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Isotopic life-history signatures are retained in modern and ancient Atlantic bluefin tuna vertebrae

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Abstract

Isotopic, tagging and diet studies of modern-day teleosts lacked the ability to contextualise life-history and trophic dynamics with a historical perspective, when exploitation rates were lower and climatic conditions differed. Isotopic analysis of vertebrae, the most plentiful hard-part in archaeological and museum collections, can potentially fill this data-gap. Chemical signatures of habitat and diet use during growth are retained by vertebrae during bone formation. Nonetheless, to fulfil their potential to reveal life-history and trophic dynamics, we need a better understanding of the time frame recorded by vertebrae, currently lacking due to a poor understanding of fish bone remodelling. To address this issue, the authors serially-sectioned four vertebral centra of the highly migratory Atlantic bluefin tuna (*Thunnus thynnus*; BFT) captured off Sardinia (Italy) and analysed their isotopic composition. They show how carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$) isotope values can vary significantly across BFT vertebrae growth-axes, revealing patterning in dietary life histories. Further, they find that similar patterns are revealed through incremental isotopic analysis of inner and outer vertebrae centra samples from 13 archaeological BFT vertebrae dating between the 9th and13th centuries CE. The results indicate that multi-year foraging signatures are retained in vertebrae and allow for the study of life histories in both modern and paleo-environments. These novel methods can be extended across teleost taxa owing to their potential to inform management and conservation on how teleost trophic dynamics change over time and what their long-term environmental, ecological and anthropological drivers are.

KEYWORDS
fish bone turnover, historical ecology, life histories of fishes, serial sectioning, stable isotope analysis

1 | INTRODUCTION

Retrospective ecological studies are increasingly analysing the stable isotopic composition of teleost vertebrae due to the predominance of these bones in the archaeological records and potential to reveal how trophic dynamics respond to environmental, ecological and cultural shifts (Barrett *et al*., 2011; Guiry *et al*., 2020; Ólafsdóttir *et al*., 2017). Nonetheless, little is known about how tissue turnover influences

isotopic variation across the growth axes of teleost vertebrae and thus whether isotope values represent short-term snapshots into the foraging ecology of fishes immediately before they were caught, or long-term averages across their entire life span (Tzadik *et al*., 2017). This information has important consequences for their interpretation, comparison with isotope measurements from other tissue types and biogeochemical data, as well as for the sampling of teleost vertebrae prior to analysis.

For highly migratory species, such as the Atlantic bluefin tuna (*Thunnus thynnus*, hereafter BFT), the ability to trace temporal changes in life histories using isotopic variation across the growth axes of vertebra represents an additional novel opportunity; otherwise it is possible only when using otoliths, which are metabolically inert and not subject to turnover (Campana & Thorrold, 2001; Tzadik *et al*., 2017). Given that otoliths are not as prevalent in the archaeological record or museum collections, and are generally only analysed for a few elements (*e.g*., $\delta^{13}C$, $\delta^{18}O$ and $^{87/86}Sr$) due to being composed primarily of calcium carbonate, vertebrae might offer a practical solution to obtaining isotopic life-history signatures from protein including those of additional elements (*e.g*., $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$: Andrews *et al*., 2022b; Barrett *et al*., 2011; McCormack *et al*., 2021; Nehlich *et al*., 2013). Importantly, this would enhance investigations into how feeding patterns and migrations change in the marine environment over time and what their environmental, ecological and anthropological drivers are (Guiry & Hunt, 2020).

Two factors will influence the degree of isotopic variation within teleost bone: (a) the variation in isotope signatures between habitats and diets utilised across the individual's lifespan and (b) how rapidly the protein containing those signatures is remodelled and reabsorbed. Teleost bone is mainly composed of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), a type of calcium phosphate which is deposited onto collagen fibrils. Teleost bone turnover has long been considered slow relative to soft tissues such as muscle and liver (Buchheister & Latour, 2010; Madigan *et al*., 2012; Tzadik *et al*., 2017) following assumptions from better-studied taxa [*e.g*., mammals and birds (Hobson & Clark, 1992; Tieszen *et al*., 1983)]. Because fish growth continues throughout life, resorption (the repair of damaged tissue by osteoclasts) and remodelling (the incorporation of new tissue by osteoblasts) are ongoing processes, which are expected to vary between bone elements and species, depending on their growth and damage rates, but these are largely unknown (Bas & Cardona, 2018; Davesne *et al*., 2019; Witten & Villwock, 1997). Complications further arise as these two processes may function differently depending on whether fishes have acellular bone (lacking osteocytes, *e.g*., Atlantic cod, *Gadus morhua*) or cellular bone (containing osteocytes, *e.g*., BFT), despite osteocytes being more heavily involved in mineral homeostasis, rather than remodelling, in teleosts (Davesne *et al*., 2019; Meunier, 2011; Witten *et al*., 2000; Witten & Huysseune, 2009).

The cellular bone of BFT can be further distinguished by two types: cortical (dense, surface) bone, which retains annuli (annual growth layers) and cancellous (spongy, interior) bone, where growth layers are more rapidly resorbed (Matsubayashi & Tayasu, 2019; Santamaria *et al*., 2018; Turner Tomaszewicz *et al*., 2016). Cortical vertebral bone is well evidenced to retain life-history isotopic signatures in elasmobranchs (*e.g*., sharks). These are *c*. 1–2‰ variation in both $\delta^{13}C$ and $\delta^{15}N$ values over the life span of 10–15-year-old individuals, which studies generally agree represents a multi-year signal instead of a perfect chronology (Carlisle *et al*., 2015; Estrada *et al*., 2006; Kerr *et al*., 2006; Shen *et al*., 2022). In teleosts, investigations are more recent and are as yet restricted to mostly anadromous Salmonidae spp., Pleuronectidae spp. and Clupeidae spp. from a confined Pacific locality (Kato *et al*., 2021; Matsubayashi *et al*., 2017, 2019, 2020), reporting variability of *c*. 1–5‰ in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ across growth axes of adult fishes, suggesting that juvenile signatures can be fully or partially retained into adulthood.

A range of ecological and environmental variables will affect the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values retained at each life-history stage. $\delta^{15}N$ values increase with each trophic level and are thus used to estimate the trophic position of an organism in a food web (Sigman *et al*., 2009). In contrast, $\delta^{13}C$ and $\delta^{34}S$ values pass between primary producers and consumers with low levels of fractionation. This lends them to being good indicators of provenance because distinct $\delta^{13}C$ and $\delta^{34}S$ signatures are generally maintained across trophic levels (DeNiro & Epstein, 1978; Guiry, 2019; Thode, 1991). Typically, coastal habitats (and those heavily influenced by cold or low-salinity water) are lower in $\delta^{13}C$ than oceanic habitats, partially due to increased terrestrially derived (low-$\delta^{13}C$) carbon inputs and lower quantities of resuspended (remineralised, high-$\delta^{13}C$) carbon from the benthos and deep-ocean (Barnes *et al*., 2009; Magozzi *et al*., 2017). For these reasons, pelagic consumers often contain lower $\delta^{13}C$ than benthic ones (Amiraux *et al*., 2023; DeNiro & Epstein, 1978).

It is important to note that multiple factors govern the variation in $\delta^{15}N$ and $\delta^{13}C$ between consumers, including the environmental conditions, levels of benthic-pelagic coupling and the production in each habitat foraged (Barnes *et al*., 2009; Jennings *et al*., 2008; Sigman *et al*., 2009). One useful technique is to use additional isotopes (such as $\delta^{34}S$) to disentangle these effects. For example, low $\delta^{34}S$ values often reflect increased foraging on benthic or neritic prey, whereas higher values indicate a greater degree of energy incorporated from pelagic production (Fry & Chumchal, 2011; Szpak & Buckley, 2020). Distance from shore also influences $\delta^{34}S$ values, not because of freshwater input (even brackish water is dominated by marine high $\delta^{34}S$ signatures) (Cobain *et al*., 2022; Fry & Chumchal, 2011; Guiry *et al*., 2022), but rather the greater extent of sulphide-rich (low $\delta^{34}S$) production in coastal areas, such as in seagrass beds, salt marshes and mudflats (Guiry *et al*., 2022; Szpak & Buckley, 2020; Thode, 1991). Because ecological and environmental variables governing production change over time (*e.g*., following production dynamics, and sources such as pollution), there is often intra- and inter-annual variation at the base of marine food-webs, which one needs to be aware of when drawing conclusions from temporal isotopic data – especially over the long-term (Jardine *et al*., 2014; Solomon *et al*., 2008). Nonetheless, some degree of temporal variation can be accounted for, like the long-term decrease in oceanic $\delta^{13}C$ following industrialisation (Suess Effect: Gruber *et al*. (1999).

BFT is a large [≤3.3 m in length and ≤725 kg in weight: Cort *et al*. (2013)] pelagic predator, which spawns from ages 3 to 4 (Mather

TABLE 1 Details of the modern Atlantic bluefin tuna (*Thunnus thynnus*) specimens serially sectioned in the current study

Sample	Location	Capture date	Coordinates	FL (cm)	Age (years)	Element analysed	Height (mm)	Width (mm)	Length (mm)	No. sections
CF_2020_588	Carloforte tonnara, Sardinia	July 2020	39.18 E, 8.31 N	196	9	35th vertebra	33.01	43.42	46.89	18
CF_2020_810	Carloforte tonnara, Sardinia	July 2020	39.18 E, 8.31 N	158	8	35th vertebra	25.10	33.4	37.12	12
CF_2020_667	Carloforte tonnara, Sardinia	July 2020	39.18 E, 8.31 N	149	5	35th vertebra	20.12	27.26	28.96	9
CF_2020_673	Carloforte tonnara, Sardinia	July 2020	39.18 E, 8.31 N	124	7	35th vertebra	17.74	22.5	25.05	8

Note. Age was estimated by counting annuli following Lee *et al*. (1983) and Andrews *et al*. (2023), as described in the methods. Height, width and length correspond to 35th vertebra measurements on the posterior centra as per Andrews *et al*. (2022a).

et al., 1995; Piccinetti *et al*., 2013), predominantly in the Mediterranean and in the Gulf of Mexico. The majority of individuals undertake diverse feeding migrations to a range of habitats throughout the Atlantic (Druon *et al*., 2016; Mariani *et al*., 2016; Wilson & Block, 2009) from as early as age 1 (Dickhut *et al*., 2009), though tagging evidence suggests that a portion of the population resides in the Mediterranean year-round (Cermeño *et al*., 2015). Juvenile and adult BFT primarily inhabit the upper 200 m of neritic habitats (Druon *et al*., 2016; Walli *et al*., 2009; Wilson & Block, 2009), feeding mostly on varied combinations of teleost fishes, cephalopods and crustaceans (Karakulak *et al*., 2009; Logan *et al*., 2011) and occasionally undertaking divergent offshore feeding strategies at great depths (Battaglia *et al*., 2013; Ólafsdóttir *et al*., 2016; Wilson & Block, 2009). The greatest shift in BFT foraging strategy appears to be at age 2, once the predation of zooplankton ends and the predation of fishes begins, after which, year classes become more isotopically similar (Rumolo *et al*., 2020; Sarà & Sarà, 2007).

The aim of this study was to determine isotopic variation across the growth axis of BFT vertebrae to explore if the degree of tissue turnover is sufficiently slow to expand chronological studies of isotopic life-history signatures in retrospective ecological contexts. The authors hypothesised that BFT vertebrae would document limited isotopic variation because the diets and habitats of BFT are rather isotopically homogeneous (Brophy *et al*., 2020), and the cellular bone of BFT in addition to their endothermy and rapid growth rates would promote more rapid resorption and remodelling than other teleosts (Davesne *et al*., 2019). The objectives of this study were to (a) serial-section modern BFT vertebral cortical bone, (b) sample inner and outer archaeological BFT vertebrae centra and (c) determine and analyse the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ collagen isotope values of samples.

2 | MATERIALS AND METHODS

2.1 | Serial-sectioning of modern specimens

Modern BFT were sampled off Isola Piana (Carloforte, Sardinia, Italy) in July 2020 by tuna trap (Carloforte Tonnare PIAM srl, Table 1). Vertebrae were mechanically cleaned of soft-tissues, macerated in ambient-temperature water for up to 3 months to remove the remaining soft-tissues by microbial decomposition, then rinsed using distilled water and dried.

The 35th vertebra of each specimen was isolated, pertaining to one of the penultimate vertebrae in BFT which have a total of 39 vertebrae [for a guide on BFT vertebra identification see Andrews *et al*. (2022a)]. The 35th vertebra was selected for consistency between specimens and due to the fact that annuli (growth rings) are often more clearly interpreted in these vertebrae. The authors estimated the age of specimens by counting annuli (Figure 1a) on the posterior centra of the 35th vertebra for each specimen, without staining and with illumination only, following Lee *et al*. (1983) and Andrews *et al*. (2023), where 1 year pertained to one groove (summer growth) and one ridge (winter growth).

Vertebrae (Table 1) were prepared for sectioning, whereby the outer surface of each specimen was mechanically cleaned to remove exogenous residues. Specimens were then cut using a band-saw, by halving first along the lateral plane (Figure 1a), and second along the ventral plane to obtain a posterior centrum cross-section, such that all annual layers of growth in each specimen were represented (Figure 1b). A cross-section sample (*c*. 250–1000 mg) was obtained for each specimen by again cutting along the lateral plane. The band saw was then set to cut *c*. 1 mm (0.1 mm error) increments starting at the centre of the centrum (age 0) (Figure 1b). Each increment was trimmed using a scalpel to remove cancellous bone, retaining cortical bone for analyses (Figure 1c). The first and second increment sections from the centre were combined in all instances to provide sufficient mass for analyses.

2.2 | Incremental sampling of archaeological specimens

A total of 13 archaeological BFT vertebrae were analysed from a Byzantine-era site in the Yenikapi neighbourhood of Istanbul, Turkey (Supporting Information Table S2). The Port of Theodosius operated at this site from the 4th to 11th centuries CE before being filled in

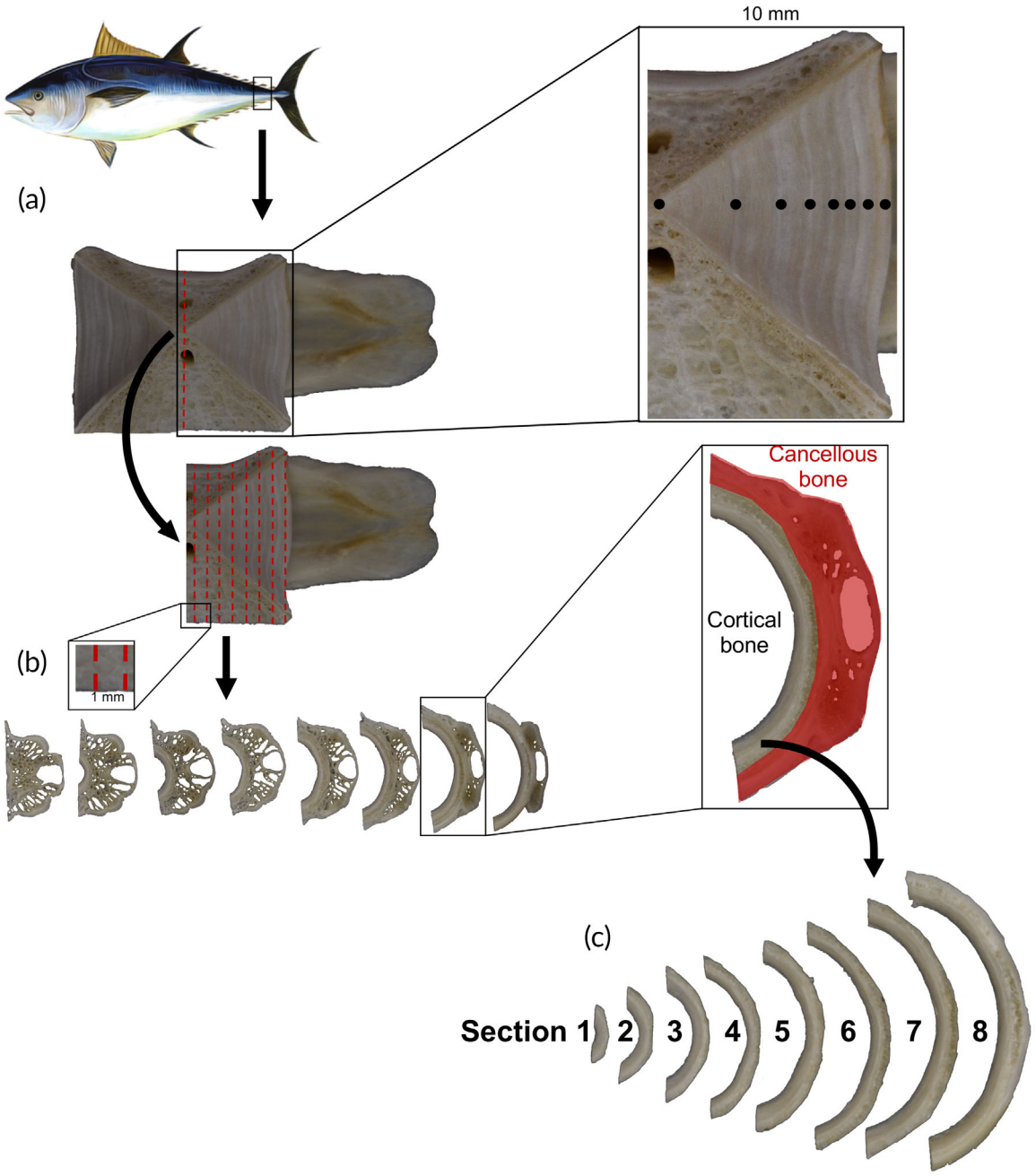

FIGURE 1 A schematic of the serial-sectioning procedure for modern Atlantic bluefin tuna (*Thunnus thynnus*) vertebra specimens, using the individual CF_2020_673 as an example. Growth axes of the 35th vertebrae (a) were sectioned in 1 mm increments from inner (early) to outer (later) bone growth before cancellous bone and (b) were removed prior to obtaining numbered sections for isotopic analyses (c). N.B. Sections 1 and 2 were pooled for each individual to provide sufficient material for analyses

during the 15th century CE (Onar *et al*., 2008). The 9th-13th century origin of the samples is proposed from ^{14}C dating taking into account reservoir effects for habitats foraged (Andrews et al., in review). Fork length (FL) of archaeological specimens was estimated following Andrews *et al*. (2022a) using the online resource https://tunaarchaeology.org/lengthestimations. Briefly, vertebrae were identified to rank or type (see Andrews *et al*., 2022a), vertebral centrum length, width and height was measured using digital callipers to the nearest 0.01 mm and the best-fitting power regression model was applied for each specimen (Supporting Information Tables S1 and S2). Age estimation was not attempted due to the poor visibility of annuli in these archaeological specimens. Size estimation was not attempted for three individuals due to inability to reliably identify vertebrae rank or type due to preservation (Supporting Information Table S2).

Due to national restrictions in the invasive sampling of archaeological materials in Turkey, the cutting of archaeological vertebrae was prohibited, and thus the authors drilled into each vertebra at inner (close to the centrum centre) and outer (close to the centrum edge)

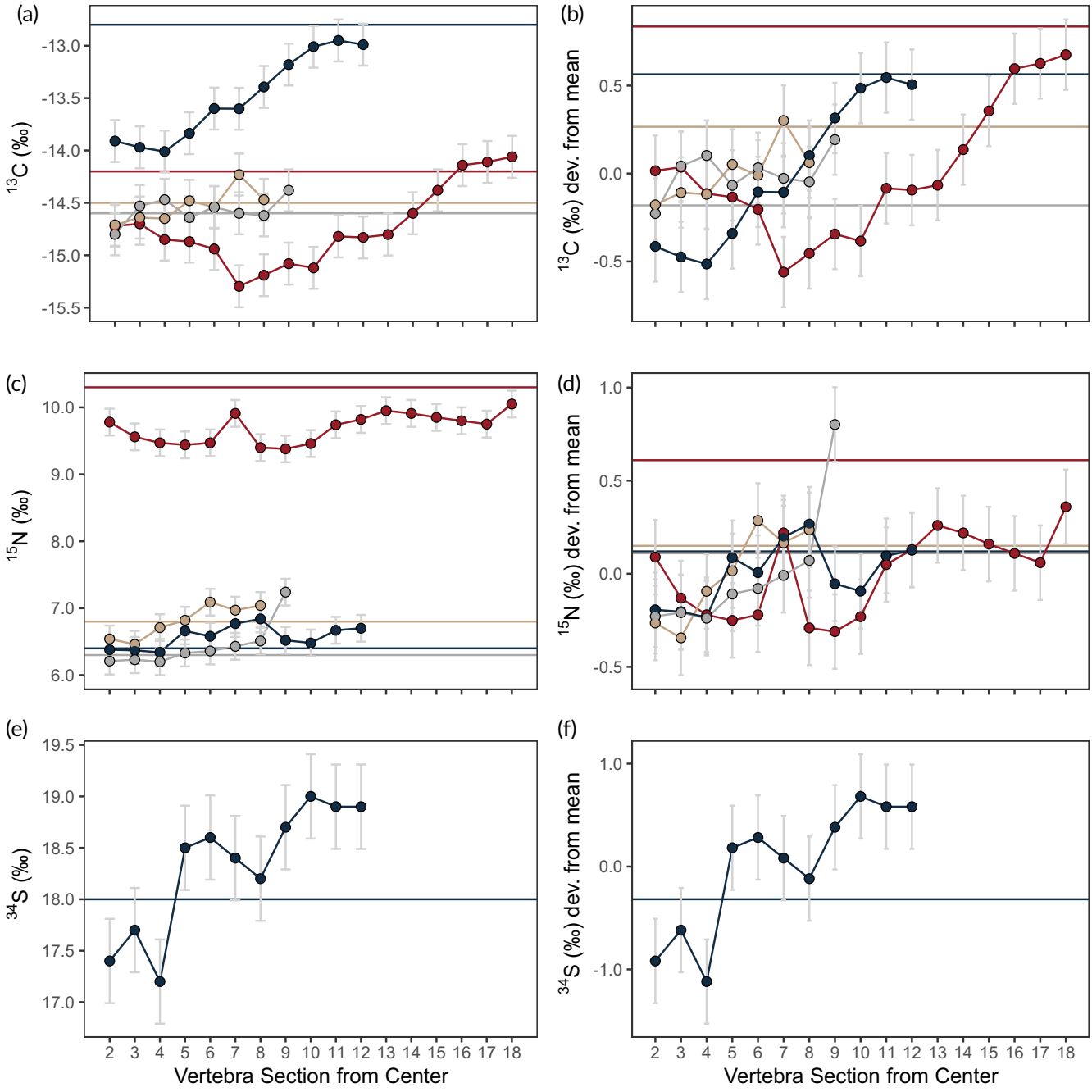

FIGURE 2 Variation in stable isotope values across vertebrae growth axes of modern Atlantic bluefin tuna (*Thunnus thynnus*; BFT). Carbon (‰, a, b), nitrogen (‰, c, d) and sulphur (‰, e, f) stable isotope ratios of vertebra centra serial sections from four specimens; CF_2020_588 (red), CF_2020_810 (black), CF_2020_667 (grey), CF_2020_673 (beige), captured off Carloforte, Sardinia. Isotope values (‰) represent absolute ratios (δ^{13}C: a, δ^{15}N: c, δ^{34}S: e) and deviations (dev.) from the mean value of all sections from each isotope and individual (Δ^{13}C: b, Δ^{15}N: d, Δ^{34}S: f). Solid lines indicate the isotope values of the cross-sections of each individual. Vertebral bone sections start at the centre of the centrum (early life) and increase towards the margin (later life). Grey error bars represent measurement error

positions (Figure 3). First, drilling sites at the inner and outer edges of vertebrae centra were mechanically cleaned for each specimen to remove exogenous residues. Then, they drilled into vertebrae at these inner and outer positions with 6 mm stainless-steel drill bits, maintaining a low speed. They collected 50–150 mg bone powder from each position, pertaining to both cortical and cancellous bone in roughly similar proportions as modern cross-section samples.

2.3 | Collagen extraction

Bone collagen was extracted following a modified Longin method (Brown *et al.*, 1988). Modern samples were defatted by sonication for 15 min in a 2:1 dichloromethane/methanol solution (Colonese *et al.*, 2015), repeated a minimum of three times until the solution remained clear. Solvents were then evaporated overnight before

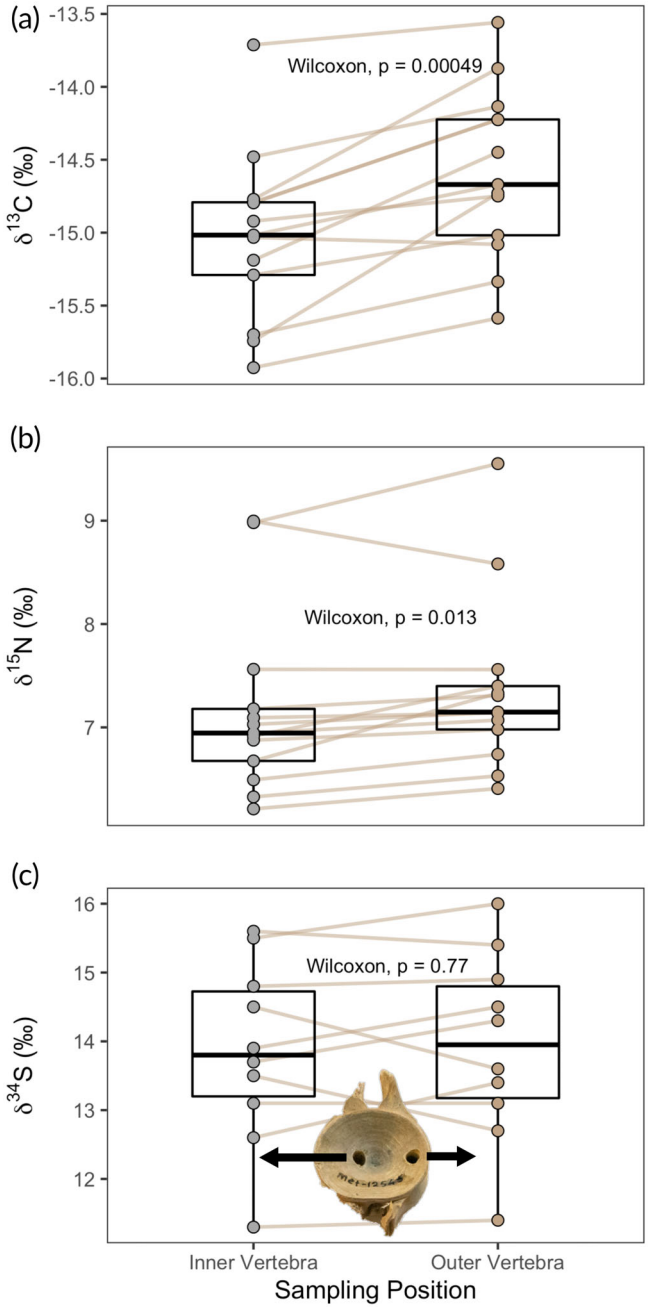

FIGURE 3 Carbon (‰, a), nitrogen (‰, b) and sulphur (‰, c) stable isotope ratios of inner vertebrae and outer vertebrae samples from 13 archaeological Atlantic bluefin tuna (*Thunnus thynnus*; BFT) specimens, dated between the 9th–13th centuries CE from the site of Yenikapi, Turkey. Inset (centre) shows approximate drilling positions of inner (left, grey) and outer (right, beige) samples, using specimen MET12545 with 41 mm centrum height, as an example. The significance (*P*) of Paired Wilcoxon tests is shown for each stable isotope

samples were rinsed thrice with milli-Q water to remove residual solvents. All samples were demineralised at +4°C, using 8 ml of 0.6 M HCl for modern samples and a gentler 0.4 M HCl for archaeological samples. Once completely demineralised, collagen was gelatinised at 80°C for 48 h in 0.001 M HCl. Gelatinised collagen was filtered (60–90 μm; Ezee filters, Elkay, UK) and then freeze-dried.

2.4 | Stable isotope analysis

To determine the carbon and nitrogen isotopic values, collagen (0.4–0.6 mg) was analysed in duplicate using a Sercon continuous flow 20–22 IRMS interfaced with a Universal Sercon gas solid liquid elemental analyser (Sercon, UK). Sulphur isotope values were determined for the serial sections of one of the specimens (CF_2020_810) by analysing collagen (0.9–1.2 mg), 20% in duplicate using a Delta V Advantage continuous-flow IRMS coupled *via* a ConfloIV to an Iso-Link elemental analyser (Thermo Scientific, Germany) at SUERC (East Kilbride, UK) as described in Sayle *et al*. (2019). The obtained values were corrected from the isotopic ratio of the international standards, Vienna Pee Dee Belemnite (VPDB) for carbon, air (AIR) for nitrogen and Vienna Cañon-Diablo Troilite (VCDT) for sulphur, using the δ (‰) notation. Accuracy was determined using a two-point calibration curve taking into account uncertainty for duplicate samples (Supporting Information Tables S1 and S2 and Supplementary Appendix).

Uncertainties on the measurements were calculated by combining the S.D.s of the sample replicates and those of International Atomic Energy Agency (IAEA) reference material according to Kragten (Kragten, 1994) for carbon and nitrogen, and (Sayle *et al*., 2019) for sulphur. The international standards used as reference material in analytical runs were caffeine (IAEA-600), ammonium sulphate (IAEA-N-2) and cane sugar (IA-Cane) for carbon and nitrogen; and silver sulphide (IAEA-S-2 and IAEA-S-3) for sulphur. International standard average values and S.D. across the runs were as follows: IAEA-600 ($n = 18$), $\delta^{13}C$ raw = −27.65 ± 0.1‰ ($\delta^{13}C$ true = −27.77 ± 0.04‰) and $\delta^{15}N$ raw = +0.77 ± 0.16‰ ($\delta^{15}N$ true = 1 ± 0.2‰); IAEA-N-2 ($n = 18$), $\delta^{15}N$ raw = +20.13 ± 0.18‰ ($\delta^{15}N$ true = 20.3 ± 0.2‰); and IA-CANE ($n = 18$), $\delta^{13}C$ raw = −11.72 ± 0.08‰ ($\delta^{13}C$ true = −11.64 ± 0.03‰); IAEA-S-2 ($n = 9$). $\delta^{34}S$ was calibrated relative to VCDT using internal standards GS2 and GAS2 (themselves calibrated to international standards IAEA-S-2 and IAEA-S-3).The maximum uncertainty across all samples was ±0.18‰ for $\delta^{13}C$ and 0.22‰ for $\delta^{15}N$ ($n = 56$), and ± 0.41‰ for $\delta^{34}S$ ($n = 21$). For more information on calibration and analytical uncertainty see Supplementary Appendix (Supporting Information Tables S3–S5).

The quality of $\delta^{13}C$ and $\delta^{15}N$ values was confirmed by assessing that atomic C:N ratios (3.0–3.3) fell within the accepted ranges for archaeological and modern samples (Guiry & Szpak, 2020, 2021), whereas $\delta^{34}S$ quality was confirmed by assessing %S, C:S and N:S against acceptable standards (Nehlich & Richards, 2009). The quality of archaeological samples was further confirmed by assessing that collagen yields (>1%, Supporting Information Table S2) which represented an adequate state of preservation. A modern bovine control which was processed and analysed alongside the archaeological sample batch yielded expected isotopic values (−23.2‰ $\delta^{13}C$, 6.2‰ $\delta^{15}N$, 3.2 C:N) and collagen yield (17.5%).

2.5 | Statistical analysis

Logarithmic regressions were performed in R (R Team, 2013) to assess the significance of the relationship between section number and isotope value, per specimen. Prior to testing, negative $\delta^{13}C$ values were transformed to positive equivalents. Paired Wilcox tests were performed in R to test the significance of difference between inner and outer isotope values. Significance was judged at the 5% level.

3 | RESULTS

3.1 | Serial-sectioning of modern specimens

Modern BFT specimens varied between 124 and 196 cm FL, ages 5–9, 18–33 mm centrum height, and yielded between 7 and 17 vertebra sections for isotopic analyses. $\delta^{13}C$ values were generally between −15 and −14‰, except for CF_2020_810, where values varied between −14 and −13‰ (Figure 2b). $\delta^{13}C$ values generally increased from the centre to the edges of vertebrae, by *c.* 1‰ for the largest individuals (CF_2020_588, 810) with 11–17 serial sections and *c.* 0.5‰ for the smaller individuals (CF_2020_667, 673) with 7–8 serial sections (Figure 2a). Cross-sections of growth axes generally had $\delta^{13}C$ values *c.* 0.2‰ greater than the outermost serial sections of each individual, the exception being CF_2020_673, which was *c.* 0.2‰ lower than outermost serial sections values. A significant relationship between $\delta^{13}C$ and vertebra sections was found for three of the four individuals, CF_2020_673 ($F_5 = 8.2$, $R^2 = 0.55$, $p < 0.05$), CF_2020_588 ($F_{15} = 12.1$, $R^2 = 0.41$, $P < 0.01$) and CF_2020_810 ($F_9 = 123.3$, $R^2 = 0.92$, $P < 0.001$).

Serial section $\delta^{15}N$ values were stochastic but overall increased from the centre to the edges of vertebrae (Figure 2c) by *c.* 0.5‰ for all individuals. $\delta^{15}N$ values of cross-sections of growth axes more closely matched the outermost serial sections of each individual except CF_2020_667, whose outermost serial section value appears to be an outlier. The oldest aged individual analysed (CF_2020_588, 9 years) had $\delta^{15}N$ value of *c.* 10‰, whereas the remaining three individuals (ages 5, 7, 8 years) had $\delta^{15}N$ values of *c.* 6–7‰ expected for juveniles (ages 1–2) (Figure 2c). A significant relationship between $\delta^{15}N$ and vertebra sections was found for three of the four individuals, CF_2020_667 ($F_6 = 11.2$, $R^2 = 0.59$, $P < 0.05$), CF_2020_673 ($F_5 = 24.3$, $R^2 = 0.79$, $P < 0.01$) and CF_2020_588 ($F_{15} = 8.3$, $R^2 = 0.31$, $P < 0.05$).

$\delta^{34}S$ values increased for the individual CF_2020_810 by *c.* 2‰ from the centre to the edge of its vertebra (Figure 2f), showing three distinct periods of feeding at *c.* 17.5‰ (sections 2–4), 18.5‰ (sections 5–8) and *c.* 19‰ (sections 9–12) throughout its 8 years of life (Figure 2e). $\delta^{34}S$ and $\delta^{13}C$ demonstrated strong covariance throughout the growth axis (Figure 2b,f), suggesting a stepwise enrichment in ^{34}S and ^{13}C after the fourth and eighth serial sections, respectively. A significant relationship between $\delta^{34}S$ and vertebra sections was found for this individual ($F_9 = 23.8$, $R^2 = 0.69$, $P < 0.001$).

3.2 | Incremental sampling of archaeological specimens

Archaeological BFT vertebrae specimens varied between 166 and 242 cm estimated FL and corresponded to post-cranial, abdominal and pre-caudal positions in the vertebral column. Specimens yielded 13 inner and outer centrum samples for $\delta^{13}C$ and $\delta^{15}N$ isotope analysis and 10 inner and outer centrum samples for $\delta^{34}S$ isotope analysis. There was no significant difference between C:N ratios of inner and outer vertebra samples ($P = 0.26$; Supporting Information Figure S1).

In agreement with observations on modern serial sections, $\delta^{13}C$ values were significantly greater at the outer of archaeological vertebrae, by *c.* 1‰ when compared with inner samples ($P < 0.001$; Figure 3a). Overall, this resulted in a mean *c.* 0.5‰ increase in $\delta^{13}C$ values across all outer samples (mean *c.* −15.0 to −14.5‰). Likewise, $\delta^{15}N$ values were significantly greater in outer samples (mean *c.* 7.2‰) compared with inner samples (mean 6.9‰, $P = 0.01$; Supporting Information Figure 3b), yet to a smaller degree than $\delta^{13}C$, reflecting the lower degree of $\delta^{15}N$ variation observed across modern serial sections. $\delta^{34}S$ values were not significantly different between inner and outer vertebrae samples, around a mean distribution of 14‰ (Figure 3c). This mirrored the observation of $\delta^{34}S$ values in modern serial sections where the cross-section sample (containing cancellous bone) presented $\delta^{34}S$ values at approximately the mean of cortical bone serial sections.

4 | DISCUSSION

The results show $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ isotope values can vary significantly throughout the growth axes of BFT vertebrae, probably reflecting the environments and prey foraged by fish over several years prior to capture. Clearly, like elasmobranch vertebrae (Carlisle *et al.*, 2015; Shen *et al.*, 2022), BFT vertebrae do not record a perfect isotopic chronology because juvenile bone collagen and muscle signatures (*e.g.*, *c.* 16‰ $\delta^{13}C$, 5‰ $\delta^{15}N$) reflecting Mediterranean planktonic diets (Rumolo *et al.*, 2020; Sarà & Sarà, 2007) were not measured in serial sections. Therefore, the authors propose, using their age 5 specimen, that a maximum of 4 years of life-history signatures may be retained, whereas early-life signatures are lost due to resorption during bone growth. In support, the degree of significant variation they observed across vertebral centra (*e.g.*, in $\delta^{13}C$) was similar to those published across multiple annuli in the otoliths and fin-spines of tunas (Fraile *et al.*, 2016; Luque *et al.*, 2020), and indeed the recent but few serial-section isotope studies corroborate a theory of >3 year isotopic retention in teleost vertebrae (Kato *et al.*, 2021; Matsubayashi *et al.*, 2017, 2019, 2020). In addition to these studies, the authors show that significant isotopic variation can be observed across archaeological and modern vertebral centra of a highly mobile species inhabiting a wider range of marine habitats than those previously studied in this fashion. This methodology offers an important advance to investigating diet and habitat use dynamics as spatial variation in marine isotopes is highly complex (Guiry, 2019; Magozzi *et al.*, 2017;

Sigman *et al*., 2009), and residency times per life-history stage remain poorly understood for migratory teleosts like BFT (Cermeño *et al*., 2015).

Significant isotopic variation in BFT vertebrae is noteworthy because BFT is supposed to have relatively high rates of bone resorption and remodelling due to its cellular bone, endothermy and rapid growth (Davesne *et al*., 2019). Further study is therefore required to assess resorption and remodelling rates in teleost bone, and its influence on their chemical recording properties. To do this, at least two methods could be employed: a direct comparison of otolith (non-remodelling) and bone isotope values at each age [*e.g*., Matsubayashi *et al*. (2017)], or a controlled feeding experiment with regular sampling of cohorts (bone) throughout life. Until a better understanding of teleost bone remodelling is achieved, studies should be cautious in assuming which years of growth are being represented by isotopic measurements.

Isotopic variation across archaeological and modern vertebral centra reflected an increasing degree of oceanic (higher $\delta^{13}C$), pelagic (higher $\delta^{34}S$) and higher trophic level (higher $\delta^{15}N$) foraging throughout the life history of individuals. This is well supported by knowledge on BFT biology shown by diet and migration studies (Druon *et al*., 2016; Karakulak *et al*., 2009; Logan *et al*., 2011; Walli *et al*., 2009; Wilson & Block, 2009) in addition to isotope values published on multiple age-classes (Estrada *et al*., 2005; Logan, 2009; Medina *et al*., 2015; Rumolo *et al*., 2020; Sarà & Sarà, 2007; Varela *et al*., 2019). How these life-history patterns vary across time and space, however, remains poorly understood, and thus the methodology used herein provides a novel means with which to identify this variation. Modern cross-section $\delta^{13}C$ and $\delta^{15}N$ values were more similar with outer, than inner, serial-section $\delta^{13}C$ and $\delta^{15}N$ values. This may be best explained by cancellous bone, which was present in cross-sections but not serial sections, being more rapidly remodelled compared with cortical bone (Matsubayashi & Tayasu, 2019; Santamaria *et al*., 2018; Turner Tomaszewicz *et al*., 2016). Although it is challenging to draw conclusions on a single sample, a rapid remodelling of cancellous bone might also explain why modern cross-section $\delta^{34}S$ values were approximately the mean of serial-sections $\delta^{34}S$ values if the individual sporadically foraged on neritic or benthic prey during its final period of life. Such dynamic seasonal foraging behaviours are well evidenced in BFT (Battaglia *et al*., 2013; Ólafsdóttir *et al*., 2016). In general, these findings suggest that vertebrae are suitable candidates for life-history signatures; nonetheless, caution should be taken to standardise sampling methodologies to minimise technical effects.

4.1 | Consequences for paleoecological isotopic studies

As has long been the best practice, the authors reiterate that isotope studies must sample teleost vertebrae using methodologies which obtain similar proportions of cortical and/or cancellous bone across growth axes to avoid a minimal (but significant) bias which might result from comparing samples representing different life stages. This bias is likely to be less significant in species with rapid remodelling rates and less variation in habitat and diet use throughout life such as BFT [than *e.g*., salmonids: Matsubayashi *et al*. (2017)]. The authors recommend cross-sectioning bone as achieved herein to obtain roughly equal proportions of bone types and growth layers which ought to largely eliminate concerns that isotopic signatures from different life stages have been sampled. Studies ought to then incorporate size data to assess relationships between isotope values and body size if specimens vary in size and age. As an example, such methodological biases may have resulted in the increases in $\delta^{34}S$ and decreases in $\delta^{13}C$ between 9th-13th centuries and modern samples; and rather than draw conclusions herein, a full-scale independent study is required to assess these. It is important to note that when conclusions are based on a large degree of isotopic variation [*e.g*., Guiry *et al*. (2021)], bias from bone type/remodelling (*c*. ≤1‰ in BFT) will clearly not be an issue.

Although lower resolution is achieved when analysing vertebrae chemistry due to sample quantity requirements than that achieved by, *e.g*., micro-milling calcium carbonate in fin spines and otoliths (Fraile *et al*., 2016; Luque *et al*., 2020; Rooker *et al*., 2008), vertebrae open up the opportunity for study in spatiotemporal contexts where other tissue types are lacking (Andrews *et al*., 2022b; Guiry & Hunt, 2020). In addition, bone protein offers the potential to study additional isotopes (*e.g*., $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$) than calcium carbonate, which also extends to the analysis of individual amino acids [CSIA, *e.g*., Bradley *et al*. (2015)], meaning that in theory, source and trophic-level effects can be explored across the life history of individuals. Nonetheless, due to the sample quantity requirements (usually >0.4 mg collagen), limitations will be placed on which species are large enough to sample, and how ancient specimens can be sampled and used due to sample degradation and institutional regulations on invasive sampling.

Thus far, studies have applied isotopic analysis to archaeological and archived teleost bone to reveal the extinction of a resident trophic niche in Atlantic salmon [*Salmo salar*; Guiry *et al*. (2016)], indicate potential millennial-scale diet shifts in the highly exploited Atlantic cod (*Gadus morhua*; Ólafsdóttir *et al*., 2021) – and Atlantic hake (*Merluccius merluccius*; Llorente-Rodríguez *et al*., 2022) – and suggest potential habitat productivity or usage shifts in Atlantic and Pacific fishes during the last 500 years compared with the previous centuries (Misarti *et al*., 2009; Ólafsdóttir *et al*., 2021). Serial-sectioning of ancient and modern teleost vertebrae has the potential to advance these investigations to reveal changes in life histories, complementing modern tagging studies which is especially important for juveniles which are difficult to tag (Walli *et al*., 2009). Examples of research gaps for BFT that we are confident vertebrae exist to evidence and that may stimulate thought for those studying a variety of systems are:

- Was/is there a resident Mediterranean population? Might there be an isotopic niche that is not only distinct from others but consistent throughout life? *sensu* Cermeño *et al*. (2015) and Di Natale (2015).
- What was unique to BFT that migrated to Atlantic extremes such as Norway and Brazil then disappeared during the 1960s? What

was their population origin, and which isotopic habitats did these individuals explore throughout their life? *sensu* Fromentin *et al*. (2014).

- What is the extent of BFT site fidelity across several years? How much interannual variability is there in habitats and prey foraged, and has this been shaped by industrial-era habitat degradation? *sensu* Andrews *et al*. (2022b).

5 | CONCLUSIONS

Ancient and modern BFT vertebrae appear to retain multi-annual isotopic life-history signatures across their growth axes. These findings are likely reproducible in many commercial and threatened teleosts because the biological features of BFT such as cellular bone, endothermic regulation and rapid growth rates are likely to promote more rapid bone resorption and remodelling than other teleosts. Accordingly, studies should take caution when sampling to avoid interpreting isotopic data from samples which contain varying components of cortical and cancellous bone across the growth axis. The authors recommend that the analysis of cortical-bone, serial-sectioned across vertebrae centra is likely to provide the highest resolution in reconstructing life histories. The ability of vertebrae to record life-history signatures highlights their potential as environmental and ecological archives and suggests that teleost bone chemistry is a powerful tool to investigate the drivers of ecological change to support management and conservation. Nonetheless, further study is required to estimate bone remodelling rates and accurately define the isotopic time-frame being recorded.

AUTHOR CONTRIBUTIONS

A.J.A., D.O. and M.A. designed the study. A.J.A., V.O., P.A. and F.T. collected vertebrae samples for analysis. A.J.A. conducted the laboratory work. A.J.A. and M.A. analysed the data. A.J.A., F.T., D.O. and M.A. wrote the paper.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest exist.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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