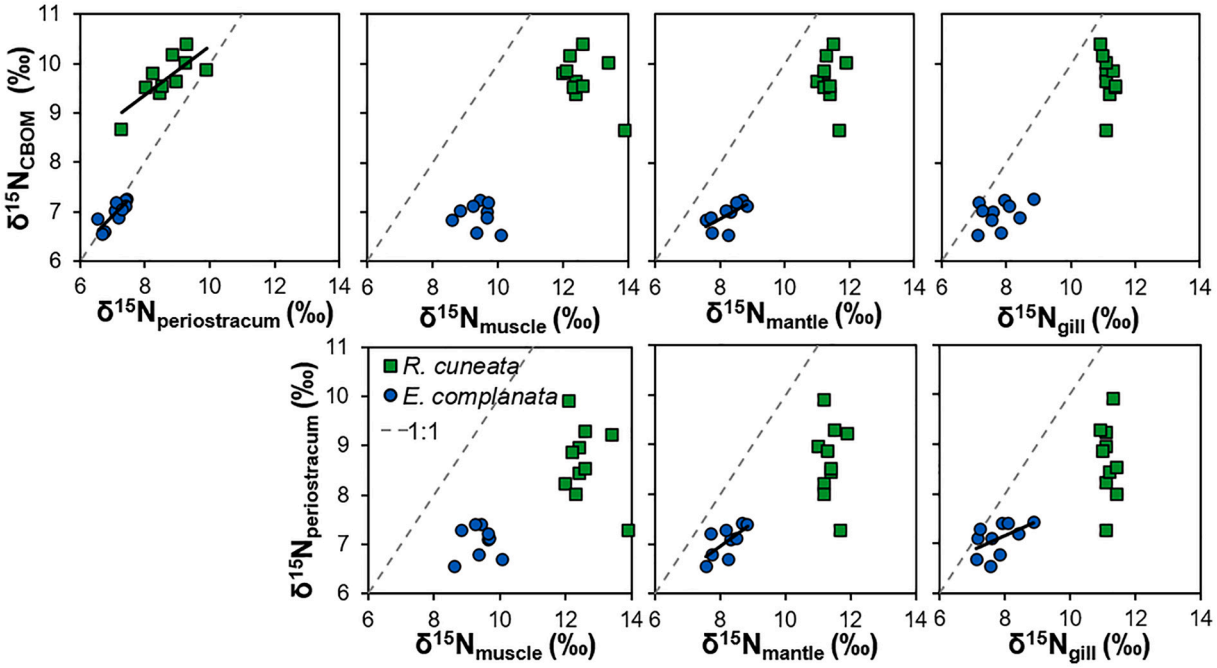

Fig. 4. Summary of correlations between $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{tissue}$ values and between $\delta^{15}N_{periostracum}$ and $\delta^{15}N_{tissue}$ values. Significant linear correlations are denoted with solid black lines and occur in *E. complanata* between $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ ($y = 0.65x + 2.38$, $R^2 = 0.67$, $p = 0.001$), $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{mantle}$ ($y = 0.36x + 4.00$, $R^2 = 0.41$, $p = 0.047$), and $\delta^{15}N_{periostracum}$ and $\delta^{15}N_{mantle}$ ($y = 0.48x + 3.08$, $R^2 = 0.46$, $p = 0.030$). A weak, positive correlation exists between $\delta^{15}N_{periostracum}$ and $\delta^{15}N_{gill}$ ($y = 0.29x + 4.81$, $R^2 = 0.27$, $p = 0.057$) in *E. complanta* as well. For *R. cuneata*, a significant positive correlation exists between $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ ($y = 0.50x + 5.39$, $R^2 = 0.60$, $p = 0.004$).

Table 2
Summary of average $\Delta^{15}N_{tissue\text{-}CBOM}$ and $\Delta^{15}N_{tissue\text{-}periostracum}$ values for E. complanata and R. cuneata.

	Tissue type	$\Delta^{15}N_{tissue\text{-}CBOM}$ (‰)	SD	n	$\Delta^{15}N_{tissue\text{-}periostracum}$ (‰)	SD	n
Elliptio complanata	Muscle	2.5	0.5	9	2.3	0.5	9
	Mantle	1.3	0.3	9	1.2	0.3	9
	Gill	0.8	0.5	10	0.7	0.4	10
	Periostracum	0.1	0.2	10			
Rangia cuneata	Muscle	2.9	0.9	10	3.9	1.0	10
	Mantle	1.7	0.5	10	2.7	0.7	10
	Gill	1.5	0.5	10	2.5	0.7	10
	Periostracum	−1.0	0.5	10			10

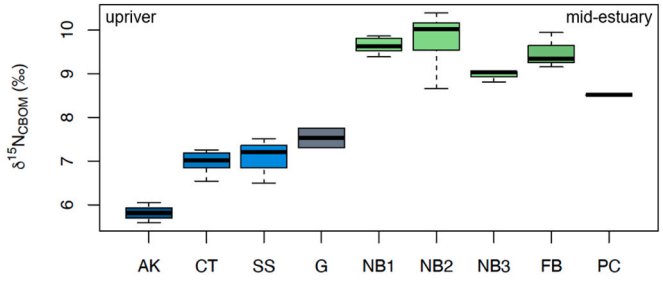

Fig. 5. Average $\delta^{15}N_{CBOM}$ values from study sites arranged from the upper Neuse River Basin (blue) toward the mid-Neuse River Estuary (green). Site abbreviations are listed in Table 1. Samples collected from New Bern are further broken down by their collection date as NB1 (12 August 2015), NB2 (05 October 2015), and NB3 (17 July 2017). Shells analyzed from the riverine portion of the Neuse River Basin are *E. complanata*, whereas shells analyzed from the estuarine portion are *R. cuneata* (see Table 1).

6. Discussion

The main findings of this study indicate that the $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in bivalves *E. complanata* are archives of $\delta^{15}N_{PN}$ values in aquatic environments. Thus, $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values can be used in a similar manner to soft tissues in this species to reconstruct biogeochemical processes in historical and fossil shells. These findings are in support of previous studies that have found similar

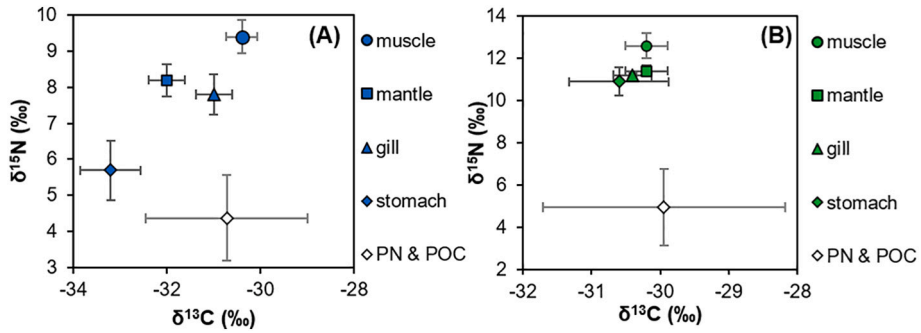

Fig. 6. The average $\delta^{15}N$ and $\delta^{13}C$ values of muscle, mantle, gill, and stomach for (A) *E. complanata* and (B) *R. cuneata* compared to the average $\delta^{15}N$ and $\delta^{13}C$ values of PN and POC, respectively. All error bars represent ±2SD.

results for other bivalve species (Gillikin et al., 2017; Whitney et al., 2019; Das et al., 2020). In addition, the $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in both species are within the range of the trophic-corrected $\delta^{15}N_{PN}$ values, suggesting that CBOM and periostracum can be used to estimate the base of aquatic food webs (Fig. 3). However, caution must be taken when interpreting the $\delta^{15}N$ values in *R. cuneata* because there is evidence of low trophic fidelity in this species. Finally, the $\delta^{15}N_{CBOM}$ values vary along a freshwater-estuarine gradient as a reflection of land-use change and differences in $\delta^{15}N$ values between species.

6.1. Suitability of $\delta^{15}N$ values in soft tissues as baselines for trophic-level reconstructions

Our findings indicate that *E. complanata* are primary consumers that mainly consume and assimilate PN from the water column. In *E. complanata*, the average muscle, mantle, and gill $\delta^{15}N$ values are within the range of trophic-corrected $\delta^{15}N_{PN}$ values for primary consumers (Fig. 3). For our comparison, we used a commonly applied trophic-level enrichment factor, +3.4‰, which is within the typical 3 to 4‰ range used in other studies (e.g., DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Peterson and Fry, 1987; Nichols and Garling, 2000; Post, 2002; Dubois et al., 2007). Similar results were reported by Gustafson et al. (2007) who found that although there was no correlation between $\delta^{15}N_{PN}$ and the $\delta^{15}N$ values of *E. complanata* foot and hemolymph, the average difference between the $\delta^{15}N$ values of PN and foot and hemolymph was within the mean trophic range of +3 to +4‰. This relationship suggests that the $\delta^{15}N$ values in *E. complanata* muscle, mantle, and gill tissues can also be used as indicators of the baseline $\delta^{15}N_{PN}$ values in rivers. This finding has applications for trophic-level reconstructions as well as studies of historical nutrient loading to rivers.

The $\delta^{15}N$ values of stomach tissues in *E. complanata* were slightly lower than trophic-corrected $\delta^{15}N_{PN}$ values, more closely reflecting uncorrected $\delta^{15}N_{PN}$ values. The similarity between the $\delta^{15}N$ values of the stomach and $\delta^{15}N_{PN}$ values demonstrates that, as expected, the $\delta^{15}N$ values of the stomach contain a high proportion of unassimilated PN material. There appears to be little to no trophic-level enrichment between $\delta^{13}C_{POC}$ values and muscle, mantle, and gill $\delta^{13}C$ values in *E. complanata* (Fig. 6). However, given that only bulk PN and POC were examined in this study, we cannot isolate specific food sources for this species (see for example, Gillikin et al., 2006).

For *R. cuneata*, the relationships between $\delta^{15}N_{tissue}$ values and trophic-corrected $\delta^{15}N_{PN}$ values are less straightforward. Muscle, mantle, gill, and stomach tissues exceed $\delta^{15}N_{PN}$ values corrected for one trophic-level increase. There are several reasons that $\delta^{15}N_{tissue}$ values may not record trophic-corrected $\delta^{15}N_{PN}$ values. First, the bulk $\delta^{15}N_{PN}$ values used to approximate the $\delta^{15}N$ values of the diet may not be representative of the actual diet of the organism (Gustafson et al., 2007). Previous studies suggest that bivalves ingest a combination of deposited and suspended materials or that they may selectively assimilate certain food sources that are enriched in ^{15}N (Raikow and Hamilton, 2001; Lorrain et al., 2002) or depleted in ^{15}N (Kovacs et al., 2010). For example, deposited material including epipsammon (microscopic organisms living on grains of sand) and/or detritus have higher $\delta^{15}N$ values than those for suspended matter (Raikow and Hamilton, 2001).

Although bivalves are often assumed to be primary consumers, recent studies suggest that their trophic position ranges from 1 to 2 (e.g., Nichols and Garling, 2000; Vokhshoori and McCarthy, 2014; Ek et al., 2018; Whitney et al., 2019). In a previous study, the relatively wide ranges in the $\delta^{15}N$ and $\delta^{13}C$ values of tissues of invasive species, *Corbicula fluminea,* were indicative of low trophic fidelity and the assimilation of a broader variety in food sources than unionid *Elliptio crassidens* (Atkinson et al., 2010). We suggest that *R. cuneata* exhibit low trophic fidelity, or a low ability to maintain the same feeding habits continuously (Atkinson et al., 2010). In a previous study, Bucci et al. (2007) reported that *R. cuneata* and *C. fluminea* had similar $\delta^{15}N_{tissue}$ values to blue crabs, which are scavengers. In their study, *R. cuneata* were enriched by +3 to +4‰ relative to the $\delta^{15}N$ values of particulate organic matter, while at other sites, they were both above and below this range (Bucci et al., 2007). Their data, in combination with our own, lead us to hypothesize that *R. cuneata* have low trophic fidelity ranging from primary to secondary consumers depending on environmental conditions. This may also explain the unconventional $\Delta^{13}C_{consumer\text{-}diet}$ value of about −1.6‰ in this species. Accordingly, caution must be taken when using *R. cuneata* soft tissues for trophic-level reconstructions because their trophic position may vary.

6.2. Evaluation of $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values as environmental proxies

Overlap between trophic-corrected $\delta^{15}N_{PN}$ values and the $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in both species suggests that CBOM and periostracum can be used to reconstruct $\delta^{15}N_{PN}$ values in freshwater and estuarine environments (Fig. 3). However, one must proceed with caution when using shells of *R. cuneata* for environmental reconstructions due to their low trophic fidelity. The average $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in *R. cuneata* likely reflect average trophic-corrected $\delta^{15}N_{PN}$ values because of time-averaging associated with sampling the CBOM and periostracum for analysis. Therefore, we suggest that *R. cuneata* may be primary consumers most of the time, but not always.

Differences in the relationships between the $\delta^{15}N$ values of tissues and $\delta^{15}N_{PN}$ values occur because of differences in tissue turnover rate, time averaging, and composition. The significant, positive correlations between $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{mantle}$ values, and $\delta^{15}N_{periostracum}$ and $\delta^{15}N_{mantle}$ values, in *E. complanata* demonstrate that $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ can be used like mantle tissue for tracing biogeochemical processes. The correlation is significant for mantle tissue, but not other tissues due to the differences in tissue turnover rates between the compared tissues (Gillikin et al., 2017). Shell and soft-tissue growth are asynchronous and vary with bivalve metabolic rate; hence, shell and

tissue may not grow simultaneously (Hilbish, 1986; Borrero and Hilbish, 1988; Lewis and Cerato, 1997). For instance, while tissues turnover constantly throughout the lifetime of the organism, once the CBOM and the underlying periostracum are formed, they are "set" in time and do not experience metabolic turnover (Gillikin et al., 2017; Whitney et al., 2019). Soft tissues are continuously renewed via the replacement of cells throughout the lifetime of the organism (Bender, 1975; Tieszen et al., 1983) and different tissues turnover at different rates (Tieszen et al., 1983; Gustafson et al., 2007; Fukumori et al., 2008; Lorrain et al., 2002). Considering that soft tissues continuously turn over, they may be better indicators of chronic nutrient loading, rather than discrete events (e.g., Gustafson et al., 2007).

In contrast to soft tissues, the CBOM sampled in our study represents recently formed material because it was sampled along the shell growth margin. Due to time averaging associated with sampling large volumes of material for $\delta^{15}N$ analyses, the ~1 mm thick increments of CBOM and periostracum sampled in this study represent anywhere from 1 to 12 months of growth in *E. complanata* ($n = 9$) and 2 weeks to 3 months of growth in *R. cuneata* ($n = 6$) based on the oxygen isotope time series established by Graniero et al., *in review*. The range in time represented by these samples is large for *E. complanata* because of irregular growth rates due to seasonal growth cessation during winter shutdown when water temperatures fall below ~12 °C (Dettman et al., 1999) and during periods of extreme weather (Graniero et al., *in review*). As a result, many of the anomalously high and low $\delta^{15}N_{PN}$ values, which occur during winter and during the hurricane season, would not be expected to be observed in the $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ record.

Compositional differences between different tissues may also explain the absence of a significant relationship between the $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values and other tissues. Previous studies indicate that bivalve muscle tissues are enriched in ^{15}N compared to other soft tissues (Lorrain et al., 2002; O'Donnell et al., 2003; Piola et al., 2006; Poulain et al., 2010). Differences in protein content may explain why muscle tissues are enriched in ^{15}N compared to other soft tissues (Lorrain et al., 2002). For instance, muscle tissues in *E. complanata* and *R. cuneata* contain a higher percentage of nitrogen than other soft tissues. This is because muscle is composed primarily of proteins, whereas mantle and gill contain higher proportions of lipids and glycogen (Berthelin et al., 2000; Ojea et al., 2004). The seasonal changes in the proportion of these organic components are relatively small in the muscle, mantle, and gill (Ojea et al., 2004). Examining the difference between average $\delta^{15}N$ values between tissues, may provide valuable information about how to utilize $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ for paleoenvironmental reconstructions.

The $\Delta^{15}N_{tissue\text{-}CBOM}$ values in *E. complanata* and *R. cuneata* are similar to previously reported values for other aragonitic bivalves (Gillikin et al., 2017). Different carbonate polymorphs have been demonstrated to contain different amino acids which have distinct $\delta^{15}N$ values (McClelland and Montoya, 2002; Vokhshoori and McCarthy, 2014; Whitney et al., 2019). Calcite and aragonite are carbonate polymorphs that are made up of different proteins and amino acid compositions (Marie et al., 2012; Gillikin et al., 2017). Marie et al. (2012) analyzed the calcite prismatic layer and aragonite nacreous layer from pearl mussels, *Pinctada* spp., and found that 94% of prism-associated shell matrix proteins and 91% of nacre-associated proteins were restricted to their respective shell layer. These amino acids associated with layers of different mineralogies have distinct $\delta^{15}N$ values (McClelland and Montoya, 2002; Vokhshoori and McCarthy, 2014; Whitney et al., 2019). Therefore, in bivalves, the $\Delta^{15}N_{tissue\text{-}CBOM}$ offset tends to be positive for aragonite shells and negative for calcite shells (Gillikin et al., 2017).

In general, the $\Delta^{15}N_{tissue\text{-}CBOM}$ values are higher in *R. cuneata* than in *E. complanata* (Table 2). Both precipitate aragonite shells, so mineralogy can be ruled out as a cause for this difference. Differences in organic compounds or time-averaging likely play a role, but more work, such as compound-specific isotope analysis, is needed for confirmation. For future studies, it is necessary to consider the appropriate $\Delta^{15}N_{tissue\text{-}CBOM}$ and/or $\Delta^{15}N_{tissue\text{-}periostracum}$ offset when using $\delta^{15}N_{CBOM}$ and/or $\delta^{15}N_{periostracum}$ values as a proxy for $\delta^{15}N_{tissue}$ values. Nevertheless, these results stress the need to conduct species-specific calibration studies before using bivalve shell $\delta^{15}N$ values for paleoenvironmental and trophic-level reconstructions.

6.3. Spatial variations along a freshwater-estuarine gradient

To evaluate whether the $\delta^{13}C$ and $\delta^{15}N$ values in bivalve tissue can be used to demonstrate spatial heterogeneity along the freshwater-estuarine gradient, we first examine the variability in $\delta^{13}C_{POC}$ and $\delta^{15}N_{PN}$ values between study sites. The $\delta^{13}C_{POC}$ values generally increase from upriver to mid-estuary, which may be partially explained by changes in the relative contribution of allochthonous and autochthonous organic matter sources in the Neuse River and its estuary (Figure 2). Allochthonous sources of organic matter include terrestrial C_3 (average − 28.5‰; Kohn, 2010) and C_4 vegetation sources (−12 to −14‰; Fry and Sherr, 1989). Therefore, the general increase in $\delta^{13}C_{POC}$ values from upriver to mid-estuary may be explained by decreasing contributions of terrestrial C_3 vegetation (^{12}C-enriched) to the watershed approaching the estuary (Matson and Brinson, 1990). Matson and Brinson (1990) reported similar results in the estuarine portion of the Neuse River Basin. On the other hand, inputs of autochthonous organic matter to the Neuse River and its estuary are primarily controlled by primary production, respiration, and mixing between freshwater and marine endmembers (Matson and Brinson, 1990). Though primary production in the river is relatively low due to high suspended particle concentrations in the water column (e.g., Vähätalo et al., 2005), the cumulative effects of primary production from upriver to mid-estuary causes an increase in $\delta^{13}C_{POC}$ values (e.g., Peterson and Fry, 1987). In addition, mixing between the $\delta^{13}C_{POC}$ values of freshwater (average at Auburn-Knightdale = −30.9 ± 1.3‰) and marine (−20.7‰; Matson and Brinson, 1990) endmembers explains why $\delta^{13}C_{POC}$ values increase toward the estuary (e.g., Benninger and Martens, 1983; Fry, 2002). So, variability in $\delta^{13}C_{POC}$ and $\delta^{15}N_{PN}$ values in the Neuse River Basin reflect variations in the relative amounts of allochthonous and autochthonous organics to the surface waters and mixing between freshwater and marine endmembers.

In general, the $\delta^{15}N_{PN}$ values in the Neuse River Basin are within the ranges of synthetic fertilizers (−1 to +4‰; Showers et al., 1990; Bateman and Kelly, 2007), urban runoff (about +2 to +7‰; Bucci et al., 2011), municipal sewage effluent (+4 to +5‰ in lime-stabilized sludge, +10 to +12‰ in dewatered sludge; Showers et al., 2006), forested drainage (about +4 to +8‰; Bucci et al., 2011), and agricultural drainage (about +5 to +10‰; Showers et al., 1990; Bucci et al., 2011). Bulk $\delta^{15}N_{PN}$ sampling made it challenging to assess spatial trends because of the rapid response of the $\delta^{15}N$ and $\delta^{13}C$ values of primary producers to environmental change (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1999; Post, 2002; Gustafson et al., 2007; McMahon et al., 2013) and contributions from a variety of organic sources. Therefore, sharp changes in $\delta^{15}N_{PN}$ values can be attributed to rapid temporal changes in the type and concentration of N-bearing sources (Cabana and Rasmussen, 1996) and processes that affect the fractionation of nitrogen isotopes during uptake by primary producers (e.g., Mariotti et al., 1984; Velinsky et al., 1989). The $\delta^{15}N_{PN}$ values overlapped substantially between sites, making identifying relative contributions from any specific source difficult (Anderson and Cabana, 2006). Relative changes in $\delta^{15}N_{CBOM}$ values sampled in this study better track prolonged changes in nutrient sources due to their lower sensitivity to rapid changes, in contrast to $\delta^{15}N_{PN}$ values which are point samples of rapidly changing conditions. Then, the integration of N-bearing nutrients on different temporal scales likely accounts for the differences between $\delta^{15}N_{PN}$ and $\delta^{15}N_{CBOM}$ values.

The $\delta^{15}N_{CBOM}$ values in *E. complanata* generally increase from Auburn-Knightdale to Grifton. There are multiple explanations for the observed patterns in $\delta^{15}N_{CBOM}$ values between sites, including land-use

type, proximity to a wastewater treatment facility, and sampling resolution. First, results from a previous study analyzing foot tissues in *E. complanata* from the Neuse River Basin demonstrated that agricultural watersheds had significantly higher $\delta^{15}N_{tissue}$ values than forested and urban ones (Bucci et al., 2011). Yet, these $\delta^{15}N_{tissue}$ values were unable to distinguish between forested and urban watersheds (Bucci et al., 2011). This may explain why $\delta^{15}N_{CBOM}$ values are similar between Clayton (urban) and Seven Springs (forested) sites, while values at Grifton (agricultural) are higher (Fig. 5). Therefore, $\delta^{15}N_{CBOM}$ values can be used in a similar manner to soft tissues to distinguish to between watersheds dominated by urban and/or forested land use and those dominated by agricultural land use.

Second, there is a notable increase in average $\delta^{15}N_{CBOM}$ values between adjacent locations Auburn-Knightdale and Clayton, which are both characterized as urban (Fig. 5). The $\delta^{15}N_{CBOM}$ values at Auburn-Knightdale are consistent with $\delta^{15}N_{tissue}$ values reported for *E. complanata* collected from urban portions of the upper Neuse River Basin, whereas the $\delta^{15}N_{CBOM}$ values from Clayton are higher (Bucci et al., 2011). This may be due to the proximity of Clayton to the Neuse River Resource Recovery Facility (NRRRF; formerly the Neuse River Wastewater Treatment Plant) which borders about 5.8 km of the 6.6 km reach between Auburn-Knightdale and Clayton (Showers et al., 2006). Since the 1980s, biosolids produced at the plant have been applied to the surrounding farmland, known as Waste Application Fields, to produce animal feed (Showers et al., 2006). Biosolids produced at the NRRRF have a range in $\delta^{15}N$ values depending on how the waste is generated and stored (Showers et al., 2006). Biosolid application to landscapes and the dewatering of sludge results in ammonia volatilization, leaching, and/or denitrification that increases $\delta^{15}N$ values (Showers et al., 2006; reviewed by Denk et al., 2017). Treated wastewater effluent is discharged directly into the Neuse River, as well (Showers et al., 2006). This effluent is highly enriched in ^{15}N, having $\delta^{15}N$ values of +35‰, due to volatilization and microbial processes during the treatment process (Showers et al., 2006). Contributions from these ^{15}N-enriched products from the NRRRF may explain the increase in $\delta^{15}N_{PN}$ and $\delta^{15}N_{CBOM}$ values from Auburn-Knightdale to Clayton given that the land use at these sites is comparable.

Finally, it is difficult to distinguish spatial trends in $\delta^{15}N_{CBOM}$ values along the freshwater-estuarine gradient because of the large difference in $\delta^{15}N_{CBOM}$ values between the species. These species have apparent differences in trophic position and diet, which makes comparing $\delta^{15}N_{CBOM}$ values between freshwater and estuarine sites equivocal.

7. Conclusions

In this study, we determined that the $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in the compared species are reliable recorders of $\delta^{15}N_{PN}$ values in aquatic environments, but that low trophic fidelity in *R. cuneata* may complicate this relationship. Consequently, $\delta^{15}N_{CBOM}$ and $\delta^{15}N_{periostracum}$ values in historical and fossil shells can be used in a similar manner to soft tissues to extend records of biogeochemical processes beyond instrumental records. These findings are in support of previous studies that have found similar results for other bivalve species (Gillikin et al., 2017; Whitney et al., 2019). In addition, the $\delta^{15}N$ values of soft tissues in *E. complanata* are within the range of the trophic-corrected $\delta^{15}N_{PN}$ values, suggesting that soft tissues in this species can be used to estimate the baselines for trophic-level reconstructions. However, caution must be used when interpreting the $\delta^{15}N$ values of *R. cuneata* because the $\delta^{15}N$ values of soft tissues provide evidence of low trophic fidelity. Lastly, the $\delta^{15}N_{CBOM}$ values vary along a freshwater-estuarine gradient as a reflection of land-use change as well as differences in $\delta^{15}N$ values between species due to diet.

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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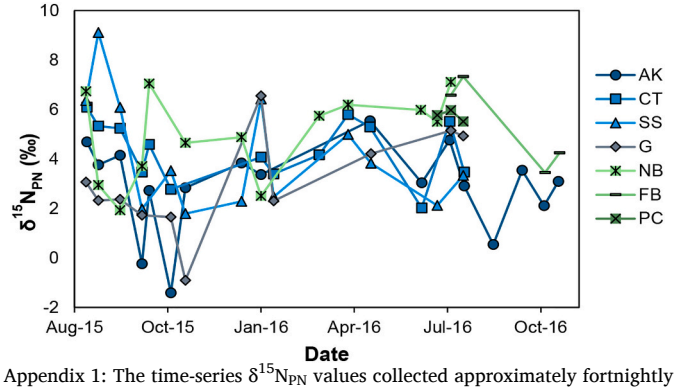

Appendix 1: The time-series $\delta^{15}N_{PN}$ values collected approximately fortnightly from Auburn-Knightdale (AK; circle), Clayton (C; square), Seven Springs (SS; triangle), Grifton (G; diamond), New Bern (NB; asterisk), Flanners Beach (FB; dash) and Pinecliff (PC; x).

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