

Influence of spatial and temporal variation on establishing stable isotope baselines of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ in a large freshwater lake

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Abstract

1. It is essential to establish a baseline in studies using stable isotopes to interpret trophic relationships across ecosystems and through time. Studies in freshwater ecosystems struggle to quantify baseline stable isotopes due to difficulties collecting representative samples, particularly from pelagic habitats. We assessed temporal and spatial variation in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ in a commonly used pelagic baseline, seston ($n = 156$), in Lake Erie to understand mechanisms that correlate with baseline stable isotope dynamics in large lakes. Seston contains a wide range of material which can confound stable isotope interpretation, and we examined the utility of element content and ratios to account for variation in sample source.
2. Seston was collected in each of the three basins of Lake Erie from May to October in 2017–2019 at nearshore (<10 m depth) and offshore (>10 m depth) sites. General linear models were conducted on each stable isotope ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$) and sample composition (variables: %N, %C, %S, C:N, C:S, and N:S) to assess how basin, month, and collection year influenced seston stable isotopes and composition.
3. Sample composition (variables: %N, %C, %S, C:N, C:S, and N:S), which is rarely reported for organisms in stable isotope studies, was constant throughout the sample period with no temporal or spatial trends except for small variations in %C, C:N, C:S, and N:S. This indicated that the temporal and spatial trends observed within the stable isotopes were related to seasonal changes in system processes and plankton community dynamics, with few or minimal changes in the amount of detrital and inorganic material within seston.
4. Values and trends of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ in seston were comparable to those measured previously in Lake Erie and other Laurentian Great Lakes. All three isotopes increased from May to October of each sample year and varied spatially, $\delta^{15}\text{N}$ was higher, $\delta^{34}\text{S}$ was lower, and $\delta^{13}\text{C}$ was the same in the west basin compared to the central and eastern basins of Lake Erie, which did not differ. These trends probably reflect seasonal changes in plankton community composition and

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nutrient cycling throughout the lake and are potentially linked to the presence of *Microcystis* blooms in the western basin during the late summer and autumn.

5. Seston turns over quickly, as shown by the rapid changes in stable isotope values throughout the study, which confounds the investigation of stable isotopes in upper trophic levels, and especially in organisms that have slower tissue turnover and move throughout the lake seasonally. Additionally, the variable composition of seston (e.g., % C, % N, % S, C:N, C:S, N:S) necessitates analysing sample composition to determine the degree of abiotic (e.g., detritus, sediment, particulate organic matter) and biotic (e.g., phytoplankton, zooplankton) content in it.

KEYWORDS

ecological stoichiometry, lake systems, nutrients, primary production, seston

1 | INTRODUCTION

Stable isotope analysis has emerged as a powerful tool that can be used to help delineate ecosystem processes and structure, largely owing to the predictable way in which isotopic ratios vary among environmental and biological community compartments (Peterson & Fry, 1987; Phillips et al., 2014). Stable isotopes are used as tracers of diet (Colborne et al., 2016), movement (Rubenstein & Hobson, 2004), habitat use (Croisetière et al., 2009), and trophic position (Post, 2002), in organisms from a wide variety of ecosystems (e.g. terrestrial, aquatic) and taxa (e.g. birds, fish, mammals; Boecklen et al., 2011). In freshwater ecosystems, nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) are the two most commonly analysed elements for stable isotope analysis, although there is a growing body of literature using isotopes of sulfur ($\delta^{34}\text{S}$), hydrogen ($\delta^2\text{H}$), and oxygen ($\delta^{18}\text{O}$) in ecological research (Colborne et al., 2016; Heuvel et al., 2019; Ofukany et al., 2014; Solomon et al., 2011).

Stable isotopes fractionate within different compartments of the food web (e.g., trophic fractionation) and behave in ways that reflect an organism's ecology. This fractionation often occurs in a predictable way that reflects patterns of resource consumption by an individual or species, which allows researchers to use them as tracers of a species' resource consumption (Peterson & Fry, 1987). For example, $\delta^{15}\text{N}$ is normally higher in consumers than their direct prey by a trophic discriminant factor of approximately 3.4‰ and is therefore often used to quantify trophic position (Post, 2002; Quezada-Romegialli et al., 2018; Vander Zanden et al., 1997). In lower trophic levels, the pool of nitrogen (e.g., $\text{NO}_2^-/\text{NO}_3^-$, NH_4^+) being consumed by primary producers also has an influence on stable isotope values, and basal (e.g., phytoplankton) $\delta^{15}\text{N}$ is strongly influenced by changes to biogeochemical processes and inputs of inorganic nitrogen to the system (e.g., fertiliser, sewage; Leggett et al., 2000; Steffy & Kilham, 2004; Teranes & Bernasconi, 2000). A consumer's $\delta^{13}\text{C}$ is less affected by trophic increase (c. 0.5‰–1‰ per trophic level; Peterson & Fry, 1987), and generally conserves the value in its prey, which represent the habitats and carbon used for feeding (Schindler & Scheuerell, 2002). For example, organisms that

use nearshore littoral habitats in lakes tend to have higher $\delta^{13}\text{C}$ than those that use offshore pelagic habitats (France, 1995; Post, 2002). Similar to $\delta^{13}\text{C}$, $\delta^{34}\text{S}$ shows minimal trophic increase (<0.5‰ per trophic level; Barnes & Jennings, 2007; Peterson & Fry, 1987), and has been used to differentiate freshwater and marine resource use (Croisetière et al., 2009; Moreno et al., 2010; Ofukany et al., 2014). While limited research has been conducted with $\delta^{34}\text{S}$ in freshwater ecosystems, lower $\delta^{34}\text{S}$ values are associated with use of more wetland or sediment derived resources (i.e., benthic) compared to planktonic (i.e., pelagic; Croisetière et al., 2009). Mixing models can be used to determine a species' diet or resource use since a species' $\delta^{15}\text{N}$ will be c. 3.4‰ higher than its prey, and its $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ will be an average of the values of its dominant prey items (Colborne et al., 2016; Fry, 2007; Stock et al., 2018).

A stable isotope baseline (hereafter isotopic baseline) is necessary to describe the natural distribution of isotopes within a study system. Isotopic baselines are usually compiled through collections of primary consumers that are relatively sessile and have a known trophic position (e.g., mussels, benthic invertebrates), to portray the ranges of values expected in different habitat types so that the trophic relationships and ecology of organisms higher in the food web can be interpreted (Cabana & Rasmussen, 1996; Layman et al., 2012). These baselines should also provide an estimate of the degree of variation in the ecosystem that might occur due to spatial or temporal factors, as stable isotopes in organisms typically used for isotopic baselines can vary spatially or change over time (Matthews & Mazumder, 2003; Smyntek et al., 2012; Woodland et al., 2012). Since stable isotopes move through an ecosystem and up food webs, any change in stable isotope values in the baseline could lead to incorrect or biased interpretations (Guzzo et al., 2011; Smyntek et al., 2012). Understanding spatio-temporal dynamics of isotopic baselines is most important for the study of highly mobile consumers that move between habitats (Guzzo et al., 2011; Woodland et al., 2012). To counter bias in data interpretation resulting from spatial variation, isotopic baselines must be established for all habitats that the species might use. Additionally, changes in stable isotope values in consumers tend

to lag behind those in lower trophic level species typically used for baselines (slower tissue turnover time). This may mean that baseline collections need to be started earlier than sample collections to track changes in stable isotopes in the baseline which may still influence study species during the study period (Leggett et al., 1999, 2000; Matthews & Mazumder, 2003; Woodland et al., 2012).

Aquatic ecosystems often exhibit seasonal variation in ecosystem function and structure based on climate, seasonal progression of the algal and plankton community, and fluctuations in nutrient cycling (Leggett et al., 1999, 2000; Yoshioka et al., 1994). Likewise, changes in stable isotope values within an ecosystem are the result of changing nutrient processing within ecosystems, which oscillates with changing season (Leggett et al., 1999, 2000; Xu et al., 2014). Nitrogen ($\delta^{15}\text{N}$) values in freshwater ecosystems tend to decline as the growing season progresses and will also reflect the nitrogen sources of the dominant types of primary production (Leggett et al., 2000). Carbon ($\delta^{13}\text{C}$) values will reflect the cycling of aqueous CO_2 within the system, which in turn affects the signature of primary producers (Leggett et al., 1999). Since $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in lower trophic level species (i.e., baseline) are both influenced by nutrient cycling, it is expected that $\delta^{34}\text{S}$ will also be temporally variable based on the type of primary production occurring, and primary producer community composition (Holmer & Storkholm, 2001).

Unfortunately, isotopic baselines can be difficult to establish and are rarely as extensive or well-expressed as they should be in ecological studies using stable isotope analysis (Woodland et al., 2012). Spatial variation in stable isotopes is often attributed to anthropogenic influences such as proximity to sewage outflows, agricultural land, or urban areas (Diebel & vander Zanden, 2009; Harvey & Kitchell, 2000; Savage & Elmgren, 2004) but this cannot be known unless the isotopic baseline is adequately quantified. In freshwater systems, organisms used for baselines (e.g., zooplankton, Oligochaetae, Chironomidae, Ephemeroptera) are often small-bodied (<30 mm) and it can be difficult to obtain sample masses large enough for stable isotope analysis so samples are often pooled together. Obtaining pelagic baselines can be difficult due to the small size of individual plankton (zooplankton and phytoplankton), which can have varied stable isotopes depending on the species composition of the plankton community, which makes obtaining stable isotope data for individual taxa almost impossible. Additionally, collection of baseline samples can be time-intensive, and expensive, especially in large lakes where weather and lake conditions can make it difficult to collect samples safely.

Pelagic isotopic baselines in freshwater systems are notoriously difficult to establish and often a filter feeding sessile organism such as mussels are used (Cabana & Rasmussen, 1996; Post, 2002). While sessile filter-feeders are good for smoothing out the temporal variability of stable isotopes exhibited in freshwater primary production (Matthews & Mazumder, 2003), they are restricted to feeding within their immediate vicinity, which may mean that they are not entirely reliant on pelagic plankton (Garton et al., 2005). As a result, such filter-feeders may not accurately represent the pelagic zone in a large

deep lake. An alternative pelagic baseline, seston, is a major source of energy within aquatic ecosystems, and represents suspended particulate organic matter, consisting of both biotic (e.g., bacteria, phytoplankton) and abiotic (e.g., sediment, detritus) components (Huguet, 2017). The quality of seston as a food source (e.g., lipids, nutrients) will vary depending on spatial and temporal factors, which in turn affects the community composition of the lower food web (Allard et al., 2011). Spatial factors such as bathymetry, proximity to land, point sources of nutrient loadings (e.g., wastewater sewage treatment plant outlet, agricultural greenhouse run-off), and underlying bedrock type (e.g., limestone, granite) will affect the availability of macronutrients for bioseston production (Huguet, 2017). Temporal factors such as seasonal fluctuations in nutrient availability, temperature, and diel light duration will also impact the types and amount of bioseston production (Hessen et al., 2005; Huguet, 2017). During the spring and early summer, seston composition is largely made up of biotic components consisting of zooplankton and phytoplankton (Hessen et al., 2005; Huguet, 2017). Seston quality in temperate ecosystems is typically lowest after the spring bloom when the phytoplankton community becomes dominated by grazing resistant species (e.g., *Microcystis* spp.; Hessen et al., 2005).

Seston acts as one of the main food sources for organisms within aquatic ecosystems (Huguet, 2017) and is a logical choice as a pelagic isotopic baseline. However, the variation in biotic and abiotic content is a confounding factor because it will affect the quality of seston as a food source for other organisms (Allard et al., 2011; Huguet, 2017). This variation in the abiotic and biotic content of seston will change depending on how much of it is plankton, or particulate matter such as sediment and detritus, and will ultimately be affected by environmental factors such as run-off, weather events, and algal blooms (Hessen et al., 2005; Huguet, 2017). Ultimately, the composition of seston could be assessed using %N %C, and %S. Although stable isotope analysis provides researchers with element content (i.e., %C and %N) it is rarely reported or used to understand stable isotopes beyond using C:N ratio as a proxy for lipid content (Ogorek et al., 2021; van der Lee et al., 2021).

With the inclusion of $\delta^{34}\text{S}$ in studies, there is the opportunity to investigate the utility of C:S and N:S ratios for understanding ecological processes. These ratios could shed light on the composition of samples, particularly seston, that are composed of different materials and have more variable elemental composition. Invertebrates and primary producers have a great deal of flexibility in their nutrient stoichiometry, which can result in changes to their elemental composition due to environmental variation in nutrient availability (van der Lee et al., 2021). Sulfur, like nitrogen, is an essential element in organisms for protein production (Droux, 2004; Xing et al., 2013), and so ratios of sulfur with other nutrients could shed light on variability of seston composition due to environmental shifts. Molar C:S and N:S ratios have been used in marine systems to explore the production of dimethylsulfoniopropionate in phytoplankton (Matrai & Keller, 1994; Ratti et al., 2011; Simó et al., 2009), but no research has been conducted to look at how either ratio varies in freshwater phytoplankton.

Stable isotopes of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ in seston ($n = 156$) collected from Lake Erie between May and October over 3 years (2017–2019) were used to investigate the spatiotemporal patterns in a pelagic isotopic baseline. Furthermore, the utility of using the elemental composition (%N, %C, %S, C:N, C:S, and N:S) of seston to assess spatial and temporal variation as a metric for understanding seston stable isotopes was explored. It is predicted that $\delta^{15}\text{N}$ will decrease from May until August when it will begin to increase until the end of the growing season in October as has been shown in other studies in the Great Lakes (Leggett et al., 2000). Values of $\delta^{13}\text{C}$ are predicted to be related to seasonal changes in seston composition (C:N) and the timing and succession of the spring phytoplankton bloom, with values gradually increasing over the sampling season. Seston composition is predicted to be related to the degree of detrital and inorganic matter within samples (high %C, low %N and %S, high C:N) and this is predicted to increase over the growing season as grazing resistant phytoplankton (e.g., *Microcystis* spp.) begin to dominate the plankton community.

2 | METHODS

2.1 | Description of study system

Lake Erie, the shallowest and most productive of the Laurentian Great Lakes (hereafter Great Lakes), provides a good system for investigating the temporal and spatial variation in baseline stable isotopes as it is a large lake that can be divided into three basins with differing characteristics. Water in Lake Erie flows from west to east and originates from two major tributaries: the Maumee River, which has a high nutrient but low water volume input; and the Detroit River, which has a high water volume and low nutrient input (Mortimer, 1987; Steffen et al., 2014). The west is the shallowest and smallest basin (area = 3,080 km²; mean depth = 7.4 m), the central basin is the largest (area = 16,159 km²; mean depth = 18 m; maximum depth = 25 m), and the east basin is the deepest (mean depth = 25 m; maximum depth = 64 m; Mortimer, 1987). The west basin is also generally the most productive (i.e., eutrophic) due to the high nutrient inflow from the Maumee River, but this decreases moving east through the lake (Mortimer, 1987; Steffen et al., 2014; Watson et al., 2016). As a result, there is decreasing influence from agricultural, industrial, and urban areas as you move east through the lake (Watson et al., 2016), potentially creating variable isotope signatures throughout the lake. Studies on fish ecology in Lake Erie have demonstrated both temporal and spatial variation in $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ in the western basin of the lake across multiple trophic levels (Guzzo et al., 2011; Heuvel et al., 2019). A similar study has not been conducted for plankton within Lake Erie.

2.2 | Sample collection

Seston samples were collected from six sites throughout Lake Erie between 2017 and 2019 (Figure 1). These sites included a nearshore

(depth <10 m) and offshore (depth >10 m) location in each basin (east, central, and west). Overall, 157 seston samples were collected bi-weekly at each site between May and October from 2017 to 2019. Seston was collected in plankton nets (63- μm mesh) that were pulled to sample the entire water column, filtered (63 μm) to remove excess water, and stored in the freezer at -20°C until processed for stable isotope analysis.

2.3 | Stable isotope analysis: nitrogen, carbon, and sulfur

All samples were lyophilised at -48°C and 133×10^3 mbar for 48 hr, and then homogenised into a fine powder by hand using a mortar and pestle or dissection scissors. Freeze-dried samples were weighed into tin cups for $\delta^{34}\text{S}$ and %S (6,000–6,500 μg) and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C and %N (400–600 μg) analysis. Sulfur isotopic composition was analysed on a Delta V Plus mass spectrometer (ThermoScientific) in continuous flow mode coupled to a 4,010 Elemental Combustion System (Costech Instruments), and carbon and nitrogen isotopic composition were determined using a Delta V Advantage mass spectrometer (ThermoScientific) in continuous flow mode coupled to a 4010 Elemental Combustion System. Instrument accuracy throughout the period of sample analysis was within 0.1‰ (SD, NIST 8547, NIST 8573 and NIST 8574, $n = 50$ for all) for $\delta^{15}\text{N}$, 0.1‰ (SD, NIST 8573, NIST 8542, and NIST 8574, $n = 50$ for all) for $\delta^{13}\text{C}$, and 0.3‰ (SD, NIST 8554, NIST 8555, and NIST 8529, $n = 30$ for all) of certified values for $\delta^{34}\text{S}$. Sample accuracy as measured by USGS 40 was within 0.1‰ (SD) of certified values for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, and <0.1‰ (SD) for $\delta^{34}\text{S}$. Sample precision of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, determined by four laboratory standards [NIST 1577c, internal lab standard (tilapia muscle), USGS 40 and IVA33802174 Urea ($n = 187$ for each)] run every 12 samples, was $\leq 0.2\%$ (SD), and precision of $\delta^{34}\text{S}$, measured by five internal laboratory standards [NIST 1577c, NIST 8529, NIST 8555, internal lab standard (tilapia muscle), and USGS 42; $n = 84$ for each] run every 10 samples, was $\leq 0.3\%$ (SD). Sample reproducibility of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ sample tissue replicates (measured in triplicate every 10 samples) was $\pm 0.2\%$ (SD, $n = 30$), and reproducibility for $\delta^{34}\text{S}$ (run in duplicate for every eighth sample) was $\pm 0.1\%$ (SD, $n = 35$), which was within the acceptable range for all isotopes ($\pm 0.2\%$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and $\pm 0.3\%$ for $\delta^{34}\text{S}$).

2.4 | Data analysis

Differences existed between mean isotope values of sample sites for $\delta^{15}\text{N}$ (ANOVA: $F_{5,150} = 6.6$, $p < 0.001$), but not for either $\delta^{13}\text{C}$ ($F_{5,150} = 0.93$, $p = 0.5$) or $\delta^{34}\text{S}$ ($F_{5,142} = 1.3$, $p = 0.3$). Tukey honest significant difference (HSD) tests indicated that $\delta^{15}\text{N}$ was not different at sites located in the same basin (Tukey HSD: West: W5 and W8, $p = 1.0$; Central: WC1 and WC3, $p = 1.0$; East: E2 and E3, $p = 0.9$). No differences existed between sample sites for %N (ANOVA: $F_{5,150} = 2.0$, $p = 0.08$), but did exist for %C ($F_{5,150} = 3.3$, $p = 0.01$) and

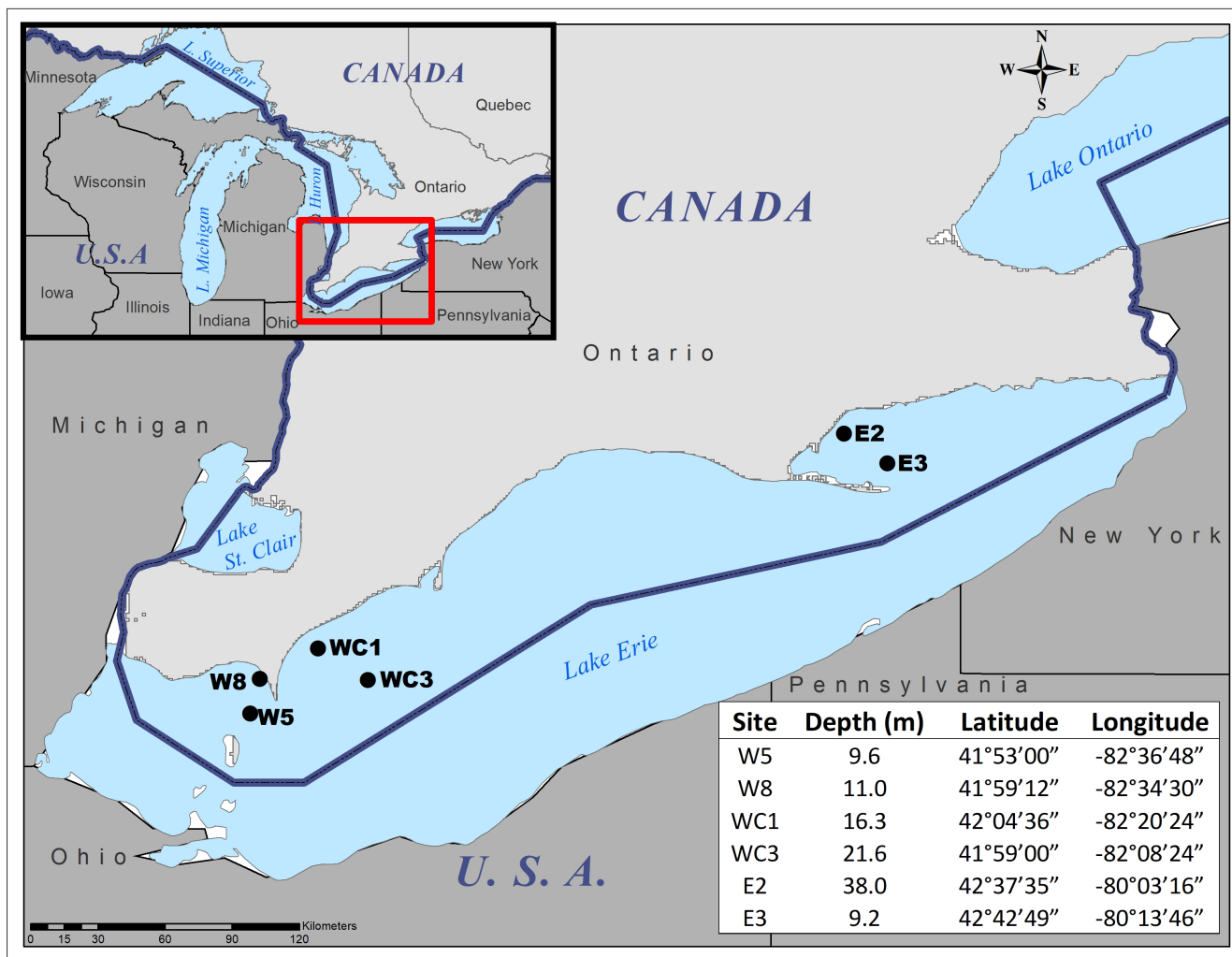


FIGURE 1 Sample sites where baseline samples were collected in Lake Erie between 2017 and 2019. Research was focused in Canada, on the north side of the lake.

%S ($F_{5,142} = 3.2$, $p = 0.01$); however, Tukey HSD tests indicated that neither %C nor %S was different at sites within the same basin (Tukey HSD: $p > 0.5$ for all). The C:N, C:S, and N:S ratios were not different at any site (ANOVA; C:N: $F_{5,150} = 2.0$, $p = 0.08$; C:S: $F_{5,142} = 1.7$, $p = 0.1$; N:S: $F_{5,142} = 1.1$, $p = 0.4$). Since no variables indicated differences between sites within the same basin, seston data was combined for a basin and was divided into groups based on month, and year collected for β regression models and general linear models (GLMs).

Combined temporal (month, year) and spatial trends (basin) in seston %N, %C, and %S were assessed using β regressions (R package: betareg; Cribari-Neto & Zeileis, 2010) which ran a regression using a β distribution and logit link function because proportional data are bounded by 0 and 1 (Crawley, 2013; Douma & Weedon, 2019; Warton & Hui, 2011). Wald Chi-squared tests were used on significant models to identify significant variables and linear hypothesis tests were conducted on pairs of significant variables to determine differences based on collection year, basin, or month. GLMs were conducted on ratios of C:N, C:S, and N:S to assess the influence of month, year, and basin collected using an inverse gaussian distribution and $1/\mu^2$ link function since these ratios are restricted to positive

numbers. Similarly, GLMs using a gaussian distribution and identity link function were performed to assess the combined temporal (i.e., month and year collected) and spatial (i.e., basin) trends in seston for each isotope ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$) within Lake Erie. Analysis of variance (ANOVA) was conducted on all significant GLMs to identify and quantify the influence of significant variables affecting isotope values and Tukey HSD tests were conducted post hoc in the event of any significant factor variables. All groups and residuals were normally distributed as evaluated through visual inspection of Q-Q, residual versus fitted, scale-location, and constant leverage plots. All data analysis was conducted in R version 4.1.0 (R Core Team, 2021).

3 | RESULTS

3.1 | Beta regression models of %N, %C, and %S

Beta regressions indicated that %N was consistent across month (β regression, Wald Chi-squared test, $\chi^2_{\text{MONTH}} = 6.1$, $p = 0.3$), and basin ($\chi^2_{\text{BASIN}} = 4.3$, $p = 0.1$) but year was a significant predictor within

the GLM ($\chi^2_{\text{YEAR}} = 11.0, p = 0.004$), albeit the effect size was small (Table 1). Linear hypothesis tests indicated that %N was lower in 2017 ($p \leq 0.02$) than it was in 2018 or 2019, which were similar ($p = 0.8$).

Month did not significantly contribute to the β regression model of seston %C in Lake Erie (β regression, Wald Chi-squared test, month: $\chi^2 = 10.9, p > 0.05$), but basin and year were significant predictors of %C (year: $\chi^2 = 8.5, p = 0.01$; basin: $\chi^2 = 9.4, p = 0.01$;

Table 1). Seston %C in the western basin (linear hypothesis test; $p \leq 0.01$) was lower than either the central or eastern basins which were similar ($p = 0.7$). Seston collected in 2018 had similar %C to both 2017 and 2019 ($p \geq 0.2$); seston %C in 2017 was higher than 2019 ($p = 0.004$).

Percent sulfur (%S) was consistent temporally (e.g., month and year) but did vary spatially (e.g., basin; β regression; Wald's

TABLE 1 Summary of β regressions investigating temporal and spatial trends in %N, %C, and %S in seston collected in Lake Erie between May and October in 2017–2019.

Variable	Variable	χ^2	p-Value	Group	Estimate	Group significance
%N	Basin	4.3	$p = 0.1$	West	-	a
				Central	0.1	a
				East	0.0	a
	Month	6.1	$p = 0.3$	May	-	a
				June	-0.1	a
				July	-0.1	a
				August	0.0	a
				September	-0.2	a
				October	-0.2	a
	Year	11.0	$p = 0.004$	2017	-	a
				2018	-0.2	b
				2019	-0.2	b
%C	Basin	9.5	$p = 0.009$	West	-	a
				Central	0.1	b
				East	0.1	b
	Month	9.1	$p = 0.1$	May	-	a
				June	0.0	a
				July	0.0	a
				August	0.0	a
				September	-0.2	a
				October	-0.1	a
	Year	8.6	$p = 0.01$	2017	-	a
				2018	-0.1	ab
				2019	-0.1	b
%S	Basin	4.8	$p = 0.01$	West	-	a
				Central	0.2	b
				East	0.0	a
	Month	16.4	$p < 0.001$	May	-	a
				June	-0.1	a
				July	0.0	a
				August	0.0	a
				September	0.0	a
				October	0.1	a
	Year	0.4	$p = 0.7$	2017	-	a
				2018	-0.1	a
				2019	-0.1	a

Note: All three models were run with a β distribution and logit link function and were fitted to the western basin for basin, May for month, and 2017 for year. Groups denoted with different letters indicate which groups significantly differed from each other.

Chi-squared test, $\chi^2_{\text{MONTH}} = 4.6$, $p_{\text{MONTH}} = 0.5$, $\chi^2_{\text{YEAR}} = 1.7$, $p_{\text{YEAR}} = 0.4$, $\chi^2_{\text{BASIN}} = 9.7$, $p_{\text{BASIN}} = 0.008$; Table 1). Seston %S in the central basin was higher than either the western (linear hypothesis test; $p = 0.003$) or the eastern basin ($p < 0.05$), which were similar ($p = 0.5$; Table 1).

3.2 | General linear models of C:N, C:S, and N:S

The C:N ratio for seston was consistent across month (GLM; $F_{\text{MONTH}} = 0.6$, $p_{\text{MONTH}} = 0.7$), but differences between years ($F_{\text{YEAR}} = 3.9$, $p_{\text{YEAR}} = 0.02$) and basin ($F_{\text{BASIN}} = 4.7$, $p_{\text{BASIN}} = 0.01$) were observed (Table 2). Seston C:N was lower in 2017 than 2018 (Tukey HSD; $p = 0.03$), C:N in 2019 was similar to both 2017 ($p = 0.1$) and 2018 ($p = 0.6$). Eastern basin C:N was higher than both the western (ANOVA, Tukey HSD, $p < 0.05$) and central basins ($p = 0.01$) which were similar to each other ($p = 0.9$).

The C:S ratio for seston was consistent across basin (GLM; $F_{\text{BASIN}} = 2.6$, $p = 0.08$) and year ($F_{\text{YEAR}} = 1.5$, $p = 0.2$), but differences between months ($F_{\text{MONTH}} = 4.4$, $p < 0.001$) were observed (Table 2). All months had similar C:S (ANOVA, Tukey HSD; $p > 0.05$ for all combinations) except for September and June ($p = 0.001$).

The N:S ratio varied between months (GLM; $F_{\text{MONTH}} = 4.2$, $p = 0.001$) and year ($F_{\text{YEAR}} = 4.3$, $p = 0.02$), but was consistent across basin ($F_{\text{BASIN}} = 0.7$, $p = 0.5$; Table 2). May N:S was similar to June (ANOVA, Tukey HSD; $p = 0.9$), July ($p = 0.3$), and August ($p = 1.0$), but was higher than September ($p = 0.02$) and October ($p = 0.02$). June had lower N:S to September ($p = 0.04$), and all other month combinations were similar ($p > 0.05$). Samples collected in 2019 had lower N:S compared to 2017 (ANOVA, Tukey HSD; $p = 0.02$), and all other year combinations were similar to each other ($p > 0.2$).

3.3 | General linear models of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$

General linear models of the three isotopes ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$) indicated that month was the main correlate of the trends observed in seston stable isotope values (GLM: $\delta^{15}\text{N}$, $F_{\text{MONTH}} = 16.3$, $p < 0.001$; $\delta^{13}\text{C}$, $F_{\text{MONTH}} = 25.5$, $p < 0.001$; $\delta^{34}\text{S}$, $F_{\text{MONTH}} = 16.4$, $p < 0.001$; Table 3, Figure 2), as year did not influence $\delta^{15}\text{N}$ ($F_{\text{YEAR}} = 1.5$, $p = 0.2$) nor $\delta^{34}\text{S}$ ($F_{\text{YEAR}} = 0.4$, $p = 0.7$), and although significant for $\delta^{13}\text{C}$ ($F_{\text{YEAR}} = 3.4$, $p = 0.04$) its F-value (and consequently effect size) was small in comparison to influence of month (Table 3). Basin significantly influenced $\delta^{15}\text{N}$ ($F_{\text{BASIN}} = 24.1$, $p < 0.001$) and $\delta^{34}\text{S}$ ($F_{\text{BASIN}} = 4.8$, $p = 0.01$) but not $\delta^{13}\text{C}$ ($F_{\text{BASIN}} = 2.4$, $p = 0.09$; Table 3, Figure 3).

Post hoc tests found that seston $\delta^{15}\text{N}$ did not differ among May, August, September, and October (ANOVA, Tukey HSD, $p \geq 0.1$), but all 4 months were higher ($p \leq 0.03$) than June and July, which were similar ($p = 0.3$; Table 4, Figure 2). Values of $\delta^{13}\text{C}$ were lower in seston during the spring (May and June) than autumn (August, September, and October; $p \leq 0.001$; Table 4, Figure 3). July had higher $\delta^{13}\text{C}$ than June ($p < 0.001$) and was lower than September

($p = 0.0003$) and October ($p = 0.02$) but was statistically similar to May ($p = 0.1$) and August ($p = 0.4$; Table 4, Figure 2). May and June (Tukey HSD: $p = 0.3$) $\delta^{34}\text{S}$ in seston was lower than July, August, and September ($p \leq 0.001$; Table 4, Figure 2). Seston $\delta^{34}\text{S}$ in October was not significantly different from any other month sampled ($p \geq 0.06$) except May ($p < 0.001$; Table 4, Figure 2).

Seston $\delta^{15}\text{N}$ was similar between eastern and central basins and both were higher than the west (Tukey HSD: $p \leq 0.02$ for both; Table 4, Figure 3). No differences in seston $\delta^{13}\text{C}$ were observed between basins ($p \geq 0.1$; Table 4, Figure 3). Values of $\delta^{34}\text{S}$ were significantly higher in the west than the central or east basins ($p \leq 0.001$; Table 4, Figure 3).

4 | DISCUSSION

Seston $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ in Lake Erie were influenced by seasonal and spatial (basin) factors, demonstrating the complexity of baseline distributions of isotopes in large freshwater lakes. Seston $\delta^{15}\text{N}$ decreased in June and July, and then increased in August, September, and October in the central and eastern basins but remained low from July to October in the western basin. Values of $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ in seston increased between May and October throughout the entire lake. These findings highlight the dynamic nature of temperate freshwater ecosystems and the need to consider season and spatial complexity in the analysis of stable isotopes within them.

Seston, in this study, represented a composite sample of small particles larger than $63\mu\text{m}$, and the biotic and abiotic components could vary significantly depending on limnological conditions and time of year (Huguet, 2017). Despite small statistical differences in % C and C:N between year collected, the magnitude of difference is small when data range (% C: minimum $\approx 16\%$ and maximum $\approx 51\%$; $\delta^{13}\text{C}$: minimum $\approx -31.0\%$, maximum $\approx -23.0\%$ for all years) is considered, and based on our current understanding of these elements, unlikely to be biologically significant. This indicates that the temporal patterns observed within the isotopes were not associated with seasonal changes in the broader elemental composition of the seston but are more likely to be associated with changes in nutrient cycling associated with primary producer/consumer composition within the ecosystem as the season progresses (Matthews & Mazumder, 2003, 2005). Ratios of C:N in all basins and months throughout were consistently between 6 and 8, consistent with the range of C:N expected for freshwater phytoplankton which is higher than that expected for primary consumers such as zooplankton, and lower than the 15–20 expected for sediment and other detritus (Finlay & Kendall, 2007; Ogorek et al., 2021). This indicates that the composition of seston throughout the study was predominantly phytoplankton. Lower %C in the western basin and higher C:N in the eastern basin could be indicative of the high productivity in the western portion of the lake compared to the central and eastern basins (Watson et al., 2016), as an excess of limiting nutrients such as nitrogen and phosphorous can result in lower C:N ratios and %C which improve phytoplankton quality for consumers (van der Lee et al., 2021).

TABLE 2 Summary of general linear models investigating temporal and spatial trends in seston C:N collected in Lake Erie between May and October in 2017–2019.

Variable	Variable	F-statistic	p-Value	Group	Estimate	Group significance
C:N	Basin	9.1	$p = 0.01$	West	–	a
				Central	0.001	a
				East	–0.005	b
	Month	1.1	$p = 0.7$	May	–	a
				June	–0.002	a
				July	–0.001	a
				August	0.0	a
				September	–0.001	a
				October	–0.005	a
	Year	7.6	$p = 0.02$	2017	–	a
				2018	–0.005	b
				2019	–0.003	ab
C:S	Basin	2.6	$p = 0.08$	West	–	a
				Central	-1.2×10^{-6}	a
				East	-2.0×10^{-5}	a
	Month	4.4	$p < 0.001$	May	–	ab
				June	-2.7×10^{-6}	a
				July	1.9×10^{-5}	ab
				August	5.3×10^{-6}	ab
				September	5.3×10^{-5}	b
				October	5.5×10^{-5}	ab
	Year	1.5	$p = 0.2$	2017	–	a
				2018	4.3×10^{-7}	a
				2019	1.6×10^{-5}	a
N:S	Basin	0.7	$p = 0.5$	West	–	a
				Central	0.003	a
				East	0.0	a
	Month	4.2	$p = 0.001$	May	–	a
				June	0.0	ab
				July	0.001	ac
				August	0.001	ac
				September	0.002	c
				October	0.003	bc
	Year	4.3	$p = 0.02$	2017	–	a
				2018	0.001	ab
				2019	0.001	b

Note: The model was run with a inverse gaussian error distribution and $1/\mu^2$ link and was fitted to the western basin for basin, May for month, and 2017 for year. Groups denoted with different letters indicate which groups significantly differed from each other.

Seasonal (monthly) patterns in stable isotopes explained most of the variation observed in seston $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$, highlighting the importance of well-defined baselines that incorporate sampling along appropriate timescales in stable isotope studies. Guzzo et al. (2011) reported similar seasonal patterns of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in seston collected within the western basin of Lake Erie between July and September, and observed that seston stable

isotopes were more variable than those of *Dreissena* spp. Isotopic baselines provide the basis for interpretation of all other stable isotopes collected within the study system (Woodland et al., 2012) and are also often important diet items exploited by consumers (Smyntek et al., 2012; Syväranta et al., 2008; Zohary et al., 1994). This means that any variation in the baseline will probably be observed in the study species and affect how their stable isotopes

Isotope	Variable	F-statistic	p-Value	Group	Estimate	Group significance	
$\delta^{15}\text{N}$	Basin	24.1	$p < 0.001$	West	-	a	
				Central	1.9	b	
				East	1.5	b	
	Month	16.3	$p < 0.001$	May	-	a	
				June	-2.4	b	
				July	-3.1	b	
				August	-1.2	a	
				September	-1.0	a	
				October	-0.5	a	
				Year	1.6	$p = 0.2$	2017
	$\delta^{13}\text{C}$	Basin	2.4	$p = 0.09$	West	-	a
					Central	-0.2	a
East					-0.8	a	
Month		25.5	$p < 0.001$	May	-	ab	
				June	-0.7	a	
				July	1.0	bc	
				August	1.8	cd	
				September	2.6	d	
				October	2.9	d	
				Year	3.4	$p = 0.04$	2017
$\delta^{34}\text{S}$		Basin	4.8	$p = 0.01$	West	-	a
					Central	-0.5	b
	East				-0.8	b	
	Month	16.4	$p < 0.001$	May	-	a	
				June	0.7	ab	
				July	1.7	c	
				August	2.2	c	
				September	1.9	c	
				October	1.3	bc	
				Year	0.4	$p = 0.7$	2017
				2018	-0.1	a	
				2019	0.0	a	

Note: All three models were run with a Gaussian distribution and identity link function and were fitted to the western basin for basin, May for month, and 2017 for year. Groups denoted with different letters indicate which groups significantly differed from each other.

should be interpreted (Woodland et al., 2012; Zohary et al., 1994). In fact, recent research within Lake Erie has shown significant seasonal variation of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ isotopes in white perch (*Morone americana*), walleye (*Sander vitreus*), and freshwater drum (*Aplodinotus grunniens*) that mimic the patterns observed in this study (Heuvel et al., 2019). Additionally, previous research in the Lake Erie's western basin has shown that mismatches between the time of sampling of baseline organisms and that of the study

species can introduce significant bias when calculating trophic position (Guzzo et al., 2011).

Seasonal succession of the Lake Erie phytoplankton community throughout the spring and summer was probably the main factor influencing the variation observed in $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ as demonstrated by similar sample composition throughout the sampling period each year. Phytoplankton community composition and fluctuations in ecosystem biogeochemical processes probably influence

TABLE 3 Summary of general linear models investigating temporal and spatial trends in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ in seston collected in Lake Erie between May and October in 2017–2019.

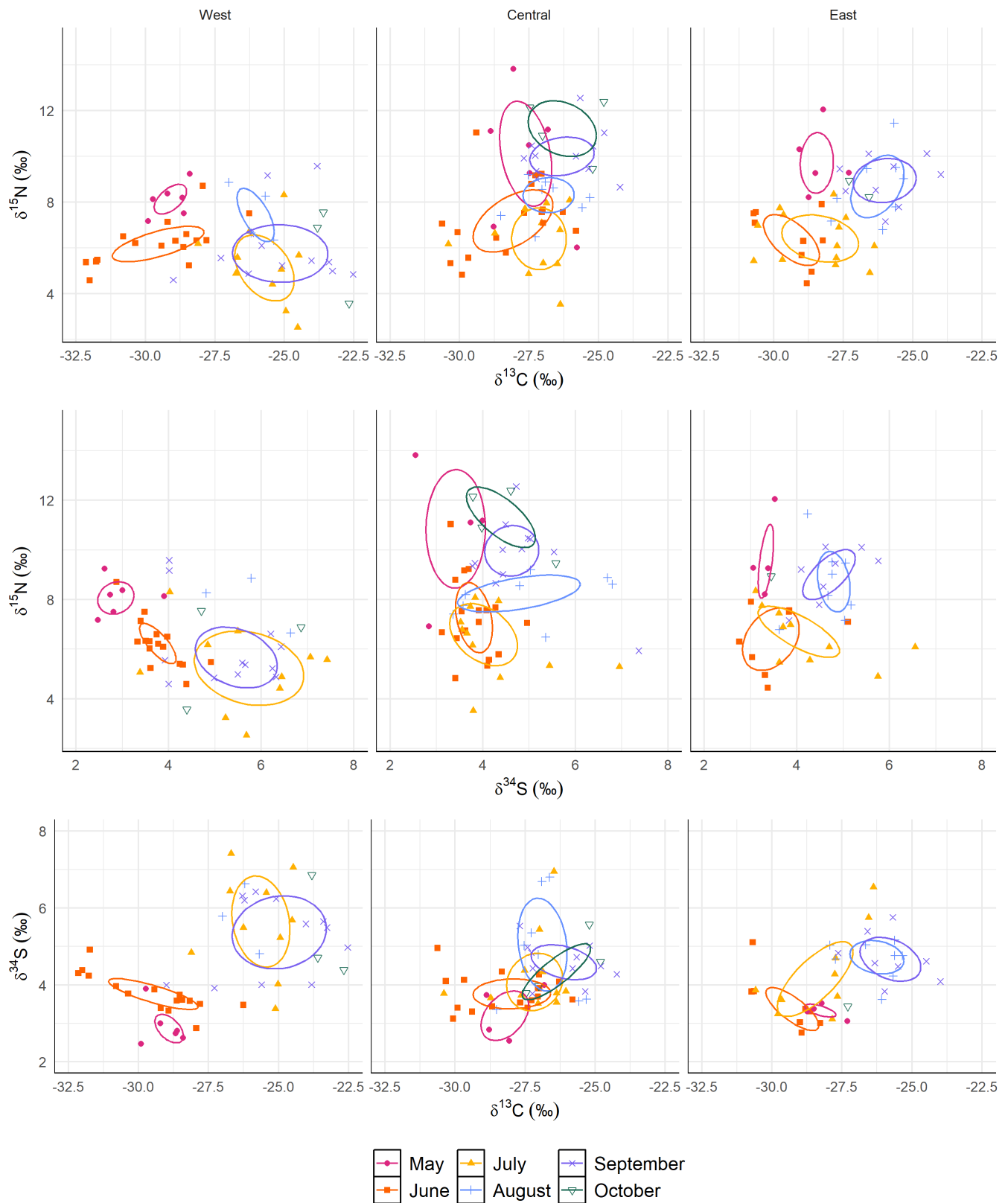


FIGURE 2 Bi-plot of (top) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, (middle) $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$, and (bottom) $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ in the (left) west, (centre) central, and (right) east basins of Lake Erie. Ellipses indicate the core (40%) spread of data for each month and were not drawn for months with fewer than four samples.

seasonal patterns of $\delta^{15}\text{N}$ in Lake Erie seston. Seasonal patterns of $\delta^{15}\text{N}$ in seston closely followed those observed in particulate organic matter and zooplankton in Lake Ontario (Leggett et al., 2000), as

well as other temperate and subtropical lakes globally (Syväranta et al., 2008; Visconti & Manca, 2011). Some variation is also probably due to changes in run-off within the ecosystem resulting in

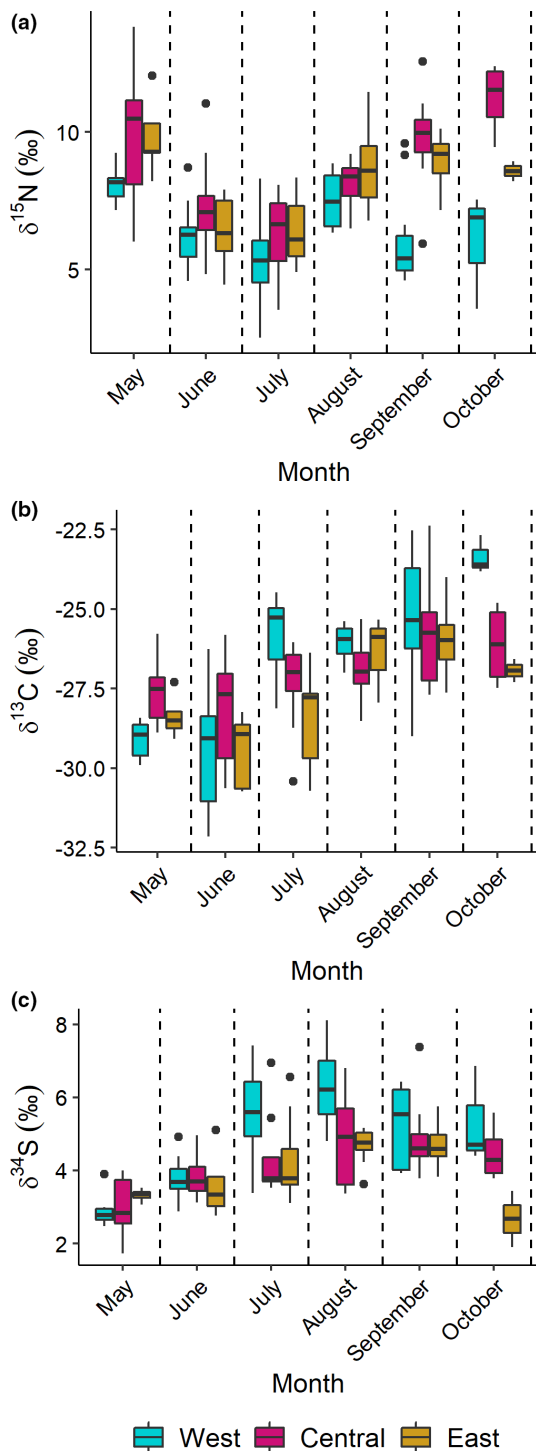


FIGURE 3 Boxplots of the distribution of values measured for (a) $\delta^{15}\text{N}$, (b) $\delta^{13}\text{C}$, and (c) $\delta^{34}\text{S}$ in seston collected in the west (blue), central (red), and east (yellow) basins of Lake Erie. Circles indicate outliers.

alterations to the biogeochemical cycling of nitrogen and the timing of the formation of the thermocline (Leggett et al., 2000).

Carbon isotope values, $\delta^{13}\text{C}$, increased steadily between May and October, consistent with trends observed in other lakes which found a cyclical trend of increasing $\delta^{13}\text{C}$ during the spring bloom followed by a decline in the autumn and winter (Leggett et al., 1999;

Zohary et al., 1994); late autumn and winter trends were not assessed here. These trends of increasing $\delta^{13}\text{C}$ between May and October are probably partially associated with seasonal community succession of plankton, which are influenced by seasonal changes in water temperature, pH, and dissolved inorganic carbon (Leggett et al., 1999; Smyntek et al., 2012; Visconti & Manca, 2011; Zohary et al., 1994). Changes in the C:N ratio seasonally could influence $\delta^{13}\text{C}$ in seston given that increased lipids (higher C:N) can deplete $\delta^{13}\text{C}$ (Matthews & Mazumder, 2005). However, evidence for this was not observed since, while we saw an increase in $\delta^{13}\text{C}$, we did not see a corresponding decrease in C:N over the sampling period, indicating that, while C:N and $\delta^{13}\text{C}$ were negatively correlated (correlation analysis, $R^2 = -0.25$, $p = 0.002$), C:N did not influence seasonal patterns of $\delta^{13}\text{C}$.

While the mechanisms driving variation of $\delta^{34}\text{S}$ in freshwater food webs are not well known (Colborne et al., 2016; Croisetière et al., 2009; Ofukany et al., 2014), this study adds to a larger body of literature that demonstrates that measuring $\delta^{34}\text{S}$ can add value to freshwater ecology research. Unlike $\delta^{13}\text{C}$, $\delta^{34}\text{S}$ demonstrated both temporal and spatial variation. In fact, $\delta^{34}\text{S}$ has been shown to be a useful tracer of food chain origin and food web dynamics in freshwater ecosystems and is possibly more effective at tracing food chain origins derived from sedimentary detritus than $\delta^{13}\text{C}$ (Croisetière et al., 2009; Finlay & Kendall, 2007; Proulx & Hare, 2014).

Ratios of C:S and N:S have never been included in a stable isotope study to our knowledge; however, the results suggest that they could be useful metrics to help assess isotopic baselines that have variable elemental composition such as seston. It is difficult to draw any conclusions from the data presented here without further research into how C:S (%) and N:S (%) ratios vary within different organisms and systems and how that relates back to stable isotope ratios of $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$. However, the patterns of decreasing seston C:S ratios observed in the western and central basins of Lake Erie here suggest that further investigation into drivers of variation in C:S and N:S ratios is merited.

Spatial patterns of seston $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ observed between basins are probably due to a combination of factors including lake morphology (e.g., nutrient availability), and bathymetry (e.g., depth). Lake Erie is divided into three morphologically distinct basins, with an increasing nutrient and decreasing depth gradient moving west through the lake (Mortimer, 1987). Nitrogen isotope values ($\delta^{15}\text{N}$) can be elevated as a result of urban and agricultural run-off (Diebel & vander Zanden, 2009), but if that was the cause of the observed differences between basins, the western basin should have had higher $\delta^{15}\text{N}$ since it has the highest influence of both in comparison to the rest of the lake (Watson et al., 2016). Increasing water depth has been shown to elevate $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in lower trophic level organisms in marine systems (Puccinelli et al., 2018), and evidence of this relationship has been observed in large freshwater systems, such as Mysis in Lake Ontario (Johannsson et al., 2001). Consequently, depth could be a factor contributing to the spatial trends observed in $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$; however, we do not have sufficient data to state this conclusively. It is more likely that the observed spatial variation in $\delta^{15}\text{N}$

TABLE 4 Mean \pm SE of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{34}\text{S}$, %N, %C, %S, and C:N in seston collected in Lake Erie between May and October in 2017–2019.

Basin	Month	n	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{34}\text{S}$ (‰)	%N	%C	%S	C:N	C:S	N:S
Western	May	6	8.1 \pm 0.3	-29.1 \pm 0.3	2.9 \pm 0.2	7.2 \pm 0.9	45.3 \pm 2.3	0.4 \pm 0.0	6.8 \pm 0.8	112.7 \pm 12.2	17.1 \pm 1.6
	June	16	6.2 \pm 0.2	-29.5 \pm 0.4	3.8 \pm 0.1	4.8 \pm 0.5	30.8 \pm 2.2	0.3 \pm 0.0	6.7 \pm 0.4	94.2 \pm 3.3	14.2 \pm 0.7
	July	10	5.3 \pm 0.5	-25.7 \pm 0.4	5.6 \pm 0.4	4.1 \pm 0.4	27.5 \pm 2.8	0.3 \pm 0.0	6.8 \pm 0.2	91.1 \pm 6.1	13.4 \pm 0.9
	August	4	7.5 \pm 0.6	-26.1 \pm 0.4	6.3 \pm 0.7	6.0 \pm 0.6	36.3 \pm 2.3	0.4 \pm 0.0	6.1 \pm 0.3	97.9 \pm 5.0	16.1 \pm 0.2
	September	12	6.0 \pm 0.5	-25.2 \pm 0.5	5.2 \pm 0.3	4.3 \pm 0.5	28.0 \pm 1.8	0.3 \pm 0.0	6.9 \pm 0.3	88.9 \pm 5.2	13.2 \pm 0.8
	October	3	6.0 \pm 1.2	-23.4 \pm 0.3	5.3 \pm 0.8	3.9 \pm 1.2	28.3 \pm 7.6	0.3 \pm 0.1	7.3 \pm 0.3	85.7 \pm 6.4	11.8 \pm 1.2
Central	May	7	9.8 \pm 1.0	-27.6 \pm 0.4	3.0 \pm 0.3 (5)	5.7 \pm 0.7	37.5 \pm 4.1	0.4 \pm 0.0 (5)	6.6 \pm 0.6	104.9 \pm 6.0 (5)	15.8 \pm 1.5 (5)
	June	17	7.3 \pm 0.4	-28.2 \pm 0.4	3.8 \pm 0.1	5.4 \pm 0.4	38.7 \pm 2.4	0.3 \pm 0.0	7.2 \pm 0.3	117.5 \pm 5.9	16.5 \pm 1.1
	July	11	6.3 \pm 0.4	-27.3 \pm 0.4	4.3 \pm 0.3	5.3 \pm 0.3	35.1 \pm 1.9	0.4 \pm 0.0	6.7 \pm 0.2	93.4 \pm 4.3	14.0 \pm 0.6
	August	8	8.1 \pm 0.3	-26.9 \pm 0.4	4.9 \pm 0.5	5.6 \pm 0.3	35.0 \pm 2.7	0.4 \pm 0.0	6.3 \pm 0.4	95.4 \pm 8.3	15.1 \pm 1.0
	September	12	9.7 \pm 0.5	-25.9 \pm 0.5	4.8 \pm 0.3	5.5 \pm 0.4	32.7 \pm 1.8	0.5 \pm 0.0	6.1 \pm 0.3	70.7 \pm 1.8	12.0 \pm 0.7
	October	4	11.2 \pm 0.7	-26.1 \pm 0.7	4.5 \pm 0.4	5.7 \pm 1.7	34.1 \pm 6.4	0.5 \pm 0.1	6.7 \pm 0.9	69.4 \pm 4.4	11.1 \pm 2.0
Eastern	May	5	9.8 \pm 0.6	-28.4 \pm 0.3	3.3 \pm 0.1 (4)	4.0 \pm 0.4	25.8 \pm 2.2	0.3 \pm 0.0 (4)	6.7 \pm 1.0	91.5 \pm 15.2 (4)	15.8 \pm 2.3 (4)
	June	9	6.4 \pm 0.4	-29.3 \pm 0.4	3.5 \pm 0.2 (8)	5.6 \pm 0.5	40.3 \pm 2.7	0.4 \pm 0.0 (8)	7.4 \pm 0.5	105.7 \pm 4.5 (8)	14.8 \pm 0.4 (8)
	July	13	6.4 \pm 0.3	-28.4 \pm 0.4	4.2 \pm 0.3 (10)	5.8 \pm 0.4	41.3 \pm 1.3	0.4 \pm 0.0 (10)	7.5 \pm 0.6	107.0 \pm 5.6 (10)	15.2 \pm 0.7 (10)
	August	8	8.7 \pm 0.5	-26.3 \pm 0.4	4.7 \pm 0.2	5.1 \pm 0.3	37.7 \pm 1.6	0.4 \pm 0.0	7.5 \pm 0.6	118.4 \pm 22.3	15.6 \pm 2.3
	September	9	8.9 \pm 0.3	-26.0 \pm 0.4	4.7 \pm 0.2 (8)	4.4 \pm 0.6	30.6 \pm 1.9	0.3 \pm 0.0 (8)	7.5 \pm 0.7	95.0 \pm 6.7 (8)	14.4 \pm 1.7 (8)
	October	2	8.2, 8.9	-27.3, -26.6	1.9, 3.4	4.2, 5.7	38.1, 40.7	0.3, 0.4	7.1, 9.1	97.0, 115.5	12.8, 13.7

Note: Numbers in brackets indicate a sample size other than that reported in the n column.

is due to differences in the composition of the phytoplankton community as the season progresses, their differing preferential uptake of nitrogen sources (e.g. NH_4^+ or NO_3^-), and spatial heterogeneity of the phytoplankton community due to basin differences in nutrient availability (Leggett et al., 2000; Syväranta et al., 2008). Lake Erie is a eutrophic lake in comparison to the other Great Lakes, and experiences large seasonal blooms of cyanobacteria (e.g., *Anabaena* spp.) in the western basin during the late summer and early autumn months (Steffen et al., 2014; Watson et al., 2016), which can fix N from the atmosphere and generally reduce $\delta^{15}\text{N}$ (Syväranta et al., 2008). This pattern can be observed in our data with $\delta^{15}\text{N}$ beginning to increase in August to September and October in the central and east basins, which are relatively unaffected by the annual cyanobacteria (*Microcystis* spp., *Anabaena* spp.) bloom, and $\delta^{15}\text{N}$ continuing to decline throughout August, September, and October in the western basin.

The spatial distribution of $\delta^{34}\text{S}$ is probably influenced by similar factors to $\delta^{15}\text{N}$ (Fry, 2007). Spatial variation in $\delta^{34}\text{S}$ in freshwater aquatic ecosystems is dependent on bedrock geology and surrounding land use, much like $\delta^{15}\text{N}$, but is also influenced by the presence and activity of sulfur-reducing bacteria (Croisetière et al., 2009; Finlay & Kendall, 2007; Proulx & Hare, 2014; Thode, 1991). The spatial patterns in $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ observed are probably due to a combination of water depth, sedimentary processes, and algal species composition.

Carbon ($\delta^{13}\text{C}$) of seston did not vary between basins, suggesting that carbon is being processed similarly throughout the lake. This could be a result of how carbon is cycled within aquatic ecosystems compared to nitrogen and sulfur. Unlike, nitrogen and sulfur, carbon has relatively few sources within freshwater ecosystems (Leggett et al., 1999). The main source of carbon for photosynthesis within freshwater systems is dissolved CO_2 , which originates from the atmosphere, and the $\delta^{13}\text{C}$ of plankton within lakes is subsequently dependent on the concentration of $\delta^{13}\text{C}$ within CO_2 (Leggett et al., 1999). As a result, $\delta^{13}\text{C}$ in seston is probably weakly associated with spatial variation and more strongly associated with temporal variation in environmental factors, as seen here.

All three isotopes showed significant temporal variation in seston and both $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ showed significant spatial variation, suggesting that any research using stable isotopes to discern trophic interactions should take both factors into consideration when determining an appropriate baseline. The temporal patterns observed in baseline isotopes here highlight the need to consider the tissue turnover time of the study species in planning collections of isotopic baselines. Assimilation of diet δ -values occurs at different rates for different taxa, and studies targeting larger-bodied, slow growing species (e.g., piscivorous fish) will assimilate isotopes from their environment more slowly than the small-bodied, fast-growing species (e.g., Dreissenidae spp., *Hexagenia*, seston) typically used for isotopic baselines (Vander Zanden et al., 2015). As such, changes in isotopes at the baseline level will not be observed in upper trophic levels (e.g., fish) until sufficient time has passed for their tissues to assimilate those changes (Cabana & Rasmussen, 1996; McIntyre &

Flecker, 2006; Vander Zanden et al., 2015). As a result, timing baseline collections to begin at an appropriate amount of time before the study species, and at an appropriate temporal and spatial scale could improve the interpretation of trophic dynamics and resource use of the study species.

AUTHOR CONTRIBUTION

Conceptualisation, developing methods, and data interpretation: C.H., Y.Z., A.F. Data analysis: C.H., Y.Z. Conducting the research, data analysis, preparation of figures and tables: C.H. All authors contributed to writing this manuscript.

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DATA AVAILABILITY STATEMENT

Data and scripts for data analysis are available upon request.

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