Freshwater wetland–driven variation in sulfur isotope compositions: Implications for human paleodiet and ecological research

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Sulfur isotope (δ^{34}S) analyses are an important archaeological and ecological tool for understanding human and animal migration and diet, but δ^{34}S can be difficult to interpret, particularly in archaeological human-mobility studies, when measured isotope compositions are strongly [34]S-depleted relative to regional baselines. Sulfides, which accumulate under anoxic conditions and have distinctively low δ^{34}S, are potentially key for understanding this but are often overlooked in studies of vertebrate δ^{34}S. We analyze an ecologically wide range of archaeological taxa to build an interpretive framework for understanding the impact of sulfide-influenced δ^{34}S on vertebrate consumers. Results provide the first demonstration that δ^{34}S of higher-level consumers can be heavily impacted by freshwater wetland resource use. This source of δ^{34}S variation is significant because it is linked to a globally distributed habitat and occurs at the bottom of the δ^{34}S spectrum, which, for archaeologists, is primarily used for assessing human mobility. Our findings have significant implications for rethinking traditional interpretive frameworks of human mobility and diet, and for exploring the historical ecology of past freshwater wetland ecosystems. Given the tremendous importance of wetlands' ecosystem services today, such insights on the structure and human dynamics of past wetlands could be valuable for guiding restoration work.

KEYWORDS

wetlands, sulfur isotopes, historical ecology, migration, archaeology

Introduction

Stable sulfur isotopes (δ^{34}S) are used widely in archaeological and ecological research (Krouse, 1989; Canfield, 2001) and their importance continues to grow, particularly in the context of understanding patterns in past human and animal migration and diet (Nehlich, 2015). With recent improvements in instrumentation, allowing smaller sample sizes and

more efficient simultaneous measurement of δ^{34}S alongside other isotopic compositions (e.g., Sayle et al., 2019), generation of these data is likely to see even faster growth moving forward. However, it is still common, particularly in the archaeological literature, for interpretation of some published δ^{34}S values to remain preliminary or tentative, especially when lower values do not appear to fit with regional expectations based on local faunal baseline isotopic compositions. In the early days of applying stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope data to archaeological contexts, a very simple interpretive framework was employed: more or fewer C_4 plants, lower or higher trophic position, and more or fewer marine resources (Schwarcz and Schoeninger, 1991). It is now apparent that a much wider range of biogeochemical processes can influence the δ^{13}C and δ^{15}N compositions throughout the biosphere, often in systematic ways, facilitating a wider interpretive context for archaeological and ecological plant, animal, and human remains. A similar widening of our isotopic interpretive framework for δ^{34}S is currently underway.

In archaeological and ecological work, δ^{34}S is typically used as a marker for mobility or diet (Nehlich, 2015; Hobson, 2019). Because δ^{34}S compositions are not thought to undergo significant fractionation between diet and consumer (Peterson and Howarth, 1987; Fry, 1988; Krajcarz et al., 2019), they can be considered as an indicator for provenance. The premise for this is that the δ^{34}S compositions of the bedrock and overlying geology and hydrology (Thode, 1991) are passed on to primary producers and up to higher-level consumers (Krouse, 1989; Canfield, 2001). This means that, across wider landscapes encompassing regions with varying underlying baseline δ^{34}S, the origin of consumers can be assessed (Vika, 2009). In this context, region-specific differences between underlying geological baselines and baselines associated with adjacent riverine environments have also been used to assess the importance of freshwater resource use (Privat et al., 2007). A second major use of δ^{34}S relies on the distinctive and homogenous isotopic composition of δ^{34}S of seawater sulfates (+20‰; Rees et al., 1978), which can differ from the (often lower) δ^{34}S characterizing baselines and food webs in adjacent terrestrial environments (Fry, 1988; Dance et al., 2018). This means that sulfate contributions from sea spray, with a marine δ^{34}S composition, can have a large impact on the δ^{34}S values of consumers in coastal areas (Zazzo et al., 2011), even masking local terrestrial sulfate contributions entirely (Guiry and Szpak, 2020). For this reason, consumer δ^{34}S is sometimes used to assess residence in coastal areas (Richards et al., 2001). This distinction between δ^{34}S in marine and non-marine ecosystems has also provided a basis for assessing the presence of marine contributions to diet, with consumers of marine foods at inland locations potentially also taking on higher δ^{34}S values (e.g., Craig et al., 2006). A third interpretation of δ^{34}S includes use as a marker for estuarine resources. It is sometimes suggested that marine consumers in estuarine areas could have lower δ^{34}S due to the influence of freshwater sulfate contributions that may have a lower baseline δ^{34}S (e.g., Nehlich et al., 2013). However, studies of consumers across modern estuarine gradients show that this will

not always be the case (Fry and Chumchal, 2011) on the basis of simple mass balance, since it takes a very small amount of (sulfate-rich) seawater added to freshwater (typically an order of magnitude poorer in sulfate; Marschner, 2011) to mask a freshwater δ^{34}S signal.

More recently, there has been a growing awareness among archaeologists (e.g., Szpak and Buckley, 2020; Rand et al., 2021; Guiry et al., 2021a; Lamb and Madgwick, 2022) of the potential for sulfides, which have a very low δ^{34}S, to influence the isotopic composition of consumer tissues, a relationship that has been noted ecologically for decades in select marine-influenced environments (Carlson and Forrest, 1982; Fry et al., 1982). Following their earlier ecological counterparts (Peterson and Howarth, 1987; Mizota et al., 1999; Oakes and Connolly, 2004; Chasar et al., 2005), recent archaeological studies have shown, for instance, that in marine-influenced settings, such as saltmarshes (Guiry et al., 2021a), seagrass beds (Guiry et al., 2021c), and benthic microalgal-subsidized areas (Szpak and Buckley, 2020), coastal and marine archaeological consumers and their broader food webs can have δ^{34}S values that are strongly impacted by sulfur with a sulfide-influenced δ^{34}S value. Although this relationship between low δ^{34}S and sulfide-rich environments is established in investigations of marine and coastal settings, it remains comparatively unexplored in higher-level consumers at inland-terrestrial and freshwater environments (though see Cornwell et al., 1995).

Here we explore the question of whether sulfur with sulfide-influenced δ^{34}S could play an important role in determining the δ^{34}S of terrestrial and aquatic consumers of resources from freshwater wetland areas. While recent work has shown that this is certainly the case for higher-level consumers (mammalian livestock) in at least some areas where primary production is dominated by coastal saltmarsh plants in the genus *Spartina* (Guiry et al., 2021a), it is possible that these and other plants in saltmarsh areas have an unusual tolerance for, or the ability to draw directly on, sulfur from otherwise highly toxic sulfides. Guiry et al. (2021a) suggest that this relationship showing [34]S-depleted isotopic compositions in terrestrial consumers of coastal wetland environments may also provide a marker for the broader use of, or proximity to, freshwater wetlands, and call for more research to establish this relationship. For its part, while the ecological literature has explored variation in δ^{34}S of coastal terrestrial plants in a variety of contexts (e.g., Stribling et al., 1998), little experimental work has been done in freshwater wetlands, although one early paper by Cornwell et al. (1995) does clearly suggest that lower δ^{34}S could also be characteristic of primary producers in some freshwater wetland areas.

We investigate δ^{34}S of an ecologically wide range of taxa to assess the extent to which δ^{34}S co-varies with use of terrestrial (land-based), lacustrian (lake-based), and wetland (slower-moving marsh- and small water body–based) environments. Using archaeological animals from southern Ontario, Canada, with differing, known ecologies, that are further constrained and verified through analyses of δ^{13}C and δ^{15}N compositions, we build

an interpretive framework for investigating the impact of wetland use on consumer δ^{34}S. By exploring the impact of wetland resource use on the isotopic compositions of non-human vertebrates, we aim to provide a better understanding of implications for δ^{34}S interpretations of both human and animal mobility and diet in contemporary, historical, and ancient contexts. Our data show that the use of resources from wetlands can have a very large impact on the δ^{34}S of higher-level consumers across a food web and indicates that archaeological and ecological interpretations in areas of the world where freshwater wetlands are present should consider wetland resource use as a potential source of variation in δ^{34}S when assessing mobility and diet. In showing that δ^{34}S compositions can be driven by wetland-oriented dietary choices, and are therefore not always an accurate provenance tracer, this study has global implications for the way archaeological δ^{34}S are interpreted. While this may be a source of interpretive uncertainty for some research questions, it opens new and potentially highly valuable avenues for others.

Context and hypotheses

Sulfur in wetlands

Sulfides can form under waterlogged, anoxic conditions as the metabolic end product of dissimulatory sulfate reducers, including both bacteria and archaea, which oxidize organic compounds using sulfate as an electron acceptor (Postgate, 1959). The redox and other conditions present in wetland sediment profiles, with slow water movement and limited oxygen, can lead to the accumulation of sulfides (Bagarinao, 1992; Marschner, 2011). These processes strongly discriminate against [34]S, with fractionations of −40‰ to −45‰ observed in natural and culture experiments (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975; Habicht and Canfield, 1997). However, a large portion of these sulfides can be re-oxidized *via* a suite of chemical and biological pathways (Jørgensen, 1982), making isotopically light sulfate available to primary producers. Furthermore, stronger depletions (up to −70‰) observed in analyses of natural sediments suggest these microbially mediated processes can cycle sulfur in ways that create even more [34]S-depleted isotopic compositions in waterlogged sediments (Canfield and Teske, 1996; Habicht and Canfield, 2001). While the processes responsible for driving the sulfur cycle in waterlogged sediments, and thus the fractionation of [34]S, are clearly tremendously complex and still under investigation (Findlay and Kamyshny, 2017; Jørgensen et al., 2019), it is widely acknowledged that they often drive δ^{34}S downwards.

It is also well documented that sulfides are both directly toxic to most plants (Lamers et al., 2013) and, by lowering soil redox potential, can lead indirectly to root oxygen deficiency stress (Koch et al., 1990). While some plants have demonstrated tolerance of sulfides (Carlson and Forrest, 1982; Fry et al., 1982), none are fully immune to higher concentrations (Koch and

Mendelssohn, 1989). The means by which sulfur with sulfide-derived δ^{34}S enters the food web remains unclear. It is possible, for instance, that plants growing in anoxic soil conditions have symbiotic relationships with sulfur-oxidising bacteria, or are themselves chemolithotrophic, allowing them to incorporate sulfide-derived sulfur and grow in areas with low soil sulfate concentrations (Morris et al., 1996). Alternatively, plants and other primary producers could incorporate sulfates that have been created from re-oxidized sulfides. In either case, aquatic and wetland primary producers would take on sulfide-influenced δ^{34}S values.

Research design and hypotheses

We analyzed bone collagen, which, owing to its slower turnover, has isotopic compositions integrating a long-term, multi-year-averaged perspective on foods consumed (Hobson and Clark, 1992; Hyland et al., 2021) (although we note that turnover rates vary within and between bones and across biological ages). Species were selected to represent different ecological groups (i.e., predominantly terrestrial, lacustrian, or wetland inhabiting), with the aim of constructing an interpretive framework that includes taxa with both more constrained and more flexible habitat preferences. Mustelids, represented by American marten (*Martes americana, n* = 3), fisher (*Martes pennanti, n* = 2), and American mink (*Neovison vison, n* = 3), are terrestrial carnivores that prey largely on smaller animals (Baker and Hill, 2003). Previously published δ^{13}C and δ^{15}N values for these samples (Guiry et al., 2021b) allowed for selection of specimens with diets typical of terrestrial predators in the region. Our terrestrial baseline also includes samples from American red squirrels (*Tamiasciurus hudsonicus, n* = 3), Eastern gray squirrels (*Sciurus carolinensis, n* = 4), turkeys (*Meleagris gallopavo, n* = 2), and ruffed grouse (*Bonasa umbellus, n* = 3). Lake Ontario's[1] now-extinct population of Atlantic salmon [*Salmo salar, n* = 16; data from Guiry et al. (2016a)] was made up of large lacustrian piscivores with a narrowly constrained pelagic niche[2] (Guiry, 2019; Guiry et al., 2020b). Both of these groups will have had diets that were minimally influenced by wetland-derived nutrients and therefore represent baselines for δ^{34}S in the two main non-wetland biomes in the region—terrestrial (with δ^{34}S influenced by local geology and hydrology) and lacustrian (with δ^{34}S influenced mainly by

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1 While there are several large lakes in the study region, the most important major water body for this study is Lake Ontario (Figure 1), in part because Niagara Falls created a natural barrier preventing key species, including Atlantic salmon and American eel, from inhabiting upstream lakes. 2 The term "pelagic" can refer to both marine and freshwater environments. Samples come from Lake Ontario's now-extinct endemic complex of Atlantic salmon populations, which were potamodromous. In other words, these fish lived their entire lives in Lake Ontario and its tributaries and did not travel to the ocean as part of their life cycle.

upstream Great Lakes, but also by local watershed inputs), respectively. At the other end of the spectrum, American beavers (*Castor canadensis, n* = 14) and a variety of turtles (snapping, *Chelydra serpentina, n* = 2; painted, *Chrysemys picta, n* = 10; Blanding's, *Emydoidea blandingii, n* = 3; map, *Graptemys geographica, n* = 1), which are primarily wetland denizens (Mac Culloch, 2002), will have had diets with a stronger influence from wetland δ^{34}S.

Together, these groups with constrained habitat preferences provide anchor points for interpreting patterns along a continuum from wetland to non-wetland (i.e., terrestrial and lacustrian) ecosystems. Natural experiments such as this come with some inherent uncertainties and we acknowledge that individuals in our wetland baseline group could have diets and habitat preferences that are not as narrowly focused as the categories into which they have been placed. Beavers, for instance consume both aquatic and terrestrial (tree bark) plant foods (Baker and Hill, 2003), while turtles may also use lacustrian environments. Although we have selected terrestrial fauna based on previous isotope work (Guiry et al., 2021b), which allowed us to target individuals that had terrestrially oriented diets, we are also aware that some of these taxa, such as the fishers and American minks, are capable of foraging in aquatic or wetland habitats. However, for the present study we expect that, while variation in individual behavior may create more isotopic variation, emergent patterns will still provide a basis for establishing general directions of isotopic shifts associated with wetland-influenced diets.

Other taxa represent a range of more flexible ecologies and we hypothesise that they will show a wider spectrum of wetland to non-wetland δ^{34}S. Muskrats (*Ondatra zibethicus, n* = 9) are capable wetland specialists, but are also well adapted for use of faster-moving lacustrian and riverine environments (Baker and Hill, 2003). We also include fish taxa with a large degree of behavioral and habitat-preference flexibility. American eels (*Anguilla rostrata, n* = 36), for instance, live for decades in Lake Ontario and its tributary watersheds before returning to the sea to reproduce and can specialize in both lacustrian and upstream wetland areas (COSEWIC, 2012). They are particularly well known for their ability to climb over obstacles and work their way inland to access habitats in slower-moving wetland areas (Allen, 2010; Jellyman and Arai, 2016). It is also worth pointing out that given that the vast majority of their growth (and life span) occurs after migration into freshwater, we do not expect a marine isotopic signal for the earliest phases of their catadromous life cycle to be retained in adult tissues (Guiry, 2019). Lastly, lake sturgeon (*Acipenser fulvescens, n* = 21) are benthic feeders and while they do travel and forage up smaller tributaries (meaning they have potential to show some wetland-influenced δ^{34}S values), based on the sampled specimens' larger, adult size, we might expect the majority to show a closer affinity with the broader lacustrian δ^{34}S baseline (COSEWIC, 2014).

While, ideally, we would also have a detailed contemporary isoscape for the study region to further refine our interpretations, differences observed between species with similar ecologies in the

archaeological past and their modern counterparts (Colborne et al., 2016; Guiry et al., 2016a) suggest that there may have been a δ^{34}S baseline shift due to modern atmospheric sulfur contributions (Zhao et al., 2003). While further work would be needed to resolve this, for the present study we believe that together, these baseline- (ecologically constrained terrestrial, lacustrian and wetland endpoints) and hypotheses-driven (ecologically flexible) sample selections create a strong interpretive framework for assessing the impact of wetlands resource use on consumer δ^{34}S.

Materials and methods

Samples (*n* = 132) come from 39 sites in southern Ontario, Canada (Figure 1 and Table 1), and date to between 500 CE and 1900 CE, although the vast majority (83%) date to between 1250 and 1650 CE (Supplementary Table S1). Bone samples were selected based on minimum number of individual estimates per archaeological context in order to minimise the possibility of sampling the same individual more than once. Bone collagen extractions followed well established methods (Longin, 1971). Samples were cut into small chunks, demineralized in 0.5 M hydrochloric acid (HCl), and then rinsed to neutrality in Type 1 water. For fish, prior to demineralization, samples were soaked in a bath of 2: 1 chloroform methanol to remove potential residual lipids (Guiry et al., 2016b). Following demineralization, samples were soaked in 0.1 M sodium hydroxide in an ultrasonic bath (solution refreshed every 15 min until solution remained clear) to remove base soluble contaminants. Samples were then neutralized in Type 1 water and refluxed in 10^{-3} HCl (pH 3) at 70°C for 36 h. Samples were centrifuged and the solubilized fraction was transferred into a fresh tube, frozen, and lyophilized.

Stable carbon and nitrogen isotope and elemental compositions were measured on 0.5 mg subsamples of collagen using a Vario MICRO cube elemental analyzer (EA) coupled to an Isoprime isotope ratio mass spectrometer (IRMS; Elementar, Hanover, Germany) or an EA 300 (Eurovector, Pavia, Italy) coupled to a Horizon IRMS (Nu Instruments, Wrexham, United Kingdom). Stable sulfur isotope and elemental compositions were measured separately on 8.0 mg (for mammals and reptiles) or 6.0 mg (for fish) subsamples of collagen along with a combustion enhancer (10 mg of V_2O_5) using an ANCA EA coupled to a Europa SL/20–20 IRMS (Europa, Crewe, United Kingdom). Replicate analyses were performed on *ca.* 30 and 10% of samples for $\delta^{13}C/\delta^{15}N$ and $\delta^{34}S$, respectively. Isotopic compositions were calibrated relative to VPDB and AIR for $\delta^{13}C$ and $\delta^{15}N$ and to VCDT for $\delta^{34}S$ (Supplementary Table S2). We monitored precision and accuracy with internal collagen standards (Supplementary Table S2). Long-term observed averages (check standards) or known (calibration standards) values for all reference materials are reported in Supplementary Table S3. Averages and standard deviations for calibration standards (Supplementary Table S4), check standards
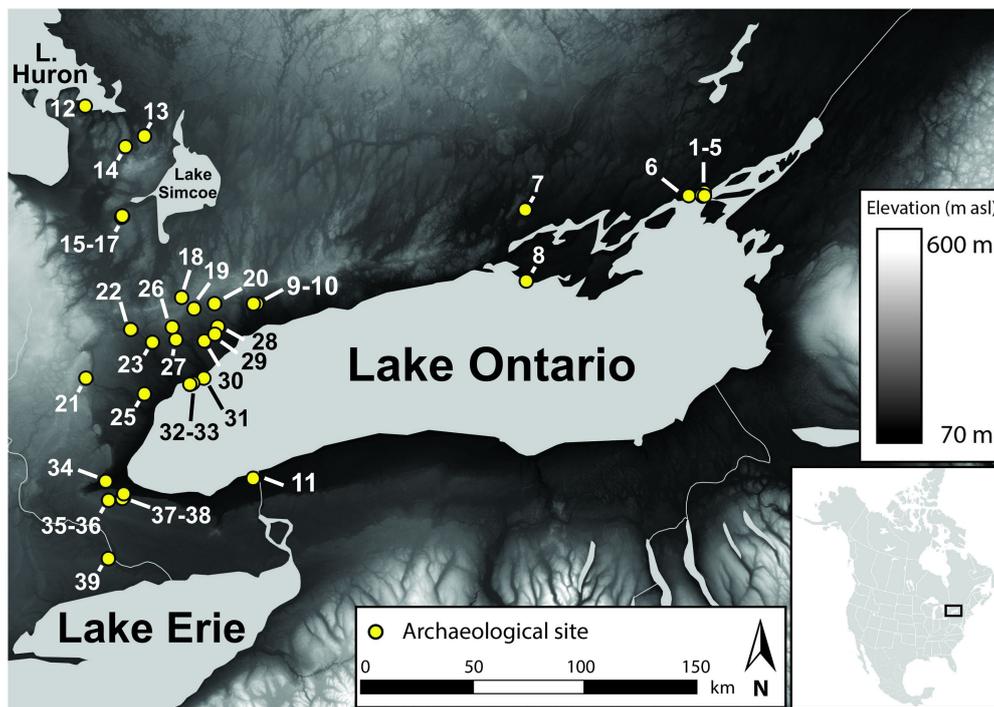
FIGURE 1
Map showing sites and study region. Numbers correspond to site contextual details listed in Supplementary Tables S1; S6.

(Supplementary Table S5), and sample replicates (Supplementary Table S6) for all analytical sessions are also available in the Supplementary material. For $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, systematic errors $[\mu_{(bias)}]$ were ±0.11‰, ±0.12‰, and ±0.23‰, respectively; random errors $[\mu R_{(w)}]$ were ±0.12‰, ±0.14‰, and ±0.12‰, respectively; and standard uncertainty was ±0.16‰, ±0.19‰, and ±0.26‰, respectively (Szpak et al., 2017). Collagen quality control (QC) was assessed using conservative C: N (Guiry and Szpak, 2021), %C (>13%), and %N (>4.8%) criteria (Ambrose, 1990). Statistical comparisons were performed in PAST version 3.22 (Hammer et al., 2001). Pearson's r tests were used to test the significance of correlations between $\delta^{13}C$ and $\delta^{34}S$.

Results and discussion

All samples passed collagen QC criteria. Stable sulfur isotope compositions show a very large range spanning 17.0‰ (−5.0 to +12.0‰; Figures 2A,B; data summarized in Table 1, full results in Supplementary Table S6). Data from baseline species map closely onto our ecological expectations based on their terrestrial, lacustrian, or wetland habitat preferences. There is a slight difference between our terrestrial (mustelids, squirrels, and ground-dwelling birds; $n=20$, mean $\delta^{34}S = +6.9 \pm 1.8$‰, range = +3.6 to +11.8‰) and lacustrian (Atlantic salmon; $n=17$, mean $\delta^{34}S = +8.8 \pm 1.6$‰, range = +5.8 to +11.4‰) taxa, suggesting that $\delta^{34}S$ baselines from these environments, while potentially variable, may rely on isotopically different sources.

Limited geological and hydrological isotopic baseline data are available for the study region (some examples exist from adjacent regions to the north; e.g., Hesslein et al., 1988). The isotopic variability in our data could, for instance, reflect broad-scale differences in the geologies (OGS, 1991; Henry et al., 2008) that underlie local terrestrial and aquatic vs. upstream aquatic habitats. The uppermost geology across much of the study region is composed of marine-derived sedimentary bedrock of various ages and compositions, which typically has relatively high $\delta^{34}S$ values (Bottrell and Newton, 2006). In contrast, the deeper bedrock underlying this, which becomes exposed immediately to the north of the study region and is an important bedrock system for some of the upstream Great Lakes (Huron and Superior), is the Canadian Shield. The Canadian Shield is a large region of Precambrian igneous and metamorphic rock, a rock type that, although variable, typically has $\delta^{34}S$ values that are lower than those found in marine environments (for review see, Thode, 1991). This latter source is, however, unlikely to be a major contributor of sulfate to biota due to the low sulfur content of silicious igneous rocks, which often means that ecosystems in these regions rely mainly on atmospheric deposition for their sulfur budget (for review see, Mitchell et al., 1998). Nonetheless, in this context, to the extent that the sulfur budget of lacustrian habitats of Lake Ontario might be subsidized by sulfate contributions from upstream watersheds, we might expect to find higher $\delta^{34}S$ values in terrestrial fauna living atop bedrock composed of ancient marine sediments compared with lacustrian counterparts, for which baselines could reflect greater

TABLE 1 Summary statistics for archaeological faunal bone collagen isotopic compositions.

Taxon	Ecology	Sample n=	Site n=	δ^{34}S (‰)		δ^{13}C (‰)		δ^{15}N (‰)	
				Range	Mean ± 1σ	Range	Mean ± 1σ	Range	Mean ± 1σ
Lake sturgeon	Flexible	21	17	8.1	+9.3 ± 2.0	9.1	−18.4 ± 2.3	2.5	+10.1 ± 0.8
American eel	Flexible	36	19	13.0	+8.5 ± 3.8	11.6	−19.6 ± 2.8	5.5	+9.2 ± 1.0
Muskrat	Flexible	9	6	10.3	+3.6 ± 3.7	6.8	−21.6 ± 2.2	7.8	+4.7 ± 2.5
Atlantic salmon	Lacustrian	16	5	5.6	+8.8 ± 1.6	1.2	−19.9 ± 0.3	4.0	+10.8 ± 1.2
American beaver	Wetland	14	6	13.1	+0.5 ± 4.1	4.7	−23.0 ± 1.3	4.8	+4.1 ± 1.3
Turtles (all)	Wetland	16	7	10.0	+4.4 ± 2.4	7.1	−24.8 ± 2.3	6.5	+7.0 ± 1.8
Snapping turtle	Wetland	2	2	2.2	+4.5 ± 1.6	0.9	−26.7 ± 0.6	0.5	+8.4 ± 0.4
Painted turtle	Wetland	10	5	8.5	+5.0 ± 2.5	5.9	−24.6 ± 2.0	3.0	+6.3 ± 0.9
Blanding's turtle	Wetland	3	3	3.0	+2.0 ± 1.7	6.0	−25.4 ± 3.2	6.2	+7.3 ± 3.1
Map turtle	Wetland	1	1		+5.1		−21.1		+10.5
Furbearers (all)	Terrestrial	8	2	2.7	+6.4 ± 1.1	5.6	−18.7 ± 1.5	3.7	+9.0 ± 1.0
American marten	Terrestrial	3	2	2.5	+6.9 ± 1.3	0.5	−19.0 ± 0.3	0.7	+8.8 ± 0.4
Fisher	Terrestrial	2	1	0.6	+5.7 ± 0.4	5.6	−18.2 ± 4.0	3.7	+9.2 ± 2.6
American mink	Terrestrial	3	1	2.4	+6.4 ± 1.2	0.1	−18.7 ± 0.1	0.4	+9.0 ± 0.2
Squirrels (all)	Terrestrial	7	6	8.2	+7.6 ± 2.6	0.9	−19.2 ± 0.3	1.6	+5.4 ± 0.6
Eastern gray squirrel	Terrestrial	4	4	3.6	+7.5 ± 1.6	0.6	−19.1 ± 0.3	1.2	+5.6 ± 0.6
Red squirrel	Terrestrial	3	3	8.2	+7.8 ± 4.1	0.5	−19.4 ± 0.2	1.4	+5.2 ± 0.7
Ground birds (all)	Terrestrial	5	3	2.1	+6.4 ± 0.9	2.9	−21.8 ± 1.1	1.2	+5.9 ± 0.5
Turkey	Terrestrial	2	2	1.3	+7.2 ± 0.9	0.4	−20.7 ± 0.3	0.1	+6.4 ± 0.1
Ruffed grouse	Terrestrial	3	3	0.8	+6.0 ± 0.4	1.3	−22.6 ± 0.7	0.5	+5.5 ± 0.2

contributions from older, non-marine geologies and/or atmosphere-dominated sources. Although the average difference we see between our terrestrial and lacustrian groups is small (<2.0‰), we observe the opposite pattern. We note, however that this basic interpretive scenario (marine vs. non-marine origins of uppermost bedrock) is highly simplified and that surface geology may be influenced by a wide range of other factors, such as the presence of glacially re-deposited, non-marine sediments and local variation in the presence of other bedrock types. It is also possible that Lake Ontario's lacustrian and terrestrial sulfur isotope baselines are simply dominated by sulfate sources endemic to the watershed (i.e., a combination of glacial re-deposition, marine-derived bedrock, atmospheric deposition, and smaller contributions from other local bedrock types). Regardless of what geological or hydrological processes are responsible for the differences we observed between our lacustrian and terrestrial fauna, we consider them to be valid, if approximate, baselines for establishing a backdrop against which to compare and contrast wetland-derived δ^{34}S variation. We also note that, although sample sizes are too small (averaging 3.3 samples per each of our 39 sites) to assess covariance between site location and δ^{34}S values for our terrestrial and lacustrian baseline groups, we see no obvious patterns between geographical locations and isotopic variation across the study region (Figure 1; Supplementary Table S6).

In comparison to these terrestrial and lacustrian baselines, our wetland taxa (beavers n = 14, mean δ^{34}S = +0.5 ± 4.1‰, range = −5.0 to +8.3‰; turtles; n = 16, mean δ^{34}S = +4.4 ± 2.4‰, range = +0.1 to +10.1‰) both indicate a lower baseline δ^{34}S for wetland denizens.

Variation among turtles includes individuals with δ^{34}S that overlap with the lacustrian and terrestrial baseline taxa, suggesting that some may have lived in larger water bodies and/or made use of terrestrial resources. Beavers show the lowest values, with the widest range (spanning 13.1‰). This matches closely with their diet range which includes both roots from wetland plants [these structures have been observed to have δ^{34}S values that are more sulfide-influenced than plant tissues that are not in contact with sediments; Frederiksen et al., 2006)] and bark from trees in adjacent terrestrial habitat. This supports the use of these groups as δ^{34}S baselines for assessing the influence of wetland (i.e., sulfide-cycled) sulfur on the δ^{34}S of more behaviorally flexible taxa.

In that context, the wide spectrum of δ^{34}S values from eels (n = 36, mean δ^{34}S = +8.5 ± 3.8‰) which spans 17‰ and matches well with their known ecology, including both high (+12.0‰) and low (−1.0‰) δ^{34}S values that closely follow our wetland and lacustrian baselines. Muskrats also show a wide, but on average lower, range of δ^{34}S values (n = 9, mean δ^{34}S = +3.6 ± 3.7‰, range = −0.8 to +9.5‰) consistent with their flexible ecology feeding along a lacustrian–wetland spectrum. In contrast, but as expected, the bulk of lake sturgeon samples produced δ^{34}S values falling at the lacustrian end of the spectrum (n = 21, mean δ^{34}S = +9.3 ± 2.0‰, range = +3.2 to +11.3‰), with only one individual producing a value significantly lower than our lacustrian baseline, suggesting significant use of wetland-influenced areas.

Considering δ^{15}N helps to further contextualize the ecology of these taxa. Each species' δ^{15}N (Figures 2B,C) is broadly consistent with its respective trophic position (DeNiro and Epstein, 1981),

FIGURE 2
(A–C) Stable isotope compositions of archaeological fauna.
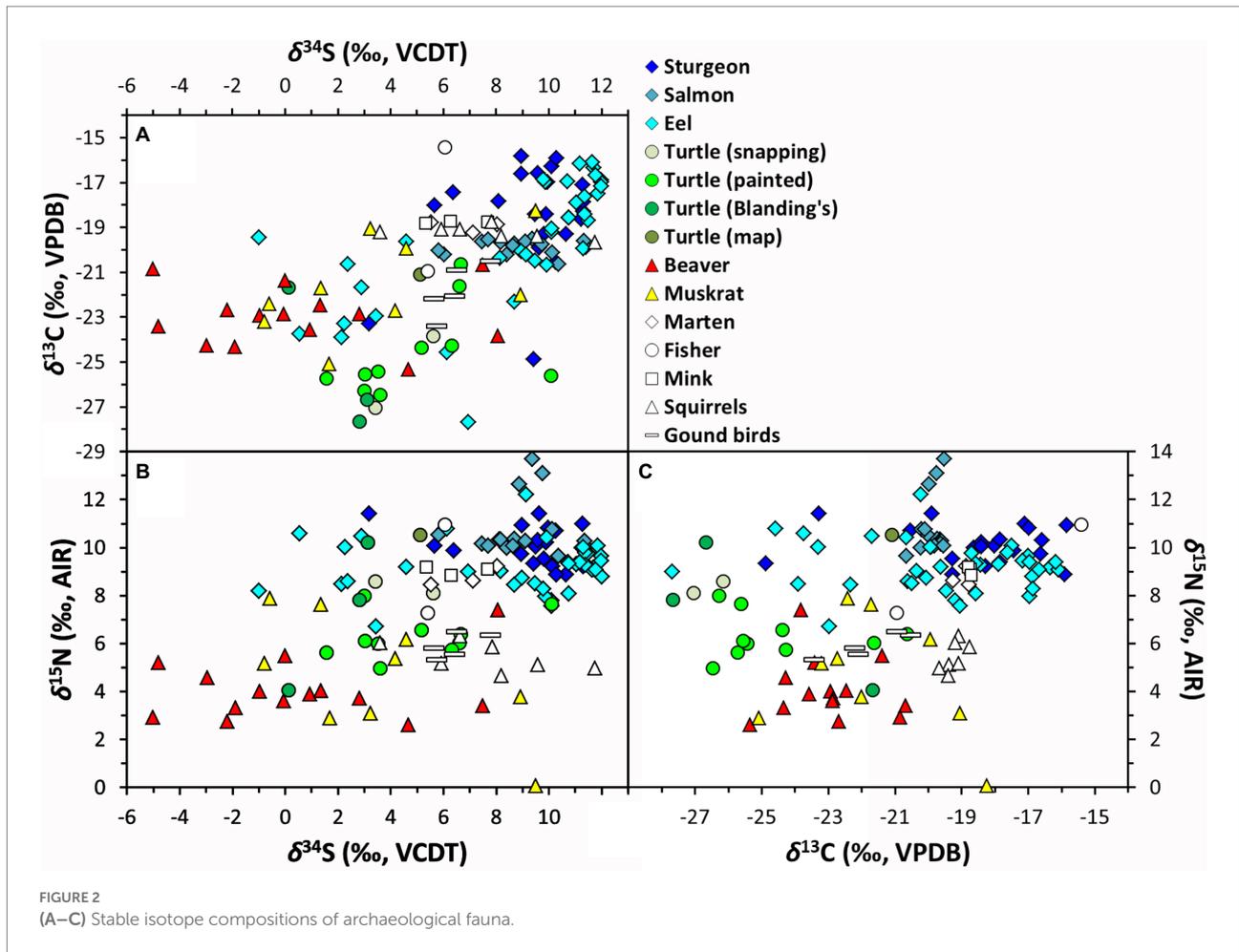
with more herbivorous aquatic (beavers, $n = 14$, $+4.1 \pm 1.3‰$; muskrats $n = 9$, $+4.7 \pm 2.5‰$) and terrestrial (squirrels $n = 6$, $+7.0 \pm 1.8‰$; ground birds $n = 5 + 5.9 \pm 0.5‰$) animals having lower values than apex predators (Atlantic salmon, $n = 16$, $+10.8 \pm 1.2‰$; mustelids $n = 8$, $+9.0 \pm 1.0‰$) in their respective habitat types. Atlantic salmon and one eel with higher $\delta^{15}N$ (> 12‰) provide an exception to this observation, but these samples come from historical contexts after 1850 CE, postdating a large-scale, early nineteenth-century isotopic shift in Lake Ontario's nitrogen cycle that followed major forestry activities in the watershed (Guiry et al., 2020a). Consistency among the $\delta^{34}S$ values of these historical Atlantic salmon with higher $\delta^{15}N$ and earlier salmon with lower $\delta^{15}N$ suggests that Lake Ontario's broader sulfur cycle did not undergo a similar isotopic shift at this time. These individuals aside, the overall consistency between $\delta^{15}N$ and expected trophic positions across our dataset suggests that, with respect to nitrogen cycling and sources, these samples are from a well-integrated wider biome with a consistent $\delta^{15}N$ baseline.

Stable carbon isotope compositions (Table 1 and Figures 2A,C) show patterns that complement and reinforce our interpretations of those observed in $\delta^{34}S$. Freshwater ecosystems such as Lake Ontario can have extremely variable $\delta^{13}C$ (Schelske and Hodell, 1991; Hodell and Schelske, 1998), owing to complex and multifaceted processes

that govern the cycling, sourcing, and partitioning of dissolved inorganic carbon (DIC) pools across varied intra- and inter-annual temporal scales and physical and biological conditions (Guiry, 2019). In this context, variation at the higher end of our observed $\delta^{13}C$ spectrum, which characterizes samples from lacustrian taxa such as Atlantic salmon ($n = 16$, $-19.9 \pm 0.3‰$) and lake sturgeon ($n = 21$, $-18.4 \pm 2.3‰$), are consistent with their expected foraging behavior in more pelagic and littoral areas, respectively (France, 1995; Guiry, 2019). In contrast, the extremely low $\delta^{13}C$ and high degree of variation among turtles ($n = 16$, $-24.8 \pm 2.3‰$) suggests a wetland DIC budget more heavily influenced by CO_2 sourced from the breakdown of allochthonous terrestrial detritus [typically ^{13}C-depleted relative to atmospheric sources used by their terrestrial and, to some extent, lacustrian counterparts (Finlay and Kendall, 2007)]. Together these data show that, for this study region, while lacustrian food webs have higher $\delta^{13}C$ values, wetlands have distinctively low $\delta^{13}C$, as might be expected in contexts where water movement is slow and CO_2 released from breakdown of allochthonous organic matter can contribute to a larger fraction of the DIC budget. In that context, we note that low $\delta^{13}C$ values are also apparent among eels (as low as $-27.7‰$, with 22% of individuals falling below the range for lacustrian taxa) which have the most ecological flexibility of our taxa, enabling them to thrive

anywhere along a wetland-to-lacustrian continuum. A strong positive correlation between $\delta^{13}C$ and $\delta^{34}S$ for eels ($n=36$, Pearson's $r=0.674$, $p=<0.000$) indicates that factors driving $\delta^{34}S$ downward occur in areas where $\delta^{13}C$ is more impacted by wetland isotopic compositions (i.e., influenced by ^{13}C-depleted carbon from allochthonous terrestrial detritus). What is more, while this correlation does not appear within other taxa (as expected given their less flexible ecologies, although see below for more detail; Supplementary Table S7), it is observed to a similar degree across the entire dataset (i.e., from all species; Figures 2A, $n=132$, Pearson's $r=0.608$, $p=<0.000$), indicating that the relationship operates at a much broader level than species-specific behavior. In other words, the known ecologies of these taxa, coupled with the observed isotopic patterns (in $\delta^{34}S$ and the correlation between $\delta^{13}C$ and $\delta^{34}S$) matching expectations based on these ecologies, strongly supports our interpretation that the primary driver of variation in $\delta^{34}S$ toward the lower end of the spectrum is an influence from ^{34}S-depleted sulfides in wetlands.

Results include another noteworthy species-specific pattern. The $\delta^{13}C$ and $\delta^{34}S$ correlation between wetland use and the most ecologically flexible taxa (i.e., eels) does not appear in beavers. In particular, although beavers are wetland specialists, with diets that include wetland plants as well as terrestrial trees, we do not see $\delta^{13}C$ co-vary with $\delta^{34}S$. Instead we see $\delta^{13}C$ values ($n=14$, $-23.0\pm1.3\text{‰}$) that appear to consistently suggest that the primary production upon which beavers relied drew CO_2 from atmospheric (terrestrial; i.e., higher $\delta^{13}C$) sources[3] rather than from sources where CO_2 came from the breakdown of allochthonous organic matter (i.e., lower $\delta^{13}C$). Dietary ecology, however, offers a clear explanation for this difference (Baker and Hill, 2003). Of the aquatic plants consumed by beavers, the favorites are lily pads (*Nymphaea* spp., *Nuphar* spp.) and other emergent aquatic vegetation (Jenkins, 1981; Novak, 1987), which, unlike other, fully aquatic primary producers (e.g., algae, macrophytes), draw a significant portion of their CO_2 directly from the atmosphere. This means that, although specialists in wetlands habitats, beavers are not necessarily expected to share low $\delta^{13}C$ values with other wetland denizens. For instance, in contrast to beavers, eels and turtles (carnivores and omnivores) consume a wider range of prey for which the basal primary production occurs in the water column (i.e., integrating isotopic compositions influenced by CO_2 from the breakdown of allochthonous materials). It therefore makes sense that beavers, while still showing a wetland diet spectrum, sit somewhat apart from other taxa. This also explains why a relationship between $\delta^{13}C$ and $\delta^{34}S$ for muskrats is weaker (and not statistically significant, Supplementary Table S7). While

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3   Note that mustelids and squirrels, although also terrestrial, are expected to have higher $\delta^{13}C$. For squirrels, this is based on their consumption of tree mast that, being composed of non-photosynthetic tissues, is relatively ^{13}C enriched (Guiry et al., 2021b) in the context of local C_3 plant foods. For mustelids, this is based on their consumption of taxa that focus on tree mast, such as squirrels.

muskrats are omnivorous, their dominant food sources are plants, with overall diets that can be more similar isotopically to beavers that focus on emergent aquatic vegetation.

Broader implications and conclusion

Our data indicate that wetland fauna can have $\delta^{34}S$ values that incorporate an isotopic signal from sulfides. This means that higher-level consumers using resources from these environments, including humans, can have $\delta^{34}S$ values that do not reflect prevailing local baselines. While this phenomenon has been documented archaeologically before for various marine and coastal settings (Szpak and Buckley, 2020; Guiry et al., 2021a,c), its observation among vertebrates in freshwater wetlands is new and is something that could have a significant, and largely overlooked, impact on interpretations of $\delta^{34}S$ data from archaeological and ecological contexts.

To date, the vast majority of archaeological studies examining past diet and mobility have done so based on the premise that $\delta^{34}S$ compositions in consumers primarily reflect underlying geology and hydrology, with the added caveat that these values could be influenced near the top end of a typical $\delta^{34}S$ range by marine sulfates (through either sea spray or direct consumption of marine primary producers and animals; Nehlich, 2015). This study shows that the use of wetland resources could influence consumer $\delta^{34}S$ towards the bottom end of a typical $\delta^{34}S$ interpretive range. In other words, where interpretations involve data from humans or animals that could have lived near and used wetland resources, $\delta^{34}S$ values could be influenced. This finding has important, global implications for the utility of $\delta^{34}S$ as a tool for reconstructing past human mobility and migration. The existence of a source of $\delta^{34}S$ variation that is not linked to location (i.e., not derived from geology or hydrology), means that human $\delta^{34}S$ compositions, even at inland areas (i.e., far from sea spray influences and where marine foods were not available), could be driven at least partly by wetland-oriented dietary choices and would, therefore, not provide a faithful provenance tracer. Because wetlands are globally distributed and provide a resource-rich area attracting human habitation, this finding could have implications for interpretation of $\delta^{34}S$ data on a broad scale. While this could make interpretation of potentially affected data more complicated, it should also help to clarify interpretations of lower $\delta^{34}S$, which are sometimes left with tentative interpretations (e.g., Pearson et al., 2016; Rand et al., 2020; Le Roy et al., 2022), by linking them with potential consumption of local wetland resources, rather than mobility to distant or unknown areas with very low $\delta^{34}S$ baselines.

It is also worth considering these data for what they can tell us about variation in wetland $\delta^{34}S$ as well as our ability to identify it archaeologically. It is apparent from the wide $\delta^{34}S$ ranges among species, even within our baseline groups, that $\delta^{34}S$ associated with ecotonal areas that include wetlands can be highly variable. It is only when we combine a wide range of ecologically diverse taxa,

constrained by other isotopic data, that a cohesive pattern emerges. The high degree of variation may simply reflect the fact that these kinds of natural experiments, particularly ones using archaeological materials that integrate longer time spans, are prone to incorporating data influenced by a wider range of biogeochemical processes. It is nonetheless the case that, for mobile, higher-trophic-level vertebrate consumers, especially in archaeological studies, in which our aim is to interpret cultural behaviors from both human and animal data, these kinds of issues with broader spatial and temporal scales will often necessarily be present. This heightened variability means that we can expect that interpreting smaller numbers of (or isolated) δ^{34}S data from bone collagen (typically the only material available to archaeologists) may be challenging or impossible and suggests that larger numbers of samples may be needed to observe clear patterns. Additional research with plants and bone collagen from contemporary samples from known species and carefully selected locations may allow this relationship to be characterized in finer detail and perhaps offer new insights to help further constrain interpretations of wetland-related δ^{34}S variation in the archaeological past.

A further point, of broader relevance, is that these data also speak to the value of including a wider variety of taxa in archaeological and ecological faunal isotopic baselines. If, for instance, we had instead examined only one or two of these species, the overall pattern we have observed might have been less clear. In other words, it is only by incorporating data from a broader suite of species across ecosystems that we gain a fuller understanding of the primary axes of isotopic variation relevant for interpreting human and animal behaviors. While the key roles that faunal baselines, particularly from animals of major economic importance, can play in isotopic research have long been recognized (Katzenberg, 1989), in this context, our results provide a clear example for the value of sampling more widely, and including species that are traditionally overlooked or seen as having less interpretive value. In order to make space for this kind of open exploration, and generation of more substantial datasets, curators may need to remain open to larger, broad-scale sampling programs. In this way we can gain a more detailed picture of the wider environmental framework in which humans and animals lived. At the same time, we acknowledge that budgetary constraints and limitations on what taxa have been preserved in relevant archaeological assemblages may mean that some archaeological projects will necessarily be limited to smaller-scale, less taxonomically diverse sampling programs.

Lastly, but perhaps most importantly, while our finding of a potentially globally significant source of variation at the bottom end of the δ^{34}S spectrum adds a source of interpretive uncertainty for some research questions, it opens the way for others. Wetlands were and are areas of tremendous cultural and ecological importance (Bernick, 2011). At the same time that wetlands continue to disappear due to human impacts (Davidson, 2014), we are learning more about the ecosystem services they provide and their value for mitigating or reversing major environmental issues, such as biodiversity loss, pollution/eutrophication, and climate change (Zedler and Kercher, 2005). In this context, having a broad-scale marker for human interactions with and use of wetlands in the past could help shed light and temporal depth on how wetlands have responded to human land management pressures through time. Moreover, such a marker for changes in the importance of wetlands for the ecology of archaeological fauna (whether wild or domestic) could provide an important source of information about the long-term ecological structure of wetlands in general, and in particular those which have long since disappeared due to human impacts. This can further inform archaeological interpretation of the importance of wetland resources for ancestors, thereby helping contemporary Indigenous peoples to reclaim part of their heritage (General and Warrick, 2012; Lesage, 2016). These findings also open the way for a range of historical-ecological research programs that could use δ^{34}S of archaeological animal remains to contribute directly to our understanding of the ecology of endangered or recovering wetland species. In turn, information from these and other lines of study have potential to help shape future conservation strategy and policy.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, and further inquiries can be directed to the corresponding author.

Author contributions

EG designed research. SN-H, TO, and EG contributed samples and background knowledge for analysis. EG performed isotopic analyses and interpreted results. EG wrote the manuscript with assistance from PS, SN-H, and TO. All authors contributed to the article and approved the submitted version.

Funding

Acknowledgments

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.953042/full#supplementary-material

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