Compound-Specific Stable Isotope Analysis of Amino Acids in Pelagic Shark Vertebrae Reveals Baseline, Trophic, and Physiological Effects on Bulk Protein Isotope Records

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Variations in stable carbon and nitrogen isotope compositions in incremental tissues of pelagic sharks can be used to infer aspects of their spatial and trophic ecology across life-histories. Interpretations from bulk tissue isotopic compositions are complicated, however, because multiple processes influence these values, including variations in primary producer isotope ratios and consumer diets and physiological processing of metabolites. Here we challenge inferences about shark tropho-spatial ecology drawn from bulk tissue isotope data using data for amino acids. Stable isotope compositions of individual amino acids can partition the isotopic variance in bulk tissue into components associated with primary production on the one hand, and diet and physiology on the other. The carbon framework of essential amino acids (EAAs) can be synthesised *de novo* only by plants, fungi and bacteria and must be acquired by consumers through the diet. Consequently, the carbon isotopic composition of EAAs in consumers reflects that of primary producers in the location of feeding, whereas that of non-essential amino acids (non-EAAs) is additionally influenced by trophic fractionation and isotope dynamics of metabolic processing. We determined isotope chronologies from vertebrae of individual blue sharks and porbeagles from the North Atlantic. We measured carbon and nitrogen isotope compositions in bulk collagen and carbon isotope compositions of amino acids. Despite variability among individuals, common ontogenetic patterns in bulk isotope compositions were seen in both species. However, while life-history movement inferences from bulk analyses for blue sharks were supported by carbon isotope data from essential amino acids, inferences for porbeagles were not, implying that the observed trends in bulk protein isotope compositions in porbeagles have a trophic

or physiological explanation, or are suprious effects. We explored variations in carbon isotope compositions of non-essential amino acids, searching for systematic variations that might imply ontogenetic changes in physiological processing, but patterns were highly variable and did not explain variance in bulk protein $\delta^{13}C$ values. Isotopic effects associated with metabolite processing may overwhelm spatial influences that are weak or inconsistently developed in bulk tissue isotope values, but interpreting mechanisms underpinning isotopic variation in patterns in non-essential amino acids remains challenging.

INTRODUCTION

Pelagic shark populations have declined regionally by >90% in the past 20 years, largely as a result of overfishing and bycatch (Worm et al., 2013), while the global abundance of oceanic sharks has declined by 70% since 1970 (Pacoureau et al., 2021). The vulnerability of sharks to marine-capture fisheries depends on individual movements and the presence of movement traits across individuals, populations, or species that may suggest a shared vulnerability (Queiroz et al., 2016, 2019; Vandeperre et al., 2016; Coelho et al., 2020). Effective management of the remaining shark populations requires a thorough understanding of their spatial ecology (Costa et al., 2012; Briscoe et al., 2016). Movements of pelagic sharks are, however, difficult to monitor or reconstruct, particularly throughout ontogeny (Graham et al., 2010; Trueman et al., 2012; McMahon et al., 2013). While physical tags have revolutionized our understanding of space use by pelagic fishes, they rarely report over the life-time of a tagged individual, and juvenile animals may be too small to support satellite-linked pop-up tags. Consequently, knowledge of juvenile movements, and connections between juvenile and adult habitats, remain poorly understood for many pelagic shark species.

Stable isotope analysis (SIA) of animal tissues has become a routine tool used in ocean ecology to reconstruct animal movements (e.g., Best and Schell, 1996; Cherel et al., 2009; Carlisle et al., 2015; Bird et al., 2018) and changes in diet and trophic level (e.g., MacNeill et al., 2005; Estrada et al., 2006; Newsome et al., 2009; Pethybridge et al., 2018; Lorrain et al., 2020) (for reviews see Post, 2002; Graham et al., 2010; Boecklen et al., 2011; McMahon et al., 2013; Trueman and St John Glew, 2019). Because the isotopic composition of primary producers varies across space and time (McMahon et al., 2013; Schmittner and Somes, 2016; Magozzi et al., 2017), animal movements can, in theory, be reconstructed retrospectively by relating variation in tissue isotopic composition to variability in isotopic baselines (McMahon et al., 2013; Trueman and St John Glew, 2019). Sequential analysis of tissues that retain a chronological record in the form of periodically deposited increments or growth bands, including shark vertebrae, may further provide movement (and trophic) information throughout life of individuals (Hobson, 1999; Newsome et al., 2010; Trueman et al., 2012; e.g., Estrada et al., 2006; Carlisle et al., 2015).

The underlying premise of the isotope tracer method is that the isotopic composition of animal tissue reflects that of primary producers at the base of the foodweb, overlain by a relatively predictable trophic offset (DeNiro and Epstein, 1978, 1981). However, this premise is a simplification of a complex suite of interacting ecological and biochemical factors. Variance in isotopic compositions from bulk tissue analyses can reflect mixed effects from variation in isotopic compositions of primary producers (baseline effects; Schmittner and Somes, 2016; Magozzi et al., 2017), changes in diet and trophic level (trophic effects), and variation in metabolic routing of dietary macronutrients to tissue synthesis (physiological effects; Trueman et al., 2005; Newsome et al., 2014; Wang et al., 2019). Interactions among these isotope effects significantly complicate the interpretation of bulk tissue isotope data alone to infer movements, diet or trophic level changes (e.g., Newsome et al., 2010; Trueman et al., 2012; e.g., Popp et al., 2007). SIA of specific structural compounds within tissues, such as individual amino acids, has been increasingly employed in animal movement (McMahon et al., 2011a,b; Seminoff et al., 2012) and trophic (Chikaraishi et al., 2009, 2014; Lorrain et al., 2009) studies to reduce uncertainty in estimates of change in location, diet and trophic level and nutrient source (e.g., Larsen et al., 2013; McCarthy et al., 2013; McMahon et al., 2015a).

A number of amino acids critical for life functions can only be synthesized *de novo* by some plants, fungi and bacteria. These essential amino acids are subsequently transferred unaltered through foodwebs (Hare et al., 1991; Reeds, 2000; Jim et al., 2006; McMahon et al., 2010). Consequently, the carbon isotope composition of essential amino acids ($\delta^{13}C_{EAA}$) in consumer tissues retains the isotopic composition fixed during biosynthesis by primary producers (Jim et al., 2006; Popp et al., 2007; McMahon et al., 2010; Wang et al., 2019). Differences in $\delta^{13}C_{EAA}$ values among consumers reflect variations in the isotopic compositions of primary production at the time and place of biosynthesis and can therefore be used to infer movement across spatio-temporal isotope gradients (Schmittner and Somes, 2016; Magozzi et al., 2017) and/or variations in the relative contribution of foodwebs fueled by different carbon sources (e.g., Larsen et al., 2009, 2013). Non-essential amino acids can either be routed directly from dietary sources to tissue or synthesized *de novo* by using metabolites derived from ingested nutrients

(McMahon et al., 2010, 2015b; Newsome et al., 2011; Berg et al., 2015). Carbon isotopes are fractionated during biomolecule degradation and synthesis (Hayes, 2001; Howland et al., 2003; Jim et al., 2006), therefore variability in $\delta^{13}C$ values of non-essential amino acids ($\delta^{13}C_{non-EAA}$) can reflect trophic and physiological effects superimposed on any baseline effects.

Non-essential amino acids can be synthesized from dietary intermediates, whether protein, lipids or carbohydrates (Berg et al., 2015). Marine pelagic predators draw energy and nutrients largely from protein and lipid sources with relatively little energy drawn from carbohydrates (Wang et al., 2019). Lipids are energy dense nutrient sources, and fish nutrition balances protein and lipid (energy) sources (Bowyer et al., 2013). Protein and energy requirements and availability vary in time and space, therefore the proportion of metabolites assimilated from protein compared to lipid precursor molecules is likely to vary considerably in time, space and among individuals depending on their nutritional status. Lipids are typically depleted in ^{13}C relative to protein, therefore the degree to which a particular amino acid is synthesized from lipid compared to protein dietary precursors should be reflected in its ^{13}C content (Wolf et al., 2015; Wang et al., 2018). Controlled-feeding experiments on salmon (Wang et al., 2019) and a study with mammals (Newsome et al., 2014; Wolf et al., 2015) have shown that the $\delta^{13}C$ values of glycolytic amino acids are highly sensitive to the lipid content of diets and lipid utilization for amino acid synthesis whereas values of Krebs cycle amino acids are not (see also Leigh et al., 2018; Wang et al., 2018; Whiteman et al., 2018). These results imply that, where diets contain no carbohydrates, glycolytic amino acids (e.g., glycine, serine, and alanine) are synthesized from glycogen predominantly contributed from the metabolism of fatty acids, whereas amino acids whose synthesis is associated with the Krebs cycle (e.g., glutamic acid, aspartic acid, and proline) draw on carbon provided by processing of both protein and lipid sources (Choy et al., 2010; Fernandes et al., 2012; Newsome et al., 2014). Variability in $\delta^{13}C$ values of glycolytic vs. Krebs cycle amino acids may therefore reflect changes in macronutrient contents of diets and/or shifts in the metabolic allocation of macronutrients for tissue synthesis. However, how carnivorous fish use dietary intermediates to synthesize amino acids is still poorly understood and may ultimately depend on their digestive physiology and nutritional requirements (e.g., Leigh et al., 2018; Whiteman et al., 2018).

The combination of essential and non-essential amino acids, the isotopic compositions of which are influenced differently by contrasting mechanisms, therefore offers an opportunity to differentiate among alternative variables contributing to variance in bulk tissue isotope values. The potential for identifying variations in animal nutrient physiology from variations in $\delta^{13}C_{non-EAA}$ values is particularly attractive but a coherent framework to interpret variability in $\delta^{13}C_{non-EAA}$ data remains elusive. In this study, we present stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope chronologies of bulk cartilage collagen from sequential vertebral samples for individual blue sharks (*Prionace glauca*, Linnaeus 1758) and porbeagles (*Lamna nasus*, Bonnaterre 1788) caught across the North Atlantic. In parallel, we present comparable carbon isotope chronologies of individual

essential and non-essential amino acids from the same sharks. We quantified variation in $\delta^{13}C$ values of bulk collagen and individual amino acids at multiple levels (between species, among areas and individuals and within-individuals) and tested for common, broad ontogenetic isotope patterns across individual blue sharks and porbeagles. We contrasted patterns in $\delta^{13}C$ values of bulk protein and essential amino acids to disentangle baseline from trophic effects on bulk tissue $\delta^{13}C$ values and test whether observed ontogenetic trends can be best interpreted in terms of movement or diet and trophic level change. Given the small number of individuals examined (nine blue sharks and six porbeagles), we clearly did not aim here to draw inferences about the ecology for species or populations. We argue that, if ontogenetic patterns in bulk protein $\delta^{13}C$ values are matched by variability in $\delta^{13}C$ values of essential amino acids, then at least a component of variance in the bulk protein signal must be associated with movement (in time and/or space) across isotopically distinct baselines. By contrast, if trends in bulk protein $\delta^{13}C$ values are solely associated with a shift in diet or trophic position, they should not be apparent in the $\delta^{13}C$ values of essential amino acids (and should match trends in bulk $\delta^{15}N$ values). We also investigated patterns in $\delta^{13}C$ values of non-essential amino acids, particularly isotopic spacing between glycolytic and Krebs cycle amino acids, to explore whether systematic variations in nutrient physiology could be encoded in the bulk or amino acid isotope values.

MATERIALS AND METHODS

Sample Description

Vertebral samples were collected from nine blue sharks and six porbeagles caught across the North Atlantic. Three blue sharks were captured in offshore waters south of Canary Islands (eastern blue sharks), three in the mid-Atlantic Ridge area northwest of the Azores (central blue sharks), and three in oceanic waters between Cape Hatteras and the Gulf of Maine (western blue sharks; **Figure 1**). Blue sharks were bycaught by pelagic longlines targeting swordfish, tunas and sharks, by research and commercial fishing vessels using pelagic longlines or during shark fishing tournaments using rods and reels. Two porbeagles were bycaught on the continental shelf in the Celtic Sea during commercial gillnet fishery targeting gadiform fish and retained under dispensation as part of a fishery bycatch study (eastern porbeagles; Ellis and Bendall, 2015; Ellis et al., 2015). One porbeagle was bycaught by a fisherman near the Faroe Islands and donated to research. Three porbeagles were caught between Massachusetts and Grand Banks (off southern Newfoundland) by commercial vessels using pelagic longlines (western porbeagles; **Figure 1**; for metadata see **Table 1**).

Sample Preparation for Bulk Tissue Analysis

Trunk vertebrae were excised from above the branchial chamber in porbeagles and western blue sharks, and vertebrae were removed posterior to the skull in central and eastern blue sharks. All vertebrae were immediately frozen after dissection. Vertebral
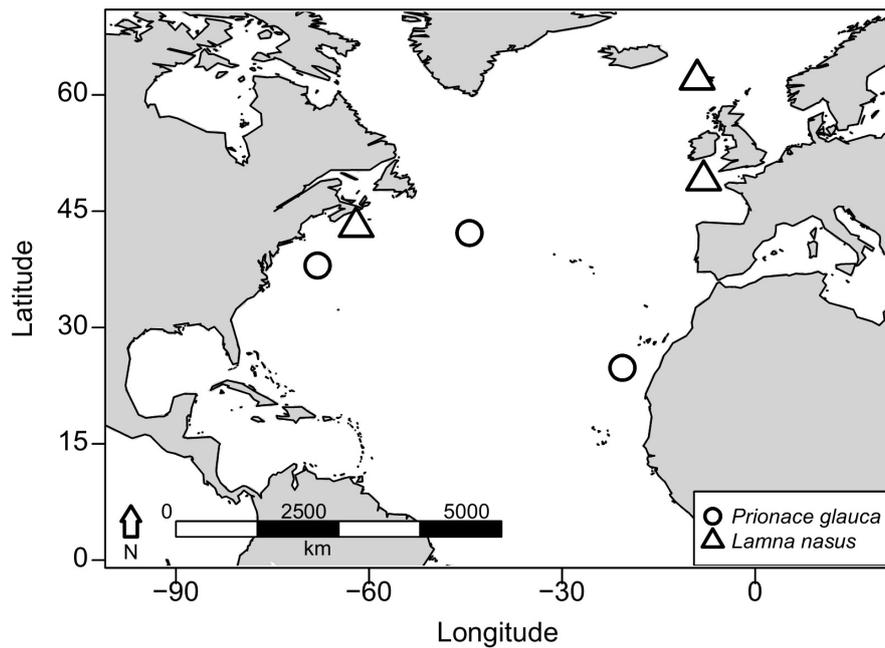
FIGURE 1 | Capture areas for blue sharks (*Prionace glauca*) and porbeagles (*Lamna nasus*) in the North Atlantic.

TABLE 1 | Metadata for individual blue sharks (*Prionace glauca*) and porbeagles (*Lamna nasus*) analyzed for stable isotopes, including individual ID, species, capture area, capture date or year, sex, maturity stage, fork length (FL, cm), vertebral radius (measured: VR, mm; adjusted VR: AdjVR, mm), number of samples collected for each vertebra (N), and (achieved) average interval between adjacent samples (AvgInt, mm).

Individual ID	Species	Capture area	Capture date	Sex	Maturity	FL	VR	AdjVR	N	AvgInt
16	*Prionace glauca*	mid-Atlantic Ridge	17/06/2014	Female	Mature	242.0	11.0	11.3	19	0.59
24	*Prionace glauca*	mid-Atlantic Ridge	20/06/2014	Female	Mature	240.0	11.0	11.2	21	0.53
12	*Prionace glauca*	mid-Atlantic Ridge	16/06/2014	Female	Mature	219.0	10.0	10.2	17	0.60
101	*Prionace glauca*	Canary Islands	07/02/2014	Female	Mature	258.0	12.0	12.0	16	0.75
131	*Prionace glauca*	Canary Islands	08/02/2014	Female	Mature	249.0	12.0	11.6	17	0.68
33	*Prionace glauca*	Canary Islands	05/02/2014	Female	Mature	244.0	11.5	11.3	21	0.54
335	*Prionace glauca*	Northwest Atlantic	21/02/1979	Female	Mature	268.0	17.0	16.4	34	0.48
415	*Prionace glauca*	Northwest Atlantic	16/05/1984	Female	Mature	265.0	15.0	16.2	31	0.52
441	*Prionace glauca*	Northwest Atlantic	29/06/1985	Female	Mature	245.0	14.5	14.9	28	0.53
11	*Lamna nasus*	Celtic Sea	2011	Female	Maturing	211.0	15.0	15.2	24	0.63
40	*Lamna nasus*	Celtic Sea	2014	Female	Immature	197.0	12.5	14.0	24	0.58
1000	*Lamna nasus*	Faroe Islands	2014	Female	Maturing	210.0	15.5	15.1	24	0.63
599	*Lamna nasus*	Northwest Atlantic	05/12/1999	Female	Mature	260.0	17.5	19.2	32	0.60
601	*Lamna nasus*	Northwest Atlantic	06/12/1999	Female	Mature	256.5	17.5	18.9	34	0.56
578	*Lamna nasus*	Northwest Atlantic	27/11/1999	Female	Mature	256.0	17.0	18.9	30	0.63

*AdjVR is calculated from FL using validated (for blue shark and porbeagle trunk vertebrae) and estimated (for blue shark cervical vertebrae) VR:FL relationships. AvgInt is calculated as AdjVR/N (for details, see **Supplementary Material**).*

centra were then defrosted, physically cleaned of excess muscle and connective tissue and air-dried for 3–10 days, depending on dimensions (Kim and Koch, 2012). A 6 mm section was cut from each centrum using a low-speed diamond-bladed Isomet® saw. This section was divided in two halves: a thinner (5– 5.4 mm) section was cut from one half and used for bulk tissue isotope analysis, the other half was used for amino acid analysis. Approximately equidistant samples (mean ± SD interval between adjacent samples among vertebrae: 0.59 ± 0.07 mm; **Table 1**)

were collected along the vertebral radius by manually cutting the thinner half section with a scalpel. Equidistant samples were chosen over sampling growth bands, as growth bands can be difficult to identify, particularly in blue sharks (Magozzi personal observation), and are compressed toward the vertebral edge due to reduced growth/accretion rates with age (Natanson et al., 2002; Skomal and Natanson, 2003), which makes it hard to sample each single growth band and obtain sufficient material for isotopic analysis. For this reason, the temporal resolution of isotope

chronologies from equidistant samples was higher (e.g., <1 year) closer to the vertebral centrum and lower toward the edge (e.g., a few years) but sufficient to depict isotopic variation at ecologically relevant time-scales for all specimens and life-history stages.

Vertebral samples were decalcified by exposure to 2 ml of 1 M HCl for 48 h to remove any potential influence of ^{13}C-enriched bioapatite on the δ^{13}C values of bulk cartilage collagen (Hussey et al., 2012; Kim and Koch, 2012; Christiansen et al., 2014; see also Tuross et al., 1988). During treatment samples were kept at a constant temperature of 4°C to reduce decalcification rates and prevent collagen dissolution and damage. Samples were then washed five times with 2 ml of Milli-Q water, frozen and freeze-dried.

Samples weighing <0.5 mg (i.e., the minimum weight required for dual carbon and nitrogen isotope analysis of bulk protein) were combined to the lighter adjacent sample(s) until the combined weight exceeded 0.5 mg. Due to the typical bowtie shape of vertebral sections, more samples generally required to be combined closer to the centrum or even further along the radius in portions of the vertebra characterized by large water or air content. Thus, the combination of samples often resulted in decreased temporal resolution at the core. Single or combined samples with a weight of >1.5 mg were powdered and ~1.25 mg of material was randomly selected for analysis. Blue shark samples were analyzed at full resolution (i.e., all adjacent samples), porbeagle samples at half resolution (i.e., one every other sample) for distances along the vertebral radius <7 mm and at full resolution afterward. This choice was made to minimize analytical costs, whilst also achieving a sufficient temporal resolution throughout ontogeny in the two species. Evidence from satellite-tagging studies indicates that, while juvenile blue sharks may undergo extensive latitudinal movements (Queiroz et al., 2010; Vandeperre et al., 2014), porbeagles tend to remain on the shelf until they conduct an ontogenetic habitat shift toward offshore waters with the onset of maturity (Biais et al., 2017) and are therefore unlikely to express large isotopic variation during early life-history stages. The vertebral radius at maturity is estimated to be ~15 mm in porbeagles from age at maturity equal to 13 years (Natanson et al., 2002); a value of 7 mm was selected here as a more conservative cutoff to account for approximations in the sampled interval.

Sample Preparation for Compound-Specific Analysis

The procedures for sample preparation for amino acid isotope analysis were similar to those for bulk tissue analysis except that the half section used for this analysis was 6 mm thick and more adjacent samples required to be combined at the core and along the vertebral radius, as the minimum weight for compound-specific analysis is 4 mg (prior to hydrolysis and derivatization; Houghton personal communication). Vertebral samples were analyzed at full resolution for central and eastern blue sharks, at half resolution for all other specimens to minimize analytical costs. Thus, the temporal resolution of amino acid analysis was

generally lower than for bulk tissue analysis but sufficient to identify breakpoints in amino acid isotope chronologies.

For compound-specific analysis, samples were initially acid-hydrolyzed (after combining adjacent samples) in 1 ml 6 N HCl at 110°C (temperature range: 105–115°C) for 20 h to liberate single amino acids from protein. Vials were tightly closed during hydrolysis to prevent sample and acid evaporation. After hydrolysis, samples were dried with a gentle stream of N$_2$ at 60°C (temperature range: 55–65°C) for 30–60 min, depending on sample weight and excess HCl volume. Samples were then dissolved in 100 µl 0.1 N HCl and preserved at 4°C until derivatization.

A total of 50 µl of the sample+HCl solution were derivatized by adding 35 µl of methanol, 30 µl of pyridine, and 15 µl of methyl chloroformate (MCF); after additions, the mixture was vortexed for 30 s. Amino acid-MCF derivates were separated from the reaction mixture by adding a volume of chloroform that depended on sample original weight: 50 µl for 4–10 mg, 75 µl for 11–22 mg, and 100 µl for >23 mg. After chloroform addition, the mixture was vortexed for another 30 s. At this stage the reaction mixture was stratified and the organic layer of amino acid-MFC derivates was separated from the aqueous layer using electrophoresis pipette tips and used for analysis.

Bulk Tissue Isotope Analysis

Carbon and nitrogen isotopic compositions of bulk cartilage collagen were measured using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom) at the UC Davis Stable Isotope Facility. Samples from Northeast Atlantic porbeagles were transported to the United States accompanied by a CITES Export Permit (No. 529713/01). All samples were analyzed in duplicate. Analytical quality-control was assessed through repeated measurement of laboratory-internal reference materials (nylon, glutamic acid, and USGS-41; for details see Magozzi, 2017), which were in turn calibrated to international reference materials. Nylon was used as a drift reference to correct for variation over the course of a run, glutamic acid to calculate the elemental totals and apply a linear correction to the isotope values. The isotope values were then scaled to two reference materials of known isotopic compositions: nylon and USGS-41. Precision was calculated as the standard deviation of isotope values for bovine liver NIST 1577 across runs (i.e., for the time period over which the samples were analyzed), accuracy as the difference between the mean isotope value for bovine liver and its accepted value. Precision was 0.03‰ for δ^{13}C values and 0.10‰ for δ^{15}N values, accuracy 0.01‰ for δ^{13}C values and 0.06‰ for δ^{15}N values. Final δ^{13}C and δ^{15}N values were expressed relative to the international reference materials V-PDB (Vienna PeeDee Belemnite) and Air, respectively. The raw ratio (R) of the heavy (^{13}C, ^{15}N) to the light (^{12}C, ^{14}N) isotope in a sample was converted to a δ-value expressed in ‰ using Eq. (1):

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right] \cdot 1000 \qquad (1)$$

where X is the heavier isotope. $R_{standard}$ refers to the raw ratio of an internationally accepted reference gas for the analyzed isotope.

Compound-Specific Isotope Analysis

Derivatized samples were analyzed with gas chromatography-combustion-isotope ratio monitoring mass spectrometry (GC-C-irm-MS) at the Woods Hole Oceanographic Institution. Samples were dissolved in dichloromethane (DCM) and injected on column in splitless mode at 260°C and separated on an Agilent VF-23ms column (length: 30 m, inner diameter: 0.25 mm, film thickness: 0.25 µm; Agilent Technologies, Wilmington, DE, United States) in an Agilent 689N Gas Chromatograph (GC). Sample concentrations were adjusted to achieve a minimum of 2 V output for all amino acids. Gas chromatography conditions were set to optimize peak separation and shape as follows: initial temperature 80°C held for 1 min; ramped to 260°C at 6°C min^{-1}; held for 3 min. The separated amino acid peaks were combusted online in a Finnigan GC-C continuous flow interface at 930°C and then measured as CO_2 on a Thermo Finnigan Mat 253 irm-MS (Agilent Technologies 689N GC). Standardization of runs was achieved using intermittent pulses of a CO_2 reference gas of known isotopic composition. All samples were run in duplicate. Carbon isotopic compositions were recovered for the essential amino acids: valine, isoleucine, leucine, threonine, and phenylalanine; and for the non-essential amino acids: alanine, glycine, proline, aspartic acid, and glutamic acid. A typical chromatogram from the analysis of carbon isotope ratios in amino acid-MCF derivates is displayed in **Supplementary Figure 2**. Values of $\delta^{13}C$ for threonine showed poor chromatography and inconsistencies among replicates, therefore were omitted from data analysis.

Two internal lab reference materials (AA1 and AA2) were created with single amino acids with known $\delta^{13}C$ values. Additionally, 20 mg of lyophilized muscle from Atlantic cod (*Gadus morhua*, Linnaeus 1758) were acid-hydrolyzed and re-dissolved in 100 µl 0.1 N HCl and 50 µl of the solution used as reference material. All reference materials were concurrently derivatized with the samples and dissolved in 100 µl chloroform. Derivatization correction factors were determined for each amino acid based on known $\delta^{13}C$ values of the amino acids in the reference materials prior to derivatization, and applied to each sample to adjust for the introduction of exogenous carbon and kinetic fractionation from derivatization. As no international reference materials are currently available for the analysis of carbon isotope ratios in individual amino acids and no large inter-laboratory calibration has yet come up with consensus $\delta^{13}C$ values, mean values across three laboratories (i.e., the Fish Ecology Laboratory, the Marine Biological Laboratory, and the UC Davis Stable Isotope Facility; **Supplementary Table 1**) were used. Reference materials were interspersed among samples and analyzed 4–6 times. Precision was determined as the standard deviation of the $\delta^{13}C$ values for each amino acid in the cod standard during the time period of sample analysis. Precision was 0.31‰ for valine, 0.34‰ for isoleucine, 0.40‰ for leucine, 0.30‰ for threonine, 0.26‰ for phenylalanine, 0.13‰ for

alanine, 0.30‰ for glycine, 0.32‰ for proline, 0.40‰ for aspartic acid, 0.22‰ for glutamic acid.

Data Analysis

To account for differential vertebral growth rates throughout ontogeny, linear distance of samples along the vertebral radius was converted to age using validated (for blue shark and porbeagle trunk vertebrae; Natanson et al., 2002; Skomal and Natanson, 2003) and estimated (for blue shark cervical vertebrae; this study) vertebral width:body size relationships and validated body size:age (Von Bertalanffy) growth curves for blue sharks (Skomal and Natanson, 2003) and porbeagles (Natanson et al., 2002; for details on age estimation see **Supplementary Material**). Clearly, due to reduced growth/accretion rates with age, the same sampled interval corresponded to "less years" integrated at smaller distances along the vertebral radius, "more years" integrated at larger distances along the radius. Thus, conversion of sample distance to age accounted for time-series expansion closer to the vertebral centrum and time-series compression toward the edge. Differences in growth and longevity between blue sharks and porbeagles were accounted for by parameters in vertebral width:body size relationships and Von Bertalanffy growth curves. Age at birth was 0 years and age at maturity was 5 and 13 years in blue sharks and porbeagles, respectively (Natanson et al., 2002; Skomal and Natanson, 2003); these values were used as cutoffs to distinguish between pre-birth, juvenile and adult life-history stages when examining isotope chronologies. Sample age is not displayed in plots for pre-birth samples, as Von Bertalanffy growth curves are not applicable to estimate age during the pre-birth stage. Age classes were determined for each species by subsetting the maximum age range across individuals by 1 year-intervals, and samples were assigned to their age classes based on their estimated age. Age classes were sufficiently small to allow the identification of relatively continuous ontogenetic patterns across individuals.

Principal component analysis (PCA) was applied to the $\delta^{13}C$ values of essential and non-essential amino acids to reduce the data to two dimensions (PC1 and PC2) explaining the majority of variance in $\delta^{13}C$ values of amino acids. For non-essential amino acids, spacing (i.e., difference) in $\delta^{13}C$ values was calculated between independent pairs of glycolytic (lipid-derived) and Krebs cycle (lipid+protein-derived) amino acids (glycine and alanine compared to glutamic acid and proline, respectively) and explored as a potential indicator for shifts in macronutrient contents of diets and/or changes in metabolic routing of macronutrients to amino acid synthesis (Wolf et al., 2015; Wang et al., 2018, 2019).

In order to test for common isotope patterns across individual blue sharks and porbeagles, the values of each variable (i.e., bulk protein $\delta^{13}C$ and $\delta^{15}N$ values, PC1 scores for $\delta^{13}C$ values of essential and non-essential amino acids and spacing in $\delta^{13}C$ values between glycolytic and Krebs cycle amino acids) in each sample were normalized to the individual mean (life history-normalization) to emphasize within-individual variation in ontogenetic profiles, whilst reducing among-individual differences. Ontogenetic patterns in life history-normalized $\delta^{13}C$ and $\delta^{15}N$ values of bulk protein and differences between species

and among areas and individuals were tested with generalized additive mixed models (GAMMs). Estimated sample age was added as a smoother, species and area as parametric fixed effects and individuals as a random effect (Pinheiro and Bates, 2000; Zuur et al., 2014). Model fits were calculated for full models and optimal models were selected with Akaike Information Criteria (AIC; Zuur et al., 2014). Generalized additive mixed models were fitted using the *mgcv* R package (Wood, 2006) and all analysis were performed using R v.4.0.2 (R Core Team, 2019).

RESULTS

Bulk Protein

Bulk protein $\delta^{13}C$ values ranged between -16.73 and -13.95‰ (mean ± SD: -15.13 ± 0.65‰) in blue sharks and between -15.80 and -13.71‰ (mean ± SD: -14.55 ± 0.38‰) in porbeagles. Values of $\delta^{15}N$ varied between 9.09 and 14.14‰ (mean ± SD: 11.47 ± 1.11‰) in blue sharks and between 9.42 and 16.29‰ (mean ± SD: 12.84 ± 1.47‰) in porbeagles. The $\delta^{15}N$ range in porbeagles was, however, strongly influenced by ^{15}N-enriched subadult and adult samples from Northwest Atlantic individuals.

Despite high among-individual variability in individual isotopic profiles (**Supplementary Figure 4**), we identified common, broad ontogenetic patterns in life history-normalized $\delta^{13}C$ and $\delta^{15}N$ values ($\delta^{13}C_n$, $\delta^{15}N_n$) of bulk protein across individuals of each species (**Figure 2**). Ontogenetic patterns in $\delta^{13}C_n$ and $\delta^{15}N_n$ values were non-linear (smoother 'estimated sample age': p-value = $2\cdot10^{-16}$, $F_{\delta^{13}C}$ = 26.46; $F_{\delta^{15}N}$ = 26.29) and differed significantly between species (maximum p-value = 0.006, F = 7.62; **Supplementary Tables 2, 3**). In blue sharks, $\delta^{13}C_n$ values increased progressively during juvenile and early adult growth, reaching a relatively steady state in the adult life stage (**Figure 2A**). Values of $\delta^{15}N_n$ increased during juvenile growth and decreased in adult stages (**Figure 2B**). Both $\delta^{13}C_n$ and $\delta^{15}N_n$ values were higher in the pre-birth stage and decreased around birth ($\delta^{15}N_n$ values to a lesser extent; **Figures 2A,B**). Pre-birth $\delta^{13}C_n$ values were highest across life-history and comparable to adult levels, whereas pre-birth $\delta^{15}N_n$ values were higher than late adult levels. Among-individual variances in both $\delta^{13}C_n$ and $\delta^{15}N_n$ values were relatively high but constant across life-history and lower than in porbeagles (**Figures 2A,B**).

In porbeagles, mean $\delta^{13}C_n$ values increased in the juvenile stage (until an age of ~7–8 years), then remained relatively constant until capture (**Figure 2C**). Pre-birth $\delta^{13}C_n$ values were higher than post-partum values but generally lower than adult levels. Among-individual variance in $\delta^{13}C_n$ values was markedly higher than in blue sharks, particularly in the pre-birth stage and early juvenile and subadult stages associated with shifts in mean $\delta^{15}N_n$ values (see below). Mean values of $\delta^{15}N_n$ showed two stepwise increases, a small one just after birth and a larger one in the subadult stage (at ~11–12 years of age), whereas values in intermediate juvenile and adult periods remained relatively constant (**Figure 2D**). The stepwise pattern in the subadult stage was driven by a step increase in $\delta^{15}N_n$ values in individuals from the Northwest Atlantic, and the plateau phase in the adult stage

was also only seen in these sharks (**Supplementary Figures 4M–O**). Similar to $\delta^{13}C_n$ values, pre-birth $\delta^{15}N_n$ values were higher than values after birth but lower than values in the adult stage (**Figures 2C,D**). Among-individual variance in $\delta^{15}N_n$ values was broadly consistent with that in $\delta^{13}C_n$ values, peaking in the pre-birth stage and early and late juvenile stages.

Amino Acids

Carbon isotope compositions of individual amino acids were analyzed for 128 blue shark vertebral samples (*n* = 196 bulk collagen samples). For porbeagles, 80 samples were analyzed for carbon isotopes in amino acids (*n* = 133 bulk protein samples). Mean ± SD $\delta^{13}C$ values for each amino acid in blue shark and porbeagle samples are presented in **Supplementary Table 4**. Individual isotope chronologies for $\delta^{13}C$ values in single amino are presented in **Supplementary Figures 5–13**.

Values of $\delta^{13}C$ of non-essential amino acids ($\delta^{13}C_{non-EAA}$) were more positive than values of essential amino acids (**Supplementary Figures 5–13**), and the bulk protein $\delta^{13}C$ value corresponded to the weighted average of $\delta^{13}C_{EAA}$ and $\delta^{13}C_{non-EAA}$ values. Co-variations between bulk protein and single amino acid $\delta^{13}C$ values are shown in **Figures 3**, **4** for essential and non-essential amino acids, respectively. Bulk protein $\delta^{13}C$ values were higher and spanned a smaller range in porbeagles. Individual blue sharks grouped by capture area, implying that spatially determined among-individual variation explained more of the variance than the within-individual term. Within individual sharks, amino acid $\delta^{13}C$ values co-varied more with bulk protein $\delta^{13}C$ values in blue sharks compared to porbeagles, implying a common mechanism influencing multiple amino acids in the same direction and therefore contributing to bulk collagen $\delta^{13}C$ values. This was especially clear for the non-essential glycolytic amino acids alanine and glycine. In porbeagles, there was very weak co-variance between bulk protein $\delta^{13}C$ values and $\delta^{13}C$ values for any single amino acids, implying that mechanisms underpinning variation in bulk protein $\delta^{13}C$ values varied among- and within-individuals, likely complicating efforts to interpret bulk collagen $\delta^{13}C$ values in an ecological or behavioral context. Aspartate/aspartic acid and, to a lesser extent, glutamate/glutamic acid $\delta^{13}C$ values separated blue shark and porbeagle individuals into two clusters but these clusters did not correspond to different capture areas.

Essential Amino Acids

Principal components 1 and 2 explained 72% and 13% of the total variance in the $\delta^{13}C$ values of essential amino acids ($\delta^{13}C_{EAA}$), respectively (**Supplementary Figures 14A,B**). Most positive PC1 scores corresponded to most positive $\delta^{13}C$ values of all essential amino acids. Shark samples laid along a gradient on the PC1 axis: central and eastern North Atlantic blue sharks had most negative PC1 (hence $\delta^{13}C_{EAA}$) values, individuals from the Northwest Atlantic had most positive values (**Supplementary Figure 14A**). Porbeagles had intermediate PC1 scores and also separated, to a lesser extent, by area and individual (**Supplementary Figure 14B**).

Despite high variability in PC1 individual profiles (**Supplementary Figure 15**), broad, common patterns in
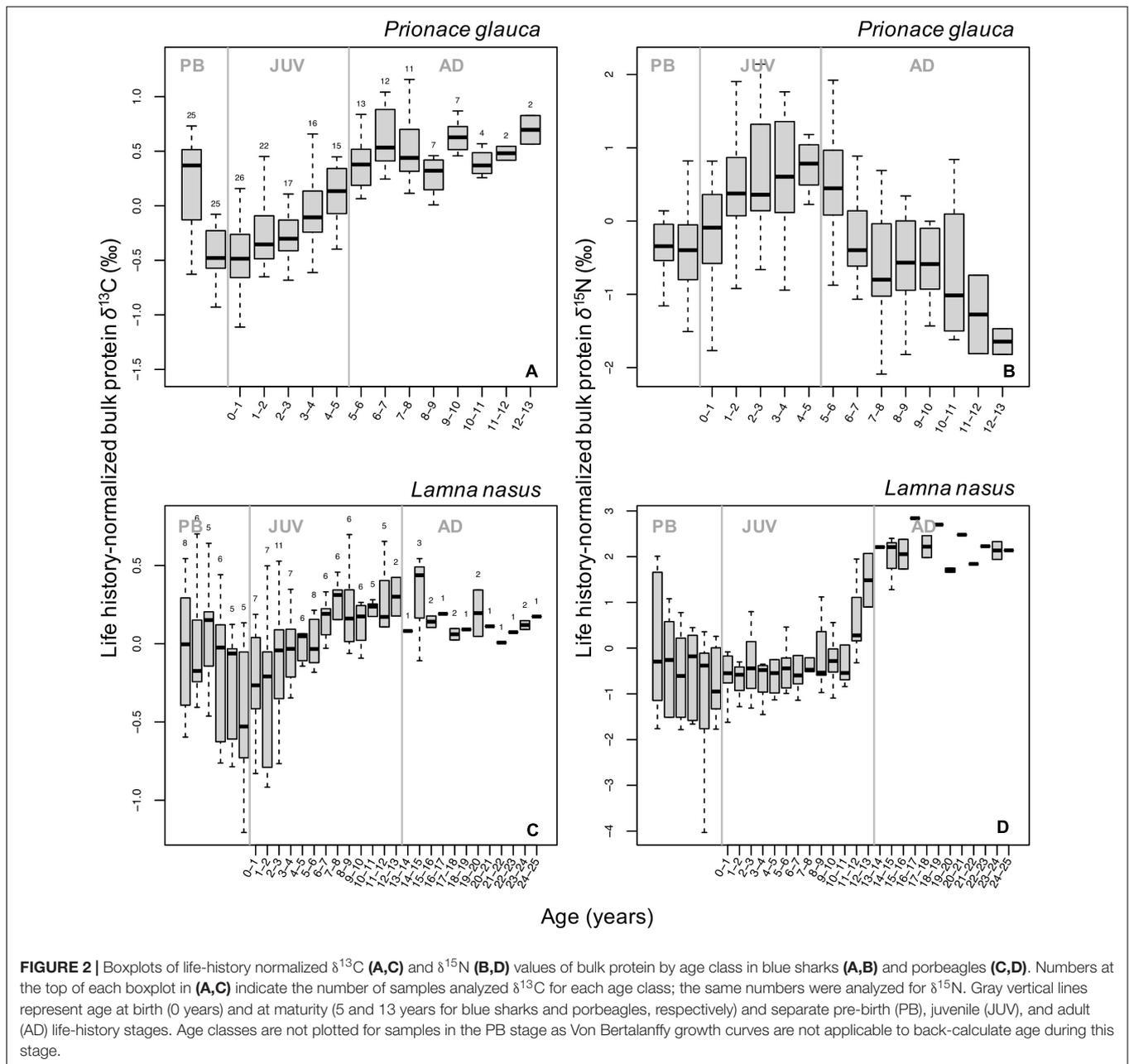
FIGURE 2 | Boxplots of life-history normalized $\delta^{13}C$ **(A,C)** and $\delta^{15}N$ **(B,D)** values of bulk protein by age class in blue sharks **(A,B)** and porbeagles **(C,D)**. Numbers at the top of each boxplot in **(A,C)** indicate the number of samples analyzed $\delta^{13}C$ for each age class; the same numbers were analyzed for $\delta^{15}N$. Gray vertical lines represent age at birth (0 years) and at maturity (5 and 13 years for blue sharks and porbeagles, respectively) and separate pre-birth (PB), juvenile (JUV), and adult (AD) life-history stages. Age classes are not plotted for samples in the PB stage as Von Bertalanffy growth curves are not applicable to back-calculate age during this stage.

life history-normalized PC1 scores for $\delta^{13}C$ values of essential amino acids ($PC1_{n-EAA}$) were observed across blue sharks and porbeagles (**Figure 5**). In blue sharks, $PC1_{n-EAA}$ (hence $\delta^{13}C_{EAA}$) values were highest in the pre-birth stage, decreased sharply around birth and increased progressively during juvenile and early adult growth but did not show any clear trend in the late adult stage (**Figure 5A**). Adult $PC1_{n-EAA}$ values were generally comparable to pre-birth levels, except for some age classes. In porbeagles, $PC1_{n-EAA}$ scores decreased during juvenile growth until an age of ~6–7 years, possibly increased back until 11–12 years of age, but showed no clear patterns in subadult and adult stages (**Figure 5C**). Scores of $PC1_{n-EAA}$ were highest in the pre-birth stage and decreased after birth.

Non-essential Amino Acids

PC1 and PC2 explained 45% and 22% of the total variance in $\delta^{13}C_{non-EAA}$ values, respectively (**Supplementary Figures 14C,D**). Most positive PC1 scores corresponded to most positive $\delta^{13}C$ values of all non-essential amino acids except aspartic acid; most positive PC2 were associated with most positive values of alanine and most negative values of glutamic acid and aspartic acid. Blue sharks had generally more negative PC1 scores for $\delta^{13}C_{non-EAA}$ values than porbeagles but more positive PC2 scores (except individual 1000; **Supplementary Figures 14C,D**). Individuals from the central and eastern North Atlantic had more negative PC1 and PC2 than Northwest Atlantic sharks in both species. Ontogenetic patterns in PC1

FIGURE 3 | Scatterplots of raw $\delta^{13}C$ values of bulk protein vs. single essential amino acids (**A**, valine; **B**, isoleucine; **C**, leucine; and **D**, phenylalanine) in comparable vertebral samples from individual blue sharks and porbeagles caught across the North Atlantic (as in figure legend).
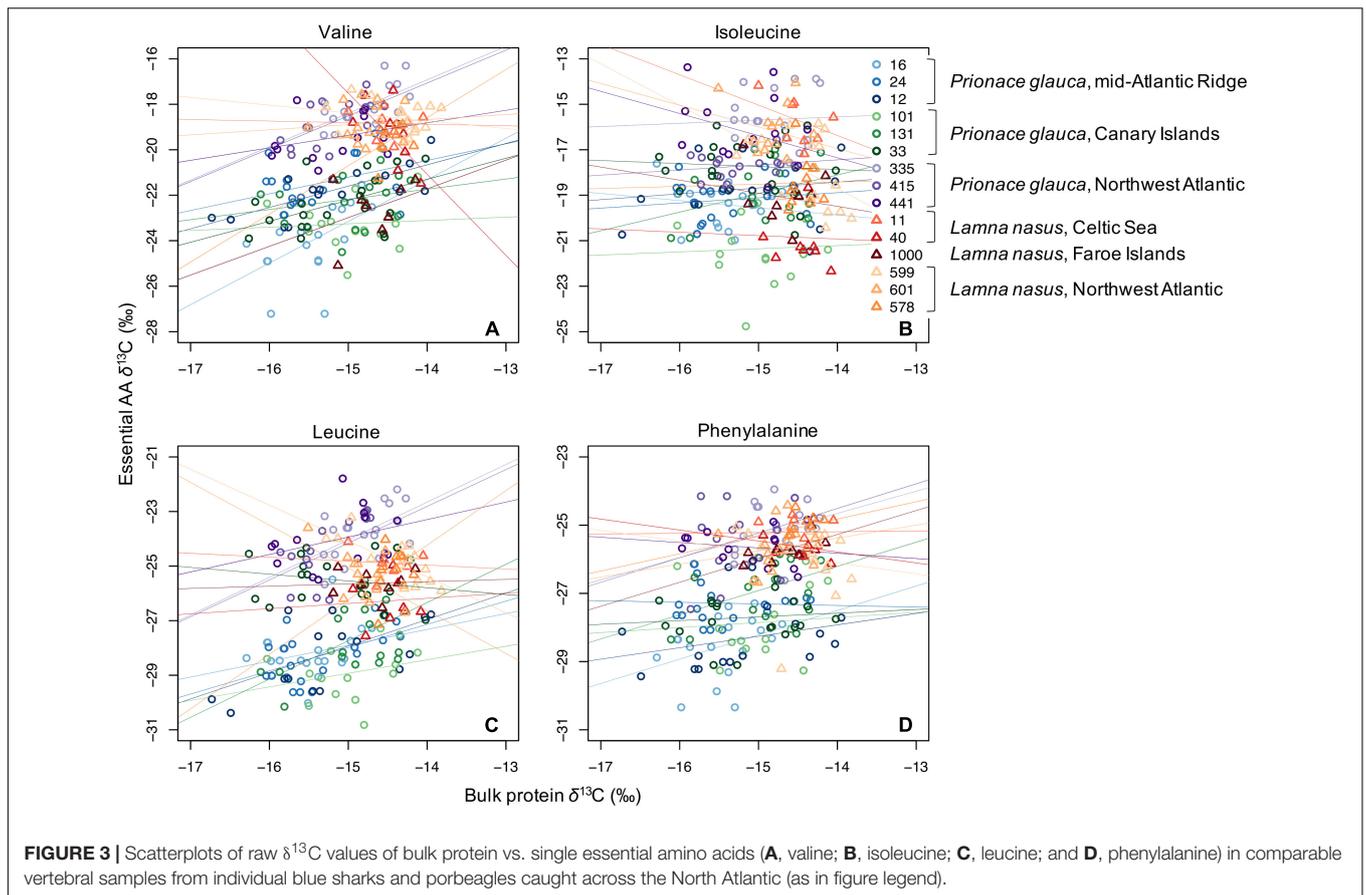
scores for $\delta^{13}C_{non-EAA}$ values were similar to patterns in $\delta^{13}C_{EAA}$ (**Figures 5B–D** and **Supplementary Figure 15**) with additional influences of aspartic acid (**Supplementary Figures 14C,D**; see also **Figure 4D** and **Supplementary Figure 12**).

Spacing Between Glycolytic and Krebs Cycle Amino Acids

In general, broad ontogenetic patterns in life history-normalized values of spacing in $\delta^{13}C$ values between pairs of glycolytic and Krebs cycle amino acids (glycine and glutamic acid compared to alanine and proline, respectively) did not match those in bulk protein $\delta^{13}C$ values (**Figure 6**). In blue sharks, spacing between both pairs of amino acids decreased slightly around birth and increased during juvenile growth, then remained relatively constant in late juvenile and adult stages (**Figures 6A,B**). In porbeagles, spacing between both amino acid pairs showed systematic patterns, with a sharp increase associated with birth and early juvenile stages, followed by broadly U-shaped profiles before declining in the most recent samples (**Figures 6C,D**). The precise timing of transitions in these profiles differed slightly between the two amino acid pairs. More or less pronounced excursions in spacing between glycolytic and Krebs cycle amino acids were observed through ontogeny in all sharks (**Supplementary Figure 16**). In general, excursions in spacing between independent pairs of amino acids showed relatively strong covariation, with a few exceptions. In many cases,

excursions in spacing coincided with those in bulk protein $\delta^{13}C$ and/or $\delta^{15}N$ values.

DISCUSSION

This study aimed to test inferences about life-history movements drawn from ontogenetic patterns in bulk collagen $\delta^{13}C$ and $\delta^{15}N$ values in shark vertebrae using complementary data for single amino acid $\delta^{13}C$ values. The study is based on a small number of individuals (nine blue sharks and six porbeagles) with highly variable isotope chronologies. We were therefore obviously unable to infer species- or population-level behavioral or ecological patterns from these data. Below we discuss possible interpretations of aspects of shark spatial and trophic ecology based on bulk protein isotopic life-histories and the extent to which these may be supported, contradicted or refined based on amino acid $\delta^{13}C$ patterns.

Inferences Based on Patterns in Bulk Protein

Bulk protein $\delta^{13}C$ and $\delta^{15}N$ values in blue sharks, normalized by life-history, showed a common pattern of increasing values during juvenile growth (**Figures 2A,B**), despite relatively high variation in individual profiles (**Supplementary Figures 4A–I**).

FIGURE 4 | Scatterplots of raw δ^{13}C values of bulk protein vs. single non-essential amino acids (**A**, alanine; **B**, glycine; **C**, proline; **D**, aspartic acid; and **E**, glutamic acid) in comparable vertebral samples from individual blue sharks and porbeagles caught across the North Atlantic (as in figure legend).
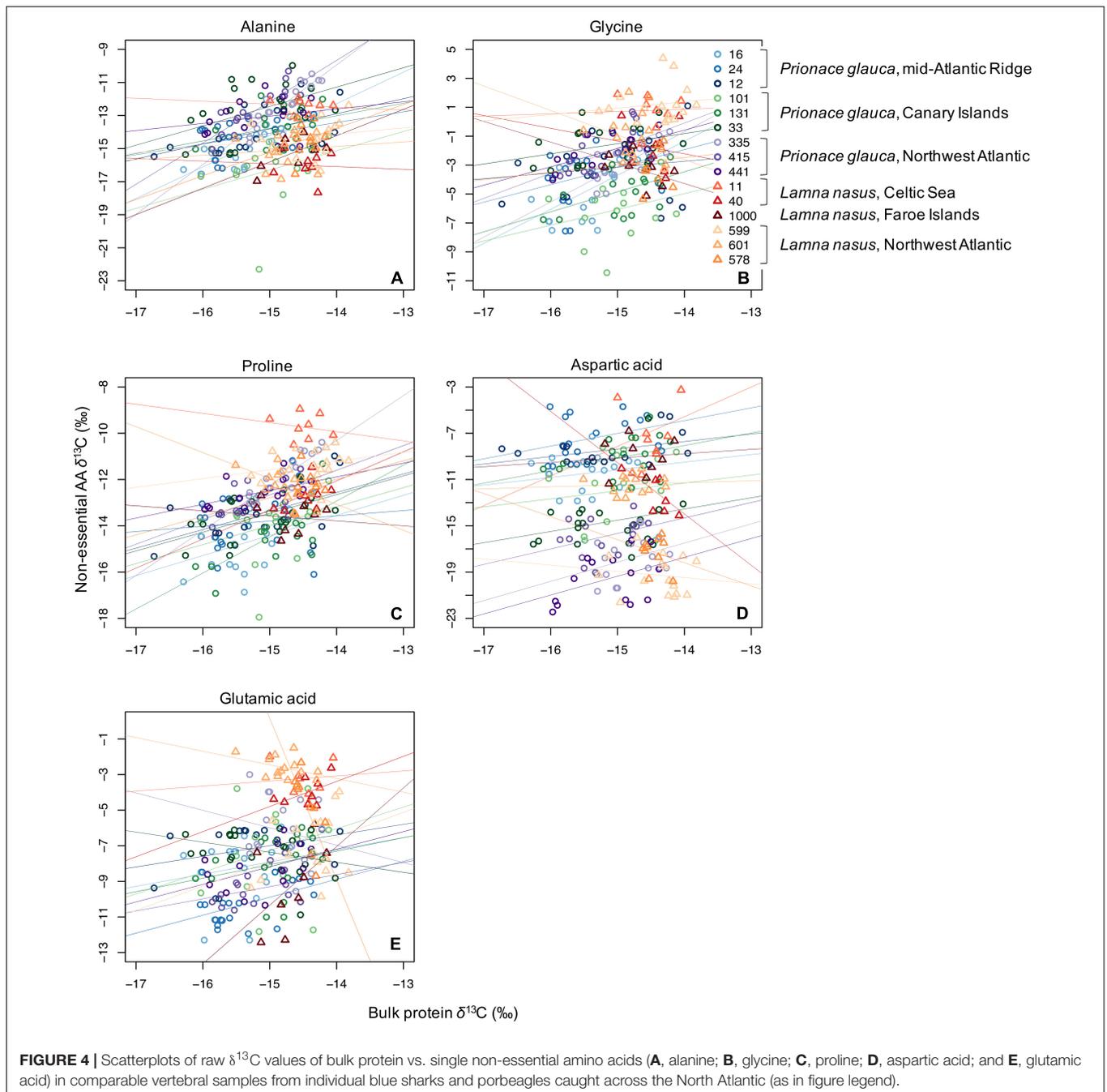
Increases in δ^{13}C values in marine animals are commonly interpreted as baseline effects resulting from increased use of warmer waters closer to the equator (McMahon et al., 2013; Magozzi et al., 2017) and/or more coastal foodwebs with higher levels of primary production (O'Learly, 1981; Miller et al., 2008). Increases in δ^{13}C values could also reflect increases in trophic position, however the lack of a corresponding trend in δ^{15}N values in blue sharks implies that trophic level-change alone could not explain the δ^{13}C pattern. In the eastern North Atlantic in particular, blue sharks are believed to be primarily oceanic sharks (Vandeperre et al., 2014, 2016; Coelho

et al., 2018), therefore ontogenetic trends in bulk protein δ^{13}C values in blue sharks are likely to be interpreted as reflecting a life-history migration resulting in increased use of resources from relatively warm southern locations through the juvenile and early adult life stages. In the Northwest Atlantic, juveniles are often observed near the shelf, particularly in summer, meaning that isotopic signals in the juvenile stages could as well be influenced by coastal processed. The increase in δ^{15}N values during juvenile growth and subsequent decrease in the adult stage (**Figure 2B**) is also consistent with baseline effects associated with an increasing

dependence on resources living in warmer waters (McMahon et al., 2013; Schmittner and Somes, 2016) rather than a strong ontogenetic trophic shift.

Ontogenetic profiles in bulk protein $\delta^{13}C$ and $\delta^{15}N$ values in porbeagles are considerably more challenging to interpret compared to blue sharks. Life history-normalized bulk protein $\delta^{13}C$ values displayed a minor ontogenetic increase with approximately half of the range seen in blue sharks, and high among-individual variance in both early and late juvenile life stages (**Figure 2C**). Bulk protein $\delta^{15}N$ values were relatively flat until the late juvenile stage, when a pronounced step increase was observed (**Figure 2D**). Similar to blue sharks, the ontogenetic profiles in bulk protein $\delta^{13}C$ values in porbeagles may imply a gradual increase in reliance on resources with higher $\delta^{13}C$ values (e.g., closer to the equator and/or more coastal resources) throughout juvenile growth but with a high degree of among-individual variance. The step increase in $\delta^{15}N$ values in late juvenile stages may potentially be linked to a change in diet or trophic level as inferred for other lamnid sharks (Estrada et al., 2006; Kerr et al., 2006), as there is no clear association with $\delta^{13}C$ values that might imply a habitat shift. Such a step increase in $\delta^{15}N$ values was, however, only observed in individuals from the Northwest Atlantic (**Supplementary Figures 4M–O**), possibly suggesting a delay in trophic shift in the Northeast Atlantic population, movement across larger nitrogen isoscapes in the eastern part of the basin, and/or differences in age estimation models not accounted for here.

In summary, bulk protein profiles in blue sharks may be interpreted as suggesting common ontogenetic changes in space use, toward greater use of oceanic and warmer waters with increasing age. For porbeagle sharks, a lack of clear, consistent, isotopic patterns, and a relatively small increase in $\delta^{13}C$ values, would likely imply no strong, common ontogenetic trend in space use, and potentially location- and population-specific variations in life-history movements.

Testing Inferences With Essential Amino Acids

Essential amino acids can only be synthesized by primary producers and bacteria, and must be acquired by consumers directly through the diet. Variations in bulk tissue isotope values reflecting horizontal movements should therefore be replicated consistently in the $\delta^{13}C$ values of essential amino acids, without confounding influences from trophic discrimination and physiology (Reeds, 2000; McMahon et al., 2010).

In blue sharks, at least a component of the positive ontogenetic trend in bulk protein $\delta^{13}C$ values was matched by a common ontogenetic pattern of increasing $\delta^{13}C$ values of essential amino acids (**Figure 5A**). Inferences drawn from bulk protein $\delta^{13}C$ values are therefore supported by patterns in $\delta^{13}C$ values of essential amino acids. That is, the ontogenetic increase in bulk protein $\delta^{13}C$ values observed in these sharks during juvenile and early adult growth can be explained by progressively increased dependence on resources from waters characterized by more positive $\delta^{13}C$ baselines (e.g., warmer waters). The interpretation of a life-history migration to southern waters

is consistent with results from satellite-tagging studies in the eastern North Atlantic, which indicate that juvenile and subadult blue sharks conduct seasonal movements between temperate and subtropical waters (Queiroz et al., 2010; Vandeperre et al., 2014; see also Campana et al., 2011), whereas adult sharks undertake a directional migration to tropical waters possibly associated with parturition and pupping (Vandeperre et al., 2014, 2016; Coelho et al., 2018).

Maternal $\delta^{13}C$ values of essential amino acids were higher than post-partum values and comparable to adult levels (**Figure 5A**) as seen in bulk protein $\delta^{13}C$ values, indicating that pupping might occur in isotopically distinct areas with more negative $\delta^{13}C$ baselines than those used by the mothers while provisioning eggs and by adult sharks. Adult $\delta^{13}C$ values of essential amino acids approached maternal levels by ~5–6 years of age, which approximately corresponds to the onset of maturity in blue sharks (Pratt, 1979; Skomal and Natanson, 2003).

In stark contrast to observations in blue sharks and bulk collagen values, $\delta^{13}C$ values of essential amino acids in porbeagles, expressed as life history-normalized $PC1_{EAA}$ scores, decreased during juvenile growth until an age of ~6–7 years (**Figure 5C**). Assuming that essential amino acids reflect baseline conditions, this trend implies a common trend of increasing assimilation of carbon from prey with low $\delta^{13}C$ values (e.g., resources from offshore and/or colder waters), as the sharks grow. The interpretation of porbeagle isotopic life-histories based on bulk protein and essential amino acid $\delta^{13}C$ values are therefore difficult to reconcile. Porbeagles are believed to conduct an ontogenetic habitat shift from shelf to shelf-edge and oceanic environments (Bendall et al., 2013; Ellis and Bendall, 2015; Ellis et al., 2015; Biais et al., 2017), and have been shown to conduct extensive latitudinal migrations (Pade et al., 2009; Saunders et al., 2011; Biais et al., 2017). Contrasting patterns in bulk protein and essential amino acid $\delta^{13}C$ values could be possibly reconciled by an increasing trophic level, if trophic level increased sufficiently to raise $\delta^{13}C$ values to counteract the decrease in $\delta^{13}C$ values of essential amino acids. However, this was not supported by the trend in bulk collagen $\delta^{15}N$ values, which would be expected to be more sensitive to changes in trophic level. Satellite-tag data are therefore consistent with the interpretations based on patterns in essential amino acid $\delta^{13}C$ values (**Figure 5C**), not with those based on bulk protein $\delta^{13}C$ values.

Pre-partum $\delta^{13}C$ values reflected carbon assimilation during maternal provision and represented the highest values that were found across life-history (**Figure 5C**). This pattern suggests that pregnant female porbeagles may exploit isotopically distinct (more positive) foraging grounds compared to those used by subadult and adult sharks and is consistent with the observation of a directional movement to subtropical waters by tagged mature (presumably pregnant) females in the Northwest Atlantic (Campana et al., 2010; see also Natanson et al., 2019).

Additional Inferences Drawn From Non-essential Amino Acids

Ontogenetic profiles in PC1 scores for $\delta^{13}C$ values of non-essential amino acids in blue sharks also showed a common

FIGURE 5 | Boxplots (in blue) of life history-normalized PC1 values from PCAs with $\delta^{13}C$ values of essential **(A,C)** and non-essential **(B,D)** amino acids by age class in blue sharks **(A,B)** and porbeagles **(C,D)**. Boxplots (in black) of life history-normalized $\delta^{13}C$ values of bulk protein are overlain for comparison. Figure description is the same as for **Figure 2**.
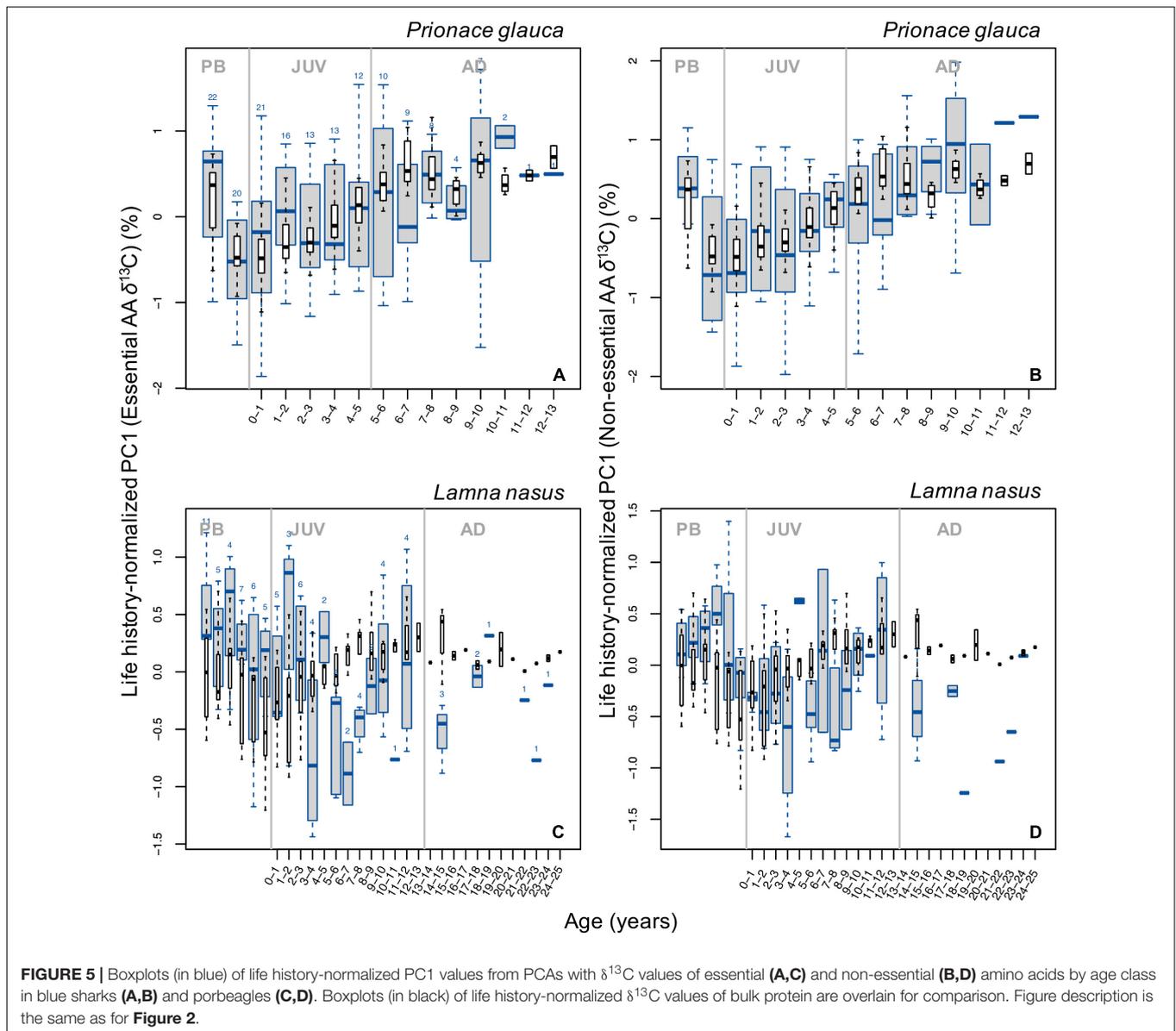
increase (**Figure 5B**), consistent with a baseline effect expressed in both essential and non-essential amino acids. As all non-essential amino acids are influenced by baseline effects, the isotopic spacing in $\delta^{13}C$ values between glycolytic and Krebs cycle amino acids to some extent controls for the baseline influence (assuming that the rate of incorporation of newly ingested carbon is consistent between the two amino acids). For blue sharks, observed ontogenetic patterns in life history-normalized isotopic spacing varied among individuals resulting in no clear common trend (**Figures 6A,B** and **Supplementary Figures 16A–I**). This pattern also suggests that the common signal seen in bulk protein $\delta^{13}C$ values in blue sharks is largely controlled by baseline spatial influences rather than dietary and/or physiological effects.

Ontogenetic trends in life history-normalized PC1 scores for $\delta^{13}C$ values of non-essential amino acids in porbeagles are unclear (**Figure 6D**) but ontogenetic profiles of normalized spacing in $\delta^{13}C$ values between glycolytic and Krebs cycle amino acids show consistent, systematic trends with a strong increase after birth, followed by U-shaped patterns throughout juvenile growth (**Figures 6C,D** and **Supplementary Figures 16J–O**). The biochemical or nutritional mechanisms underpinning differences in isotopic spacing between non-essential amino acids remain uncertain. However, the presence of systematic trends in isotopic spacing between amino acids assumed to be responsive to differences in macronutrient (lipid and protein) assimilation and use (Wolf et al., 2015; Wang et al., 2018, 2019) implies that amino acid isotope data hold information associated with individual nutrition, and that bulk protein $\delta^{13}C$ values may be very difficult to interpret reliably.

Therefore, we suggest that in porbeagles relatively minor isotopic variability associated with ontogenetic transitions between juvenile foraging in more coastal, shelf waters

FIGURE 6 | Boxplots (in blue) of life history-normalized spacing in $\delta^{13}C$ values between independent pairs of glycolytic (lipid-derived) and Krebs cycle (lipid+protein-derived) amino acids (**A,C,** glycine and glutamic acid; **B,D** alanine and proline) by age class in blue sharks (**A,B**) and porbeagles (**C,D**). Boxplots (in black) of life history-normalized $\delta^{13}C$ values of bulk protein are overlain for comparison. Figure description is the same as for **Figure 2**.

and greater dependence on cooler, offshore and slope environments in adult stages are masked in bulk protein $\delta^{13}C$ values by individual variation and by physiological influences potentially associated with differential use of lipid and protein macronutrients. In contrast, common ontogenetic movement transitions across latitudinal gradients in $\delta^{13}C$ values in blue sharks are large enough to be clearly expressed in bulk protein $\delta^{13}C$ values and overwhelm any shared ontogenetic trends in macronutrient use.

CONCLUSION

SIA can provide a useful complement to satellite archival tags to study the movements of pelagic sharks throughout their lives. However, bulk tissue isotopic signals are often confounded by mixed baseline, trophic and physiological effects. Compound-specific analyses of individual amino

acids may be required to disentangle these effects, aiding interpretations of bulk tissue isotope data to infer migration histories.

In this study we analyzed an average of 22 samples from each shark. Assuming that the cost of bulk isotope analysis is $10 per sample, high-resolution analysis of an individual costs $160–280. The cost of compound-specific isotope analysis is approximately 10 times that of traditional bulk analysis, and resources and expertise associated with sample preparation and analysis are significantly greater. Additionally, compound-specific analysis requires more sample material, resulting in decreased temporal resolution for longitudinal ecological information. A cost- and time-effective combination of bulk and compound-specific SIA, whereby individual amino acid analyses are used to test hypotheses generated from high-resolution or multiple-individual studies of bulk tissue analyses, may represent an acceptable compromise to complement tag-derived movement information.

Despite the small number of individuals examined here, and large among-individual variability in isotopic profiles, we found evidence of common, broad ontogenetic isotope patterns in blue sharks. Such isotopic life-histories appeared to be somewhat associated with ontogenetic movement across isotopically distinct baselines, despite additional influences from trophic and physiological effects. Given the large intra- and inter-individual isotopic variability we observed, these findings should, of course, be tested by increasing the sample size but, if verified, could imply a shared vulnerability of each species across life-history to fishery capture. Furthermore, comparisons of maternal and post-partum $\delta^{13}C$ values provided information on transgenerational movements and suggested that blue sharks and porbeagles might adopt different strategies for parturition and pupping, based on the analyzed individuals of each species.

Our study contributes to a growing body of work illustrating the biochemical complexity that can underpin variations in bulk tissue stable isotope compositions. Inferences drawn from bulk tissue isotope data may be relatively robust in situations where the behavior of the animal results in a consistent change in the isotopic composition of all or many macronutrients, such as a directed migration across large spatial isotopic gradients. In such cases, isotopic variation in ingested nutrients is pervasively expressed during metabolic processing, and is likely to overwhelm superimposed isotopic variation associated with more subtle changes in metabolic routing. However, where variance in bulk isotope compositions is more closely related to variations in macronutrient processing and associated biochemical routing of metabolites, high levels of among- and within-individual variation are likely to significantly complicate interpretation of bulk tissue $\delta^{13}C$ values. In the case of porbeagles studied here, it is possible that apparent population-specific trends in bulk collagen $\delta^{13}C$ values were spurious co-incidences. Attempts to interpret such trends to infer aspects of life-history ecology are therefore dangerous. We suggest that single amino acid analyses offers one of the few options to test such inferences independently.

Future research should explore how spatio-temporal variation in isotopic baselines, migration and tissue isotopic discrimination interact to influence tissue isotopic compositions in pelagic sharks. Such space-time isotope dynamics can be explored using a simulation modeling framework combining isoscape models, agent-based models of behavior and movement, and physiology-biochemistry models (e.g., Carpenter-Kling et al., 2019; Trueman et al., 2019; Magozzi et al., 2020). Furthermore, additional controlled-feeding experiments such as those by Wang et al. (2019) are required to better constrain trophic and physiological influences on $\delta^{13}C$ values of non-essential amino acids.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the study examined vertebral samples from dead sharks that were bycaught by longline fisheries primarily targeting tuna and swordfish.

AUTHOR CONTRIBUTIONS

FUNDING

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.673016/full#supplementary-material

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