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ARTICLE

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Amino acid-specific nitrogen stable isotope analysis reveals the trophic behavior of Icelandic fin whales in winter and suggests variable feeding strategies

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Abstract

Fin whales (*Balaenoptera physalus*) aggregate west of Iceland during summer, where they feed mostly on krill, but their winter distribution is poorly known. This study investigates the winter behavior of fin whales that summer off Iceland by analyzing amino acid-specific nitrogen isotope ratios in the baleen plates. This technique is increasingly used in large marine vertebrates to discriminate between changes in trophic position and changes in isotopic baseline levels, while ruling out the effect of fasting on these patterns. Analyses were conducted on samples extracted from two baleen plate segments: one corresponding to winter, another to summer. The trophic position was higher and slightly more variable in winter (3.6 ± 0.7) than in summer (3.0 ± 0.4), which indicates that during winter fin whales tend to feed on fish and are more generalist in feeding habits. Interestingly, the $\delta^{15}N_{Phe}$ values were not lower in winter than in summer (winter: 5.2‰ ± 2.4‰; summer: 4.0‰ ± 2.1‰). This was unexpected because baseline $\delta^{15}N$ values (e.g., zooplankton) tend to be lower at low

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[†]Deceased

latitudes, where fin whales are believed to spend the winter. Fin whales may remain in relatively high latitudes during winter or visit low-latitude areas with locally higher than average δ^{15}N values, such as upwelling areas.

KEYWORDS
Balaenoptera physalus, baleen plate, compound specific isotope analysis, CSIA-AA, fin whale, migration, trophic position

1 | INTRODUCTION

The fin whale (*Balaenoptera physalus*) is a cosmopolitan species that aggregates during the summer to feed in highly productive areas (Aguilar & García-Vernet, 2018). One of these areas is located between the eastern coast of Greenland and the western and southern coast of Iceland and constitutes one of the feeding grounds with the highest density of fin whales in the North Atlantic (Pike et al., 2019; Víkingsson et al., 2009). There, fin whales feed primarily on krill, mainly *Meganyctiphanes norvegica* (Sigurjónsson & Víkingsson, 1997; Víkingsson, 1997) but their stay extends well into the autumn, when they seem to shift to a capelin (*Mallotus villosus*) based diet (Pike et al., 2019). During winter, most fin whales leave the feeding grounds and migrate to their wintering grounds in low latitudes, although some individuals may remain at high latitudes even during the coldest months (Gunnlaugsson & Víkingsson, 2014). Some attempts have been made to study the migratory behavior of the fin whales in winter using stable isotopes and satellite tags (Gauffier et al., 2020; Lydersen et al., 2020; Silva et al., 2019). However, tagging is often conducted during spring or summer and the short tracking periods recorded do not inform about their location during winter, which remains largely unknown.

The analysis of the stable isotope ratios (bulk nitrogen [δ^{15}N$_{\text{Bulk}}$] and bulk carbon [δ^{13}C$_{\text{Bulk}}$] being those most widely used) is a well-established technique to study migration (Hobson et al., 2010), especially when metabolically inert tissues are used. Thus, while metabolically active tissues continuously exchange nutrients with the blood, and therefore modify their stable isotope values, metabolically inert tissues fix the stable isotope ratios once synthesized (Hobson, 1999). Furthermore, if a metabolically inert tissues grows continuously, it stores a sequential record of the stable isotopes present in the body pool of the individual over some period. In fin whales, such stable isotope sequential record can be found in the baleen plates, where the δ^{15}N$_{\text{Bulk}}$ values often display a characteristic sinusoidal pattern that can be related to the annual cycle of the whales (Aguilar et al., 2014; Best & Schell, 1996; García-Vernet et al., 2018, 2022).

The δ^{15}N$_{\text{Bulk}}$ values in animal tissues largely depend on the isotopic ratios of the environment where the animal lives, i.e., baseline stable isotope ratios (McMahon et al., 2013). However, values are modulated by diverse factors such as the tissue discrimination factor (Borrell et al., 2012; Caut et al., 2009), the trophic position (Post, 2002), and some physiological functions such as reproduction, growth, or fasting (Borrell et al., 2016; Warinner & Tuross, 2010), among others. All these factors intermingle to produce distinct bulk stable isotope signatures for each individual, tissue, and phase of the life cycle. Thus, it is often difficult or even impossible to ascertain the contribution of the various factors to the changes observed in the δ^{15}N$_{\text{Bulk}}$ values in a tissue. In particular, it is difficult to know whether an observed change in the δ^{15}N$_{\text{Bulk}}$ value is due to a change in the baseline isotopic ratios or to a change in diet. Amino acid-specific nitrogen isotope analysis (CSIA-AA) contributes to overcome this limitation.

Amino acid isotope analysis is a technique to determine the nitrogen stable isotope ratios of specific amino acids. The δ^{15}N values of each amino acid is affected differently by the factors mentioned above and these values can be combined to establish the proportion in which each factor affects the stable isotopes of the bulk tissue

(Brault et al., 2019; Matthews & Ferguson, 2013; Riekenberg et al., 2021). Phenylalanine (Phe), for example, is considered a "source" amino acid because it is not transaminated in animal tissues except during the elimination of the molecule. Thus, its $\delta^{15}N$ values (from here denoted as $\delta^{15}N_{phe}$ value) undergo a low trophic enrichment (McClelland & Montoya, 2002; Popp et al., 2007). Other amino acids, such as glutamic acid/glutamine (Glx), proline (Pro) or alanine (Ala), are considered "trophic" amino acids because their stable isotope ratios substantially vary from a trophic level to the next (McClelland & Montoya, 2002; Popp et al., 2007). Taking advantage of these differences, $\delta^{15}N_{phe}$ values can be used as a reference of the baseline $\delta^{15}N$ values, and the difference between the $\delta^{15}N$ values of a trophic amino acid and those of Phe can be used to establish the trophic position of an organism (Chikaraishi et al., 2009; McClelland & Montoya, 2002).

Another confounding factor that can be clarified using CSIA-AA technique is the potential effect of fasting. Fin whales get thinner during winter (Lockyer, 1987b; Víkingsson, 1995), which suggests that the amount of food eaten during this period does not fully cover their metabolic needs. In some populations, the decrease in food intake does not appear to induce a negative nitrogen balance and hence an impact on the $\delta^{15}N_{Bulk}$ values (Aguilar et al., 2014), although it is unclear whether this finding may be extended to all populations, particularly if they undergo more intense fasting. In other capital breeder species, such as southern elephant seals (*Mirounga leonina*), fasting induces a characteristic pattern on the amino acid $\delta^{15}N$ values: while the $\delta^{15}N$ values of aspartic acid/asparagine (Asx), proline (Pro), glycine (Gly) and serine (Ser) increase, $\delta^{15}N$ values of Ala decrease (Lübcker et al., 2020). Lübcker et al. (2020) suggested that this pattern may be a consequence of the fractionation that occurs during the glucose-alanine (Cahill) cycle. It should be noted that the underlying mechanisms to cope with fasting may be different between fin whales and southern elephant seals. However, since the glucose-alanine cycle is common in all vertebrates, a similar pattern should be detected in the baleen plates if the whales require catabolizing protein reserves to meet their energy requirements.

The current study aimed to explain the changes in $\delta^{15}N_{Bulk}$ values in the baleen plate tissue of fin whales occurring off western Iceland and, in particular, to clarify the relationship between the trophic position, the $\delta^{15}N$ baseline value, and fasting. The results obtained provide information on the winter grounds and trophic biology of this fin whale feeding stock and may assist in interpreting future studies using stable isotope analysis on this species and other Balaenopterids.

2 | METHODS

2.1 | Sample collection

No whales were killed for the purpose of these analyses; all samples were collected opportunistically from an ongoing activity outside the research community, in accordance with the *Guideline for Treatment of Marine Mammals* (Society for Marine Mammalogy, 2022). The baleen plates were collected from 15 Icelandic fin whales flensed at the Hvalur H/F whaling station (Hvalfjörður, Iceland) during the summer seasons of 2013, 2015, and 2018. Of these adult whales (17.7–21.3 m in length), 11 were females and four were males (Table S1). Each baleen plate was excised from the middle section of the right jaw at the very proximal end, in contact with the jawbone, to ensure that the whole plate was extracted. After collection and during transport, baleen plates were preserved at −20°C. The baleen plates were transported to the analytical laboratory in Spain under Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permit numbers 15IS017MA, 18ISO36MA, and ESBB00207/15I, ESBB00107/18I.

Once at the laboratory, the "gum" of the plate was removed using a scalpel and the plate surface rinsed using a chloroform/methanol (2:1) solution. Tissue sampling was conducted by scratching the surface of the baleen plate with a mini drill (Dremel 300 series) every 1 cm along the first 40 cm of the plate. Given a baleen growth rate of ~20 cm/year (Aguilar et al., 2014), 40 cm in length is roughly equivalent to 2 years of life of the animals. Subsamples for CSIA-AA analyses were collected in two different segments of the baleen plates: those synthesized in summer, and those synthesized in winter (see below for the procedure to select the sampling segments).

2.2 | Bulk and compound-specific stable isotopes analysis

All samples were analyzed for $\delta^{15}N_{Bulk}$ values at the Scientific and Technological Centre of the University of Barcelona, Spain, following procedures described in Garcia-Vernet et al. (2018). Approximately 0.3 mg of each of the powdered subsamples were weighed in tin capsules and analyzed using a continuous flow isotope ratio mass spectrometer (ThermoFinnigan Flash 1112 elemental analyzer; CE Elantech, Lakewood, NJ), coupled to a Delta C isotope ratio mass spectrometer via a ConFlo III interface (both from ThermoFinnigan, Bremen, Germany).

Nitrogen stable isotope ratios were expressed in delta (δ) notation, with relative variations of stable isotope ratios expressed as per mil (‰) deviations from a predefined international standard (Bond & Hobson, 2012). Thus, nitrogen isotope ratios were reported relative to atmospheric nitrogen (air) through secondary calibrated standards of known $\delta^{15}N$ values, supplied by the International Atomic Energy Agency (IAEA, Vienna, Austria). These materials included $(NH_4)_2SO_4$ (IAEA-N-1, $\delta^{15}N = +0.4‰$ and IAEA-N-2, $\delta^{15}N = +20.3‰$), L-glutamic acid (IAEA USGS-40, $\delta^{15}N = -4.5‰$), and KNO_3 (IAEA-N-3, $\delta^{15}N = +4.7‰$). These isotopic reference materials were assessed once every 12 analyzed samples to recalibrate the system and compensate for any drift in measurements over time. The raw data were normalized by the multipoint normalization method based on linear regression (Skrzypek, 2013). Analytical $\delta^{15}N$ precision for repeated measurements of the reference material, run in parallel with the baleen samples, was 0.05‰.

The results of the $\delta^{15}N_{Bulk}$ showed the classical oscillation pattern caused by the seasonal behavior and location change of this species (e.g., Aguilar et al., 2014; García-Vernet et al., 2018). For collection of the samples for the analyses of the amino acid-specific nitrogen isotope ratios, this oscillation pattern was used to choose, from each plate, one sample corresponding to the baleen plate segment deposited during winter (maximum $\delta^{15}N_{Bulk}$ value) and one corresponding to that of the adjacent summer (minimum $\delta^{15}N_{Bulk}$ value). The decision regarding the identification of the sampling point was based on the decreasing tendency of $\delta^{15}N_{Bulk}$ values shown in the first centimeters, which corresponds with the most recently synthesized tissues (Rita et al., 2019), in most of the baleen plates from this population (e.g., García-Vernet et al., 2018). Additionally, there appears to be an inverse correlation between $\delta^{15}N_{Bulk}$ values from fin whale skin and the day of capture (unpublished data), with minimum $\delta^{15}N_{Bulk}$ values in late summer. Both variation patterns suggest that, in this fin whale population, the $\delta^{15}N_{Bulk}$ values decrease during summer, reaching its minimum at some point during the season, while the opposite occurs during winter.

The 30 samples so collected (one winter and one summer sample from each plate) were analyzed for amino acid-specific nitrogen isotope ratios at the Leibniz Institute for Baltic Sea Research, Warnemünde, Germany. Between 10 and 20 mg of each sample was hydrolyzed with hydrochloric acid, derivatized to trifluoro-acetylated isopropyl amino acid esters (AA-TFA/IP; Hofmann et al., 2003), and cleaned as described by Veuger et al. (2005). The amino acid nitrogen-specific isotope analyses were performed with a Thermo MAT 253 isotope ratio mass spectrometer (IRMS) coupled to a Thermo Trace GC 1310 gas chromatograph (GC) fitted with a 5% phenyl polysilphenylene siloxane nonpolar column (BPX-5, 60 m, 0.32 mm inner diameter, film thickness 1.0 μm; Scientific Glass Engineering Analytical Science, Ringwood, Victoria, Australia). The combustion unit used was a Thermo Isolink fixed at the GC oven and connected to the IRMS via a ConFlo IV interface. A standard mixture of 16 individual AAs was run through the entire analysis in every 10-sample batch. These included alanine (Ala), arginine (Arg), aspartic acid + asparagine (Asx), cysteine (Cys), glutamic acid + glutamine (Glx), glycine (Gly), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), and valine (Val). Since this technique is more prone to measurement errors, each sample was measured between two and five times and the result was only considered valid if the variability among the $\delta^{15}N$ values of an amino acid was below 1.0‰.

2.3 | Baseline value, trophic position, fasting, and trophic enrichment

The $\delta^{15}N$ values of a source amino acid ($\delta^{15}N_{source}$) were used in combination with the $\delta^{15}N$ values of a trophic amino acid ($\delta^{15}N_{trophic}$) to calculate the trophic position (Chikaraishi et al., 2009). As source amino acid we used Phe (McClelland & Montoya, 2002). As trophic amino acid we used Pro. Glx has been used as the trophic amino acid because its trophic discrimination factor has little variability in low trophic position species (Chikaraishi et al., 2009; Nielsen et al., 2015). However, the estimates obtained with this amino acid are not reliable in marine mammals and other top predator species (Lorrain et al., 2009; Matthews et al., 2020; Tachikawa et al., 2014). Pro, on the other hand, has provided more reliable results in other marine mammals (Brault et al., 2019; Hückstädt et al., 2017) because its trophic discrimination appears to be less variable with variations in diet. Trophic position (TP) was estimated using the following equation:

$$\mathrm{TP} = \frac{\delta^{15}\mathrm{N}_{\mathrm{Pro}} - \delta^{15}\mathrm{N}_{\mathrm{Phe}} - \beta}{\mathrm{TDF}_{\mathrm{Pro-Phe}}} + 1$$

where β is the difference between the $\delta^{15}N$ values of the two amino acids in autotrophs ($\beta = 3.1 \pm 1.7‰$; Chikaraishi et al. 2009) and TDF is the trophic discrimination between diet and consumer for Pro, minus the same for Phe ($\mathrm{TDF} = 4.5 \pm 2.2‰$; Brault et al., 2019; supplementary material in McMahon & McCarthy, 2016). The absolute error in TP estimates, calculated by the propagation of error, ranged between 0.0 and 0.2 ($M = 0.1$) due to analytical uncertainties in the $\delta^{15}N$ measurements of Pro and Phe; and it increased to 0.3–1.1 ($M = 0.5$) when systematic uncertainties for β and TDF from literature were also included. Propagation of error was estimated using a Monte Carlo simulation.

To test the accuracy of the trophic positions estimated based on Pro, we collected the results of amino acid $\delta^{15}N$ values analyzed in marine mammals (Bode et al., 2022; Brault et al., 2019; Feddern et al., 2021; Germain et al., 2013; Hückstädt et al., 2017; Matthews et al., 2020; Matthews & Ferguson, 2013). We only used those articles that provided raw data (or at least averages) of $\delta^{15}N_{Pro}$, $\delta^{15}N_{Glx}$, and $\delta^{15}N_{Phe}$ values. Then, we compared the trophic position calculated using $\delta^{15}N_{Pro}$ or $\delta^{15}N_{Glx}$ values with an estimated trophic position. The trophic position used was that provided by the author of the study, or that obtained from Pauly et al. (1998). Because most of the references used by Pauly et al. (1998) were from the 1980s and 1990s, we only used the articles that analyzed samples collected after 1980.

Ideally, only studies in which the diet of animal was certain, such as in control feed studies or studies in well-known population, should be used. Unfortunately, studies of these characteristics are scarce since CSIA analyses typically aim to estimate the trophic position when other options to ascertain species diet are costly or impossible. However, the trophic position provided by Pauly et al. (1998) should be sufficient to provide a first approximation to the accuracy of the trophic position calculated using $\delta^{15}N_{Pro}$ and $\delta^{15}N_{Glx}$ values.

2.4 | Statistical analysis

Normality and homoscedasticity were tested using the Shapiro–Wilk and the Bartlett tests. ANOVA tests were used to compare the differences between summer and winter and between males and females of three response variables: $\delta^{15}N_{Bulk}$, trophic position and $\delta^{15}N_{Phe}$ values. Differences between summer and winter were tested using *t*-test of paired samples in the rest of the amino acids. Due to the small sample size for males (four individuals), caution should be taken when interpreting similarity and dissimilarity between the two sexes. All statistical analyses were carried out using the free software R (R Core Team, 2020) and the software Rstudio (RStudio Team, 2016).

3 | RESULTS

The δ^{15}N values were statistically different between summer and winter in all the amino acids and the bulk values except for phenylalanine, for which differences were, nonetheless, close to the statistical threshold (Figure 1; detailed model results in Table S4). The δ^{15}N values during winter were higher in all the bulk value and in all the amino acids except Thr (Figure 1). On average (± *SD*), $\delta^{15}N_{Ala}$ values were 3.3 ± 2.1‰ higher in winter than in summer. The differences between seasons in $\delta^{15}N_{Ala}$ values were similar to those of the rest of the trophic amino acids (3.3‰ ± 0.4‰), which, in turn, had a larger seasonal difference than the source amino acids (1.7‰ ± 0.4‰; Figure 1). The average trophic position of the studied animals increased from 3.0 ± 0.4 in summer to 3.6 ± 0.7 in winter (Figure 2) ($F(1,27) = 10.2$, $p = .004$). No differences were detected between the trophic position values of males and females ($F(1,27) = 3.21$, $p = .08$). No differences were detected between the $\delta^{15}N_{Bulk}$ values of males and females ($F(1,27) = 1.82$, $p = .18$). Sex, on the other hand, had a statistically significant effect on the $\delta^{15}N_{Phe}$ values ($F(1,27) = 31.37$, $p = .011$). Average $\delta^{15}N_{Phe}$ values were 6.3‰ ± 2.4‰ in males and 4.0‰ ± 2.0‰ in females (detailed results of the nitrogen isotope analysis per individual whales can be found in the Supplementary Material). It is worth noting that the standard deviations were not statistically different in any of the analyzed variables

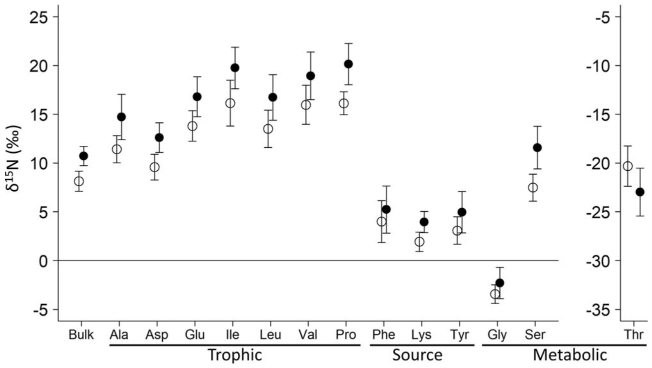

FIGURE 1 Average (*M* ± *SD*) δ^{15}N values in winter (black dots) and summer (empty dots) of bulk nitrogen and in trophic, source, and metabolic amino acids.

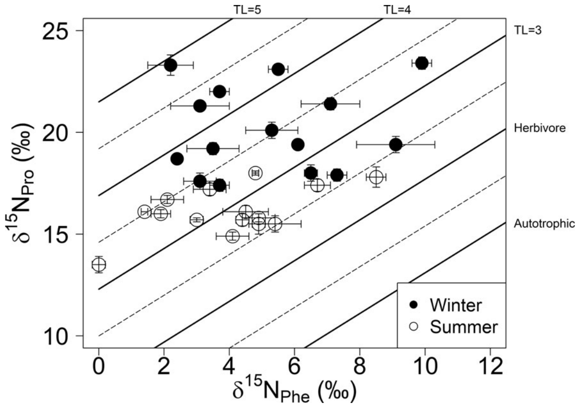

FIGURE 2 Winter (black dots) and summer (empty dots) values of $\delta^{15}N_{Pro}$ and $\delta^{15}N_{Phe}$. The diagonal lines are a visual representation of the trophic position of an organism for any given $\delta^{15}N_{Pro}$ and $\delta^{15}N_{Phe}$ combination.

TABLE 1 Average values (*M* ± *SD*) of $\delta^{15}N_{Phe}$, $\delta^{15}N_{Glx}$, and $\delta^{15}N_{Pro}$, and the estimated ($TP_{literature}$; from the authors or Pauly et al., 1998) and calculated trophic position (TP_{Glx} and TP_{Pro}). Species code stands for crabeater seal (*Lobodon carcinophaga*; LCAR), Weddell seal (*Leptonychotes weddellii*; LWED), Ross seal (*Ommatophoca rossii*; OROS), harbor seal (*Phoca vitulina*; PVIT), killer whales (*Orcinus orca*; OORC), bowhead whale (*Balaena mysticetus*; BMYS), common dolphin (*Delphinus delphis*; DDEL), beluga (*Delphinapterus leucas*; DLEU), sperm whale (*Physeter macrocephalus*; PMAC) and fin whale (*Balaenoptera physalus*; BPHY).

Study	Species	Tissue	$\delta^{15}N_{Phe}$	$\delta^{15}N_{Glx}$	$\delta^{15}N_{Pro}$	$TP_{literature}$	TP_{Glx}	TP_{Pro}
Brault et al., 2019	LCAR	Blood	6.0 ± 0.7	14.8 ± 0.4	15.4 ± 0.6	3.3	1.7 ± 0.1	2.4 ± 0.2
Brault et al., 2019	LCAR	Whisker	4.3 ± 0.3	15.0 ± 0.2	15.9 ± 0.5	3.3	1.9 ± 0.0	2.9 ± 0.1
Brault et al., 2019	LWED	Blood	5.7 ± 0.6	21.7 ± 0.7	20.3 ± 1.3	4.0	2.6 ± 0.0	3.6 ± 0.3
Brault et al., 2019	OROS	Blood	2.6 ± 0.7	26.4 ± 0.5	26.5 ± 0.6	4.1	2.5 ± 0.1	3.5 ± 0.2
Germain et al., 2013	PVIT	Muscle	15.7 ± 3.2	23.3 ± 2.6	27.4 ± 2.7	4.0	1.9 ± 0.2	2.7 ± 0.5
Germain et al., 2013	PVIT	Serum	10.5 ± 1.3	25.2 ± 1.9	25.9 ± 2.8	4.0	2.2 ± 0.3	4.1 ± 0.8
Matthews & Ferguson, 2013	OORC	Collagen	10.2 ± 3.3	18.8 ± 1.4	20.7 ± 1.8	4.5	2.5 ± 0.4	3.8 ± 0.6
Matthews et al., 2020	BMYS	Baleen	6.1 ± 0.1	23.6 ± 0.2	25.7 ± 0.1	3.2	2.2 ± 0.1	3.5 ± 0.0
Matthews et al., 2020	DDEL	Skin	9.9 ± 2.1	28.5 ± 3.7	29.3 ± 2.2	4.2	2.3 ± 0.4	3.8 ± 0.3
Matthews et al., 2020	DLEU	Collagen	10.0 ± 1.0	27.8 ± 0.9	29.0 ± 0.9	4.0	3.0 ± 0.1	4.6 ± 0.2
Matthews et al., 2020	DLEU	Muscle	9.7 ± 1.2	29.0 ± 1.6	32.4 ± 1.9	4.0	2.9 ± 0.2	4.6 ± 0.3
Matthews et al., 2020	DLEU	Skin	9.1 ± 1.3	29.7 ± 1.4	28.2 ± 1.1	4.0	3.2 ± 0.0	5.5 ± 0.1
Matthews et al., 2020	OORC	Collagen	10.5 ± 0.3	27.8 ± 0.4	28.9 ± 0.5	4.3	3.1 ± 0.1	4.3 ± 0.1
Matthews et al., 2020	OORC	Collagen	14.3 ± 1.7	23.3 ± 1.2	21.8 ± 1.6	5.0	2.3 ± 0.1	3.6 ± 0.2
Matthews et al., 2020	PMAC	Collagen	7.8 ± 1.1	25.8 ± 1.1	26.0 ± 0.6	4.4	2.6 ± 0.2	3.4 ± 0.2
Matthews et al., 2020	PMAC	Skin	10.7 ± 1.5	13.8 ± 2.2	16.1 ± 1.5	4.4	2.5 ± 0.3	3.7 ± 0.3
Hückstadt et al., 2017[a]	LWED	Hair	7.1 ± 2.1	21.0 ± 3.9	21.5 ± 5.5	4.0	2.4	3.5
Bode et al., 2022	DDEL	Muscle	6.6 ± 1.2	21.7 ± 1.9	14.2 ± 5.9	4.2	2.6 ± 0.3	2.1 ± 1.3
Feddern et al., 2021	PVIT (Salish)	Bone	12.6 ± 2.4	27.3 ± 1.8	25.9 ± 3.2	4.0	2.5 ± 0.4	3.3 ± 0.6
Feddern et al., 2021	PVIT (WA)	Bone	11.0 ± 2.4	27.3 ± 1.8	25.9 ± 3.2	4.0	2.7 ± 0.2	3.6 ± 0.8
This study	BPHY	Baleen	4.0 ± 2.1	14.8 ± 1.6	15.4 ± 1.2	3.2	1.8 ± 0.3	3.0 ± 0.4

[a] Only average $\delta^{15}N$ values were provided and, therefore, no standard deviation could be calculated for the trophic positions.

(TP, $\delta^{15}N_{Phe}$ values, and $\delta^{15}N_{bulk}$ values), but the trophic position standard deviation was twice as high in winter than in summer.

The results of the literature review are shown in Table 1. Six out of seven scientific articles show that the trophic position inferred using the $\delta^{15}N_{Pro}$ value is more representative of the expected trophic position than that inferred through the $\delta^{15}N_{Glx}$ value. The only exception to this pattern is the common dolphin (*Delphinus delphis*), where the $\delta^{15}N_{Pro}$ values were exceptionally low (in some cases even lower than the $\delta^{15}N_{Phe}$ values; Bode et al., 2022).

4 | DISCUSSION

Tissue $\delta^{15}N_{Bulk}$ values are often used as a proxy of the trophic position of the organism, but this approximation may be misleading. Both the potential change in baseline and the effects of fasting contribute to the bulk values of mammals and may have a particularly significant effect in migrating, capital-breeder species.

Our analyses on fin whale baleen did not show the effects of fasting described in southern elephant seals. In fasting southern elephant seals, the $\delta^{15}N$ values of four amino acids (Gly, Ser, Pro, and Asx) were strongly enriched during fasting, while the $\delta^{15}N$ values of Ala were depleted (Lübcker et al., 2020). Because the metabolic pathway (the glucose-alanine cycle) proposed by the authors to explain their results is common to all vertebrates, fasting would be expected to have a similar effect on baleen whale $\delta^{15}N$ values if the individuals catabolized muscle proteins. However, it is worth noting that Lübcker et al. (2020) only analyzed one species and the proposed hypothesis for their results is yet to be confirmed in other species. However, and pending this validation, we can assume that the fin whales here studied did not mobilize muscle proteins or, if they did, the byproduct of the mobilized proteins did not enter the glucose-alanine cycle. Another less plausible explanation would be that the whales did catabolize muscle protein and that the resulting amino acids were directly routed to the synthesis of baleen tissue without fractionation. However, this is unlikely given the observations of fin whales feeding during winter and far away from their summer feeding grounds (Christensen et al., 1992; Silva et al., 2013; Whooley et al., 2011), as well as the finding that bulk stable isotope ratios in spring are inconsistent with a winter fasting (Aguilar et al., 2014; Gauffier et al., 2020; Silva et al., 2019).

However, several previous studies on the Icelandic fin whale stock have shown appreciable seasonal energy deposition during summer (up to 30% increase body weight), and mobilization of these energy reserves during winter (Lockyer, 1987a,b; Víkingsson, 1995). Since the catabolization of muscle proteins would have been reflected in this and other stable isotope studies, it is proposed that the energy reserves consumed during winter are limited to those stored in the fat tissue, although further research is required to confirm this. Similarly, other cetaceans, such as striped dolphins (*Stenella coeruleoalba*) have been found to go through periods of poor nutritional condition without associated protein mobilization and subsequent $\delta^{15}N$ enrichment (Gómez-Campos et al., 2011). In summary, fasting did not seem to underlie the given changes in $\delta^{15}N_{Bulk}$ values between winter and summer months of the fin whales off Iceland.

The mean of the $\delta^{15}N_{Bulk}$ values was higher in the segments of baleen plates synthetized in winter than in those synthetized in summer. This change was likely caused by an increase in the trophic position exploited. Thus, the trophic position of the summer samples (i.e., 3.0 ± 0.4) was consistent with a diet based on an herbivorous prey, a finding supported by previous studies on the diet of this species carried out through stomach content analysis (Víkingsson, 1997), as well as the application of mixing models based on stable isotope composition (García-Vernet et al., 2021). In particular, the main food item was by large the North Atlantic krill (*Meganyctiphanes norvegica*), which is herbivorous during the spring and summer blooms. However, during winter the mean trophic position of the fin whales increased (3.6 ± 0.7), a finding that might have, at least, two potential explanations. First, fin whales may switch from a krill-based diet to a diet including, or based on, schooling fish. This would be consistent with previous studies reporting a shift of this nature in other North Atlantic fin whale stocks at the end of summer (Christensen et al., 1992; Gavrilchuk et al., 2014; Jonsgård, 1966; Nøttestad et al., 2015; Skern-Mauritzen et al., 2011). Second, fin whales may still be feeding on krill during winter, but the krill may have shifted its diet from a phytoplankton-based diet to an omnivorous or carnivorous one (Schmidt, 2010). In this scenario, the increase in krill trophic position would imply a trophic lengthening of the whole food web based on this species (Post et al., 2000). These two hypotheses are not exclusive, and we believe that the high variability of the trophic position observed in fin whales during winter may be explained by whales adjusting to different feeding strategies.

We must also point out that the variation in trophic level may be even higher than estimated due to a possible change in the trophic discrimination factor correlated with the protein quality of the diet. Thus, when the diet protein content is close to the protein requirements of the predator, the trophic discrimination factor is low (McMahon et al., 2015). Because the potential fish prey have a higher protein content than krill (~15% in capelin; Payne et al., 1999 vs. ~10.5% in krill; Saether & Mohr, 1987), the use of the same trophic discrimination factor may be underestimating the variation in trophic position during winter. The same would be true for the krill, which is mainly herbivorous in winter and becomes mainly carnivorous in winter, therefore shifting from a poor-protein food to a rich-protein food.

Contrary to expectations, $\delta^{15}N_{Phe}$ values during winter were not lower than $\delta^{15}N_{Phe}$ values during summer. Although some fin whales may remain at high latitudes during winter, as shown by the historical catches (Jonsgård, 1966; Víkingsson et al., 2013), the results of sighting cruises (Gunnlaugsson & Víkingsson, 2014), and the detection of fin whale songs in winter in northern latitudes (Ahonen et al., 2017), most of the population is thought to migrate to lower latitudes (Lydersen et al., 2020; Pereira et al., 2020; Silva et al., 2013). In the largely oligotrophic waters of these warmer latitudes, N_2 fixation causes low $\delta^{15}N$ values (0.0‰–6.0‰ in tropical and subtropical zooplankton as opposed to ~8.0‰ in high latitude zooplankton) (McClelland et al., 2003; McMahon et al., 2013). Consequently, and in contrast with the variation shown by our data, we expected $\delta^{15}N_{Phe}$ value to be lower in the winter samples than in the summer samples.

A possible explanation to this apparent contradiction may be that the $\delta^{15}N_{Phe}$ values are known to vary not only with latitude, but they may also increase on a regional scale due to the emergence of deep-water nitrate. The areas subject to such events, which typically are cold eddies and upwelling zones, have typically higher $\delta^{15}N$ values as compared to areas with greater proportion of N_2 fixation. In some extreme cases, denitrification may raise the $\delta^{15}N$ values to remarkably high values, such as it happens in the Benguela upwelling area, where $\delta^{15}N$ values are 5‰ above the global mean (Nagel et al., 2013). We hypothesize that the high variability in the winter $\delta^{15}N_{Phe}$ value is due to the use by fin whales of different strategies during winter, including the exploitation of oligotrophic sites and/or upwelling zones.

Upwelling zones are enriched in deep water nitrate and can support higher primary productivity than those zones that rely on the fixation of molecular nitrogen (McGillicuddy et al., 2003). Although many of these mesoscale features tend to be temporal (Chelton et al., 2011), their effects are long enough to attract high trophic position organisms (Godø et al., 2012), including marine mammals (Bailleul et al., 2010; Baines & Reichelt, 2014; Campagna et al., 2006; Davis et al., 2002; Garcia-Rojas et al., 2018). Fin whales from other stocks have already been associated with these oceanographic features (Baines et al., 2017; Lydersen et al., 2020; Pérez-Jorge et al., 2020) and our results suggest that at least part of the Icelandic fin whales may also use them to feed in winter.

Differences in the $\delta^{15}N_{Phe}$ values between the two sexes are difficult to interpret due to the small sample size in males, and further research is necessary to evaluate whether such differences are indeed real. If the differences were corroborated, a possible explanation might lay on differences in the phenology of male and female migrations because the baseline of nitrogen stable isotope ratios changes during the spring bloom (Tamelander et al., 2009) and such change would be reflected differently if one sex reaches Icelandic waters before the other. However, although sequential migration has been reported in fin whales for different age or reproductive classes (Aguilar & Sanpera, 1982; Lockyer, 1984), no evidence exists that males and females undergo different migratory paths.

Finally, it is worth mentioning that the trophic position calculated using CSIA-AA is strongly dependent on the amino acids and the coefficients used. The most commonly used trophic amino acid is Glx, which provides accurate measurement of trophic position in species situated at a low trophic position but tends to underestimate it in the top-predating marine mammals and birds (Germain et al., 2013; Matthews et al., 2021; McMahon & McCarthy, 2016). Different reasons have been proposed for this bias, such as the excretion mechanisms of the species (Germain et al., 2013) or the protein quality of the diet (Chikaraishi et al., 2015; Hoen et al., 2014; McMahon et al., 2015).

Whatever the case, Pro appears to be less affected by these factors and, thus, may be more reliable (Brault et al., 2019). With the only exception of the data from Bode et al. (2022), which showed exceptionally low $\delta^{15}N_{Pro}$ values, the marine mammal data previously published indicated that $\delta^{15}N_{Pro}$ values provided trophic positions close to those expected. However, it should be noted that the expected trophic position may not be the actual trophic position of that population, although it provides a first approximation to interpret results. The trophic position calculated using $\delta^{15}N_{Pro}$ values were, in general, still under the expected value, but the error was within one unit except for the common dolphin from Bode et al. (2022), and the marine mammal-eater killer whales. Furthermore, Germain et al. (2013), the only study to date that calculated under diet-controlled conditions the amino acid specific TDF in a marine mammal, showed a Pro TDF of 4.7‰ ± 2.8‰ in serum and 4.3‰ ± 2.4‰ in muscle, similar to the one used in this study. Thus, these results support the use of Pro over Glx as a trophic amino acid for trophic position calculation, although further research is needed to establish the accuracy of such calculations.

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AUTHOR CONTRIBUTIONS

Diego Rita: Formal analysis; investigation; writing – original draft; writing – review and editing. **Asunción Borrell:** Conceptualization; funding acquisition; resources; writing – review and editing. **Dirk Wodarg:** Formal analysis; methodology; writing – review and editing. **Gísli Víkingsson:** Resources; writing – review and editing. **Raquel García-Vernet:** Formal analysis; writing – review and editing. **Alex Aguilar:** Conceptualization; funding acquisition; resources; writing – review and editing. **Natalie Loick-Wilde:** Funding acquisition; methodology; resources; writing – review and editing.

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SUPPORTING INFORMATION

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