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3 **Discrimination of stable isotopes in fin whale tissues and application to**
4 **diet assessment in cetaceans**
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7 Short title: Diet-tissue discrimination factors in fin whale
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ABSTRACT

RATIONALE: In stable isotopes research, the use of accurate, species-specific diet-tissue discrimination factors (i.e., $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$) is central to estimate trophic position relative to primary consumers and to identify the dietary sources of an individual. Previous research suggested that the diet of fin whales from the waters off northwestern Spain is overwhelmingly based on krill, a trait that permits reliable calculation of discrimination values in this wild population.

METHODS: After confirming that stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in muscle from 65 aged fin whales remained constant through age classes (4-65 years), we analyzed signatures in muscle, bone protein, skin, liver, kidney, baleen plates and brain, as well as food (krill), from a subset of individuals to calculate discrimination factors. Signatures were determined by means of elemental analysis isotope ratio mass spectrometry (EA-IRMS) using a Thermo-Finnigan Flash 1112.

RESULTS: Isotopic values remained constant regardless of age. The mean $\Delta^{15}\text{N}$ values between krill and whale tissues ranged from 2.04 in bone protein to 4.27‰ in brain, and those of $\Delta^{13}\text{C}$ ranged from 1.28 in skin to 3.11‰ in bone protein. This variation was consistent with that found in other groups of mammals, and is attributed to variation in tissue composition and physiology.

CONCLUSIONS: Because discrimination factors are relatively constant between taxonomically close species, the results here obtained may be reliably extrapolated to other cetaceans to improve dietary reconstructions. The skin discrimination factors are of particular relevance to monitoring diet through biopsies or other non-destructive sampling methods. The large difference of bone protein discrimination factors when compared to other tissues should be taken into consideration when bone collagen is used to determine trophic level or to assess diet in paleodietary isotopic reconstructions.

Key words: $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, diet, cetacean, *Balaenoptera physalus*, Spain,

INTRODUCTION

Stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) have been widely used to study the diet, trophic interactions, migratory patterns, and habitat use of mammals and other vertebrates. To a lesser extent, stable isotopes have also been used to investigate physiological processes, such as nutritional stress and those associated with reproductive biology.^[1]

It is possible to use stable isotopes to study diet, and infer trophic relationships among species, because the isotopic composition of animal tissues is directly related to the isotopes present in their food resources.^[2, 3] Typically, the $\delta^{15}\text{N}$ signature increases between 2‰ and 4‰ at each trophic level, usually varying among tissues and species.^[4,5] In comparison, $\delta^{13}\text{C}$ values show little or no consistent variation with increasing trophic level;^[6] however, this value is primarily related to ecological divisions within aquatic systems. For instance, inshore, benthic, and coastal sources tend to be ^{13}C enriched (higher $\delta^{13}\text{C}$) compared to offshore or pelagic sources.^[7,8,9]

Discrimination factors between the consumer and food source ($\Delta^{3}\text{C}$ and $\Delta^{15}\text{N}$; also termed fractionation factors) are calculated as the differences between the isotopic signal of consumer tissue and those of its food source.^[10, 11] Caut et al.^[5] summarized the three main causes of variation in the magnitude of the discrimination: 1) diet composition: lipids, proteins, carbohydrates, and the different forms in which each of these components may occur in diet are metabolized in different ways, thus affecting isotope discrimination; 2) tissue type: the composition of different consumer tissues determines differential isotopic routing and assimilation; 3) taxon: different species present different discrimination factors, due to variation in excreted nitrogen and metabolic rate among other species-specific variables.

The use of accurate, species-specific diet-tissue discrimination factors is one of the most important basic requirements when applying stable isotope mixing models to predict the dietary sources of a consumer^[11]. In addition, they are also required to estimate species trophic position relative to primary consumers^[11]. Current knowledge about patterns of isotopic discrimination ($\Delta^{3}\text{C}$ and $\Delta^{15}\text{N}$) on marine mammals, particularly cetaceans, remains limited.^[12] $\Delta^{3}\text{C}$ and $\Delta^{15}\text{N}$ are known for certain species (some pinnipeds and odontocetes) that are able to live in captivity, with values being supported by experimental feeding using a controlled and constant diet during the required period for tissues to acquire the new isotopic signal. This period, often termed “turn over,” may range from days to months or years, depending on the metabolic activity of the tissues that are involved.^[5,12] To our knowledge, less than 10 studies have been carried on diet-tissue discrimination in marine mammals,^[11] with only one of these on cetaceans^[13] and none on whales. Furthermore, these studies were primarily mainly conducted on a few tissues, such as fur, vibrissa, or blood components.

The diet of fin whales (*Balaenoptera physalus*) inhabiting the Atlantic ocean off northwestern Spain is known to be primarily dependent on the euphausiid krill, *Meganyctiphanes norvegica* (hereafter krill).^[14,15] Moreover, this species was exploited in their foraging area for over 30 years (1951-1985) by three whaling factories located in Galicia, on the northwestern coast of Spain.^[16] During the 1980s, we sampled captured whales, and examined their stomach

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5 contents, which exclusively contained krill.^[17] Hence, because the diet of this
6 population appeared stable, we considered it an ideal species to study isotopic
7 discrimination between its tissues and dietary source in a wild population.

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9 The objectives of the present study were to: 1) demonstrate the absence
10 of ontogenetic variation in the food resources of fin whales, by analyzing
11 differences in the isotopic signatures of their tissues related to age, and 2)
12 determine the stable isotope discrimination of different fin whale tissues, to
13 obtain valid values that could be applied to isotopic dietary studies of other
14 taxonomically close species.

15 16 17 **MATERIALS AND METHODS**

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19 During the 1983–1985 whaling seasons, samples of fin whales were
20 obtained at the Caneliñas whaling station, which is situated on the northwestern
21 coast of Spain.

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23 Muscle samples, from the region posterior to the dorsal fin, and ear-plug
24 cores were collected from 65 individuals. The ear-plug samples were preserved
25 in 10% formaldehyde until being used to determine the age of whales in the
26 laboratory. Muscle samples, and other tissue samples, were wrapped in
27 aluminum foil, and preserved at -20 °C until analysis.

28
29 In addition, skin, bone, liver, kidney, muscle, and brain samples were
30 collected from 11 individuals (7 males and 4 females) (Table 1). Furthermore, 5
31 baleen plates and 10 krill samples from stomach content were collected from
32 other 15 fin whales in 1985.

33 34 35 **Age determination**

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37 Age was determined by counting the growth layers present on a
38 longitudinal section of the ear-plug core, according to the procedure described
39 by Aguilar and Lockyer.^[18] Each plug was assessed by more than one
40 researcher, with approximately 70% of plugs being read twice by the same
41 researcher. Where different values were obtained in multiple readings, the
42 average of all age estimates was used, unless the difference between the
43 readings was greater than 10% of the lowest reading, in which case the
44 specimen was not used in subsequent calculations.

45 46 47 **Stable isotope analyses**

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49 Approximately 1 g of tissue (except for bone) was subsampled from each
50 sample, dried for 48 h at 60 °C, and then ground to a powder with a mortar and
51 pestle. Since lipids may bias the analyses by decreasing $\delta^{13}\text{C}$ levels^[2], they
52 were removed from the samples sequentially soaking samples in a
53 chloroform:methanol (2:1) solution, and shaking them with a rotator to
54 accelerate lipid content extraction.

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56 Bone was separated into two subsamples. One subsample was treated
57 with a 0.5 M HCl solution, to eliminate inorganic carbonates and obtain protein
58 isotope signatures.^[19, 20] The second subsample was analyzed without HCl
59 treatment, to prevent alterations in nitrogen signatures. However, comparison
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3 between methodologies showed that isotope ratios did not differ between
4 demineralized and untreated samples.

5 Approximately 0.5 mg of powdered samples was weighed into tin
6 capsules, automatically-loaded and combusted at 1000°C to be analysed in a
7 continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C
8 Series EA Thermo Finnigan). Analyses were performed at the Scientific-
9 Technical Services of the University of Barcelona.

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11 The abundance of stable isotope was expressed in delta (δ) notation, while
12 the relative variations of stable isotope ratios were expressed as permil (‰)
13 deviations from the predefined international standards as:

$$14 \quad \delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000,$$

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16 where X is ^{13}C or ^{15}N , and R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$
17 ratios in the sample and standard, respectively. The standards used were
18 Vienna Pee Dee Belemnite (V-PDB) calcium carbonate for ^{13}C and atmospheric
19 nitrogen (air) for ^{15}N . International secondary standards provided by the
20 International Atomic Energy Agency (IAEA) were inserted after every 12
21 samples, to calibrate the system and compensate for any drift over time.
22 Precision and accuracy for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements were 0.1‰ and 0.3‰,
23 respectively.
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28 **Calculation of diet-tissue discrimination**

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30 Diet-tissue discrimination denotes the difference in isotopic composition
31 between a consumer and its diet. We calculated the diet-tissue discrimination
32 factor as:

$$33 \quad \Delta X_{\text{tissue-diet}} = \delta X_{\text{tissue}} - \delta X_{\text{krill}}$$

34
35 where X is ^{13}C or ^{15}N . The notation $\Delta X_{\text{tissue-diet}}$ was abbreviated to ΔX in
36 this study. Because consumers are typically enriched in the heavy isotope
37 relative to diet, such discrimination values are generally positive, with some
38 exceptions in $\Delta^{13}\text{C}$, probably due to rich lipid diets.^[21]
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42 **Data analyses**

43 The data were first checked for the occurrence of possible outliers. Out of
44 142 datapoints in total, three $\delta^{15}\text{N}$ values (those of bone protein from whale 1
45 and of brain and skin from whale 4; table 1) were treated as outliers, which
46 comprised values differing by at least three standard deviations from the overall
47 tissue mean. These values were excluded from the analysis.
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49 The normality and homoscedasticity of the data were tested using
50 Kosmogorov-Smirnov and Levene's tests, respectively. All groups followed a
51 normal distribution, and presented homogeneity of variances.
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53 Regression analyses were performed to assess the relationship between
54 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and age.
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56 GLMs (general linear models) with individual identity (individual as a
57 random factor) and tissue as a fixed factor were used to test differences in
58 discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$) among tissues. Following a significant
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3 fixed effect, differences between tissue types were analyzed by a Post Hoc
4 Tukey's pairwise comparisons test. All statistical calculations were carried out
5 using the statistical package SPSS15 (*SPSS Inc.*).
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8 **RESULTS**

9 **Age-related variations in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values**

10 The estimated age range based on ear-plug readings was 4 to 65 years.
11 The $\delta^{15}\text{N}$ values in fin whale muscle ranged from 8.15‰ to 11.03‰, while the
12 $\delta^{13}\text{C}$ values ranged from -17.43‰ to -19.29‰.
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15 No relationship between $\delta^{15}\text{N}$ ($R^2 = 0.060$, $p = 0.076$) or $\delta^{13}\text{C}$ ($R^2 = 0.001$,
16 $p = 0.850$) values and the age of the individuals was observed (Fig. 1).
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19 **Discrimination between fin whale tissues and krill**

20 The $\delta^{15}\text{N}$ values of fin whale ranged from 8‰ in bone protein to 11.9‰ in
21 the brain, whereas the $\delta^{13}\text{C}$ values ranged from -19‰ in muscle and liver to -16
22 ‰ in bone protein.
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25 As krill was identified as the sole diet of fin whale, diet-tissue
26 discrimination was always calculated relative to the delta values of krill. The
27 $\delta^{15}\text{N}$ values in krill ranged from 5.12‰ to 8.48‰ ($n=10$, $\text{mean}\pm\text{SD}$: $7.15 \pm$
28 1.10%), while the $\delta^{13}\text{C}$ values ranged from -18.56‰ to -20.02‰ ($n=10$,
29 $\text{mean}\pm\text{SD}$: $-19.57 \pm 0.47\%$). $\Delta^{15}\text{N}$ ranged from 0.85‰ in bone protein to 4.75
30 ‰ in brain, whereas $\Delta^{13}\text{C}$ ranged from 0.57 ‰ in muscle and liver to 3.57 ‰ in
31 bone protein (Fig. 2). Table 2 presents the mean stable isotope and diet-tissue
32 discrimination values for each tissue type.
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35 GLMs indicated that both tissue ($p<0.001$) and individual ($p<0.001$)
36 affected $\Delta^{15}\text{N}$, with a non-significant interaction term ($p=0.122$). Figure 2 shows
37 the relationship of each discrimination factor per individual and tissue. $\Delta^{15}\text{N}$ in
38 fin whale was highly different among tissues, ranking brain > liver > kidney >
39 skin > baleen > muscle > bone protein (Table 2, Fig. 2). Pairwise differences
40 between tissues are presented in Table 3.
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42 GLMs indicated that both tissue ($p<0.001$) and individual ($p<0.01$) affected
43 $\Delta^{13}\text{C}$, with a non-significant interaction term ($p=0.292$). $\Delta^{13}\text{C}$ values in fin whale
44 ranked: bone protein > baleen > brain > liver > kidney > muscle > skin (Table 2,
45 Fig. 2). Pairwise differences between tissues are presented in Table 3. $\Delta^{13}\text{C}$ in
46 bone protein was significantly higher compared to all other tissues. Skin and
47 muscle showed the lowest levels of $\Delta^{13}\text{C}$, which differed to the brain and
48 baleen. Those of liver and kidney only differed to bone protein.
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51 **DISCUSSION**

52 As anticipated, the stable isotope ratios of muscle did not change with age
53 for animals older than 4 years, thus indicating no ontogenic dietary change in fin
54 whales after that age. We could not evaluate younger individuals because
55 yearlings and calves were not caught in commercial whaling operations, as a
56 protocol to minimize threat to the population. In theory, calves should present
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3 higher $\delta^{15}\text{N}$ values compared to older age groups, due to their receiving milk
4 nourishment from their mothers, which is typical for most mammals.^[22–25]

5 Differences in isotopic signatures related to age have been documented
6 for several marine mammals, including pinnipeds,^[26, 27] odontocetes,^[28] and
7 even baleen whales.^[29, 30] These differences have generally been attributed to
8 ontogenetic dietary shifts due to variations in feeding area or in habitat use, or in
9 the nutritional requirements of the individual throughout its lifespan. The lack of
10 ontogenetic variations in $\delta^{13}\text{C}$ values reinforces that fin whales inhabiting the
11 Atlantic ocean off northwestern Spain consistently aggregate at the same
12 summer foraging grounds, as previously suggested.^[15] However, the larger
13 $\delta^{13}\text{C}$ variability in whales younger than 15 years relative to that of older whales
14 may reflect a wider geographical dispersion of younger whales in the feeding
15 grounds.
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18 Similarly, the consistency of $\delta^{15}\text{N}$ values with age shows that fin whales
19 forage on the same prey throughout their life span, in agreement with stomach
20 content analysis^[31, 32] and observations of feeding aggregations.^[33]

21 Moreover, the $\delta^{15}\text{N}$ signature in krill ($7.15\text{‰} \pm 1.10$) showed that krill is
22 located one trophic level below fin whales (mean of all tissues $10.27\text{‰} \pm 0.9$).
23 This result verifies that krill is the major dietary source for fin whales, which
24 supports existing of stomach contents and diet studies of these animals.^[14, 31–33]
25 Thus, the results obtained in the present study reflect a diet-tissue isotope
26 enrichment of 2–4 ‰ in $\delta^{15}\text{N}$ values and of 1–3‰ in $\delta^{13}\text{C}$ values (Table 2).
27 These ranges are similar to estimates of overall trophic-level enrichment in
28 aquatic and terrestrial food webs.^[2, 3, 4, 6, 8, 22, 34, 35]
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31 Isotope discrimination between consumer tissues and its dietary source
32 has been extensively used to reconstruct diets. However, the absence of
33 studies quantifying factors that might potentially alter these values has resulted
34 in researchers generally using fixed global mean discrimination factors, which
35 are obtained from published reviews that mix different tissues and consumer
36 classes. The use of such datasets potentially cause biased results in dietary
37 studies^[5, 10] that might be easily resolved using more adequate discriminating
38 factors, depending on the species or tissue.
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41 Caut et al.^[5] reviewed available isotope discrimination factors in different
42 species, and analyzed the causes that influence this discrimination. The authors
43 found that tissue type and consistency, diet, and consumer taxonomic group
44 were the most important factors that contributed to its variation. In the current
45 study, we focused on discrimination differences among fin whale tissues. Other
46 factors may also influence the magnitude of tissue-diet discrimination, such as
47 protein quantity or quality in the diet, form of nitrogen excretion, or other
48 species-specific differences. However, these factors did not affect the results
49 and interpretations of the present study, because they remained constant as we
50 used tissues of just one species that had a stable dietary source (i.e., krill).
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52 Previous studies have identified that differences in diet-tissue
53 discrimination may arise due to the consistency (or nature) of consumer tissue
54 being evaluated.^[5, 10, 12, 13, 36–38] Tissue nature is primarily the biochemical
55 composition of proteins and lipids, along with metabolic properties. Generally,
56 lipids are ^{13}C depleted,^[2, 39, 40] and tissues rich in fat content have lower $\delta^{13}\text{C}$
57 discrimination compared to those with lower fat content. To avoid the influence
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3 of lipids in $\delta^{13}\text{C}$ values, we extracted lipid from tissues, following general
4 recommendations.^[41] However, differences in the protein composition (i.e.,
5 different proteins contain distinct proportions of amino acids) and metabolic
6 routing of dietary components among tissue types may yield dissimilar isotopic
7 compositions, irrespective of other factors.^[10,11] For example, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
8 values of different amino acids in a single tissue may vary by more than 15%.
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10 [42]

11 The tissue-diet discrimination values found in fin whale revealed
12 consistent differences among tissues, which were comparable to those reported
13 for other mammals. The results of a review by Caut et al.^[5] showed that mean
14 tissue $\Delta^{15}\text{N}$ values in mammals ranked: plasma (3.8‰) > entire blood (3.6‰) >
15 liver (3.3‰) > muscle (2.7‰) > hair (2.5‰) > red blood cells (2.1‰). The
16 coincident tissues analyzed in the current study were liver and muscle, which
17 ranked similarly to that showed previously; liver (3.9‰) > muscle (2.7‰).
18 Unfortunately, we did not collect fin whale hair (because whales have very little)
19 or blood for comparison. However, Hobson et al.^[12] found that the red blood
20 cells of seals was the tissue with lowest $\Delta^{15}\text{N}$ (1.7‰). They suggested that this
21 low value might be the result of the high hemoglobin and hematocrit levels in
22 the blood of diving mammals. Furthermore, $\Delta^{15}\text{N}$ in bottlenose dolphin (*Tursiops*
23 *truncatus*) and in killer whale (*Orcinus orca*) red blood cells showed lower levels
24 (0.7‰ and 1.4‰ respectively) compared to plasma (2.6‰ and 2.3‰
25 respectively).^[13] To our knowledge, besides these two species and associated
26 tissue samples, other discrimination factors are not available for cetaceans.
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29 In the current study, fin whale bone protein $\Delta^{15}\text{N}$ (2.03‰) values were
30 significantly lower as compared to all other tissues. They were twofold lower
31 compared to the brain (4.27‰). Skin, baleen, and muscle ($\Delta^{15}\text{N} \approx 2.8\%$) showed
32 very similar $\Delta^{15}\text{N}$ values, all of which were higher than bone protein. The
33 similarity in both discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$) between skin and
34 muscle indicates that skin may serve as an adequate tissue in place of muscle
35 in studies that use stable isotopes. This is particularly important when following
36 non-destructive approaches to investigate feeding ecology. However, the utility
37 of skin should be evaluated by comparing the stable isotope ratios between skin
38 and muscle in other cetacean species (even though discriminations have not
39 yet been calculated). For instance, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values are similar between
40 muscle and epidermis in beluga whales (*Delphinapterus leucas*); however, $\delta^{15}\text{N}$
41 is significantly higher in the skin of bowhead (*Balaena mysticetus*) and gray
42 whales (*Eschrichtius robustus*) compared to muscle, whereas $\delta^{13}\text{C}$ was similar
43 for the two tissue types in both species,^[30] and for humpback whales
44 (*Megaptera novaeangliae*).^[43]
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48 Fin whale brain exhibited the highest estimate of $\Delta^{15}\text{N}$ (4.51‰). This value
49 was similar to that (4.78‰) reported by Vanderklift and Ponsard^[38] for mice and
50 rats. This high value is possibly due to the nature of the cerebral proteins. Liver
51 and kidney had the next highest values, and were very similar to one another
52 (Table 3). In mammals, $\Delta^{15}\text{N}$ in liver is often higher than in other tissues^[5, 12, 38],
53 which has been attributed to its high metabolic activity. Kidney is not usually
54 analyzed in mammals; therefore, there are no comparative references available.
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56 Although the majority of fin whale tissues showed only minor $\Delta^{13}\text{C}$
57 enrichment (1–2‰) compared to the dietary source (krill), differences among
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3 tissues were important. Bone protein had significantly higher values compared
4 to all other tissues (Table 3). This parameter was twofold higher (3.11‰)
5 compared to most tissue types, except brain (2.22‰) and baleen (2.26‰).
6 These values support those found in other mammals.^[5] Koch^[1] explained that
7 the high $\Delta^{13}\text{C}$ discrimination in collagen (principal bone protein) is due to its
8 distinctive amino acid composition, i.e. collagen contains 33% glycine, a ^{13}C
9 enriched aminoacid (+8‰) which implies it to be enriched relative to other
10 tissues ^[44]. The large variation between bone protein and other tissues with
11 respect to $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ should also be considered in paleodietary isotopic
12 reconstructions, for which bone is often used.^[19]
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15 Aside from collagen, the integument generally shows the highest $\Delta^{13}\text{C}$
16 relative to other tissues in mammals.^[2, 12, 45–47] Hair, whiskers, nails, skin and
17 baleen are all keratinized tissues that have similar biochemical composition.
18 Hobson et al.^[12] found that some of these tissue types in seals have relatively
19 high $\Delta^{13}\text{C}$ (2.8–3.2‰). Similarly, Tieszen et al.^[45] reported that hair had the
20 highest $\Delta^{13}\text{C}$ among tissues examined in laboratory gerbils raised on a
21 monotonous diet.
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24 In the current study, baleen plates had a significantly higher $\Delta^{13}\text{C}$ value
25 compared to muscle, based on biochemical composition. However, a much
26 lower $\Delta^{13}\text{C}$ value (1.28‰) in skin was recorded than that expected, compared to
27 that reported for keratinized tissues by Hobson et al.^[12]. The $\Delta^{13}\text{C}$ value of
28 whale skin was similar to that of muscle, kidney, and liver (Table 3). Observed
29 differences in skin $\Delta^{13}\text{C}$ between cetaceans and other mammals may be due to
30 large differences in skin composition. Some previous data supports these
31 results. For instance, Horstmann-Dehn et al.^[30] found no differences in the $\delta^{13}\text{C}$
32 values of muscle and skin of three Arctic cetaceans (i.e., bowhead, gray, and
33 beluga whales). Similarly, Todd et al.^[43] found no significant difference in the
34 $\delta^{13}\text{C}$ values for the muscle and epidermis of humpback whales (*Megaptera*
35 *novaeangliae*).
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38 Therefore, additional studies are needed to discern whether the low $\Delta^{13}\text{C}$
39 value of cetacean skin is a generalized result. The acquisition of such
40 information is important because skin can be easily obtained of free ranging
41 wild animals through biopsy sampling, a non-destructive technique.^[44]
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43 CONCLUSIONS

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45 Given that discrimination factors are relatively constant between
46 taxonomically close species, the results of the current study on fin whales may
47 be extrapolated to other cetaceans to improve dietary reconstructions. Attention
48 should be focused on the discrimination factors of skin (not previously
49 available), which are of particular relevance towards monitoring diet through
50 non-destructive biopsy sampling techniques. Furthermore, the large difference
51 of bone protein discrimination factors as compared to other tissues should be
52 taken into consideration when bone collagen is used to determine trophic level
53 or to assess diet in paleodietary isotopic reconstructions.
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TABLES:

Table 1. Biological variables and sampled tissues of fin whales (n = 11)

Code	Sex	Length (m)	Skin	Bone	Liver	Kidney	Muscle	Brain
1	male	19.5	*	*	*	*	*	*
2	female	18.9		*	*	*	*	
3	male	19.0	*	*	*	*	*	
4	male	18.0	*	*	*	*	*	*
5	male	15.0	*	*	*	*	*	*
6	female	17.1	*		*	*	*	
7	male	17.8	*	*	*	*	*	*
8	male	19.3	*	*	*		*	*
9	male	16.6	*	*	*	*	*	
10	female	18.7	*	*	*	*	*	*
11	female	18.5		*	*	*	*	*

Table 2. Stable isotope values for each tissue and krill-tissue fractionation factor (Δ) (mean \pm SD)

Tissue	n	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\Delta^{15}\text{N}$	$\Delta^{13}\text{C}$
Skin	8/9	9.97 \pm 0.30	-18.29 \pm 0.38	2.82 \pm 0.30	1.28 \pm 0.38
Muscle	11	9.88 \pm 0.58	-18.27 \pm 0.56	2.73 \pm 0.58	1.29 \pm 0.56
Kidney	10	10.63 \pm 0.85	-18.04 \pm 0.42	3.48 \pm 0.85	1.53 \pm 0.42
Bone protein	9/10	9.19 \pm 0.71	-16.46 \pm 0.47	2.03 \pm 0.71	3.11 \pm 0.27
Liver	11	11.09 \pm 0.42	-17.88 \pm 0.61	3.94 \pm 0.42	1.69 \pm 0.61
Brain	5/6	11.42 \pm 0.36	-17.35 \pm 0.51	4.27 \pm 0.36	2.22 \pm 0.51
Baleen	5	9.92 \pm 0.22	-17.31 \pm 0.30	2.77 \pm 0.22	2.26 \pm 0.30

Table 3. Statistical differences in $\Delta^{15}\text{N}$ values (above the divide) and $\Delta^{13}\text{C}$ values (below the divide) between tissues (n.s. non significant).

$\Delta^{15}\text{N} / \Delta^{13}\text{C}$	Baleen	Skin	Liver	Bone protein	Kidney	Muscle	Brain
Baleen		n.s.	p<0.01	p<0.05	p<0.05	n.s.	p<0.01
Skin	p<0.05		p<0.01	p<0.05	p<0.05	n.s.	p<0.01
Liver	n.s.	n.s.		p<0.001	n.s.	p<0.01	n.s.
Bone protein	p<0.05	p<0.001	p<0.01		p<0.001	p<0.05	p<0.001
Kidney	n.s.	n.s.	n.s.	p<0.01		p<0.05	p<0.05
Muscle	p<0.05	n.s.	n.s.	p<0.001	n.s.		p<0.01
Brain	n.s.	p<0.05	n.s.	p<0.05	n.s.	p<0.05	

Figure Legends

Figure 1. Relationship in the age of male (n = 55) and female (n = 10) individuals with (a) $\delta^{15}\text{N}$ (‰) and (b) $\delta^{13}\text{C}$ (‰) values in muscle.

Figure 2. (a) $\delta^{15}\text{N}$ (‰) and (b) $\delta^{13}\text{C}$ (‰) fractionation factors plotted against tissue of the sampled fin whales (n = 11). Each individual is represented by a different colour.

Figure 1

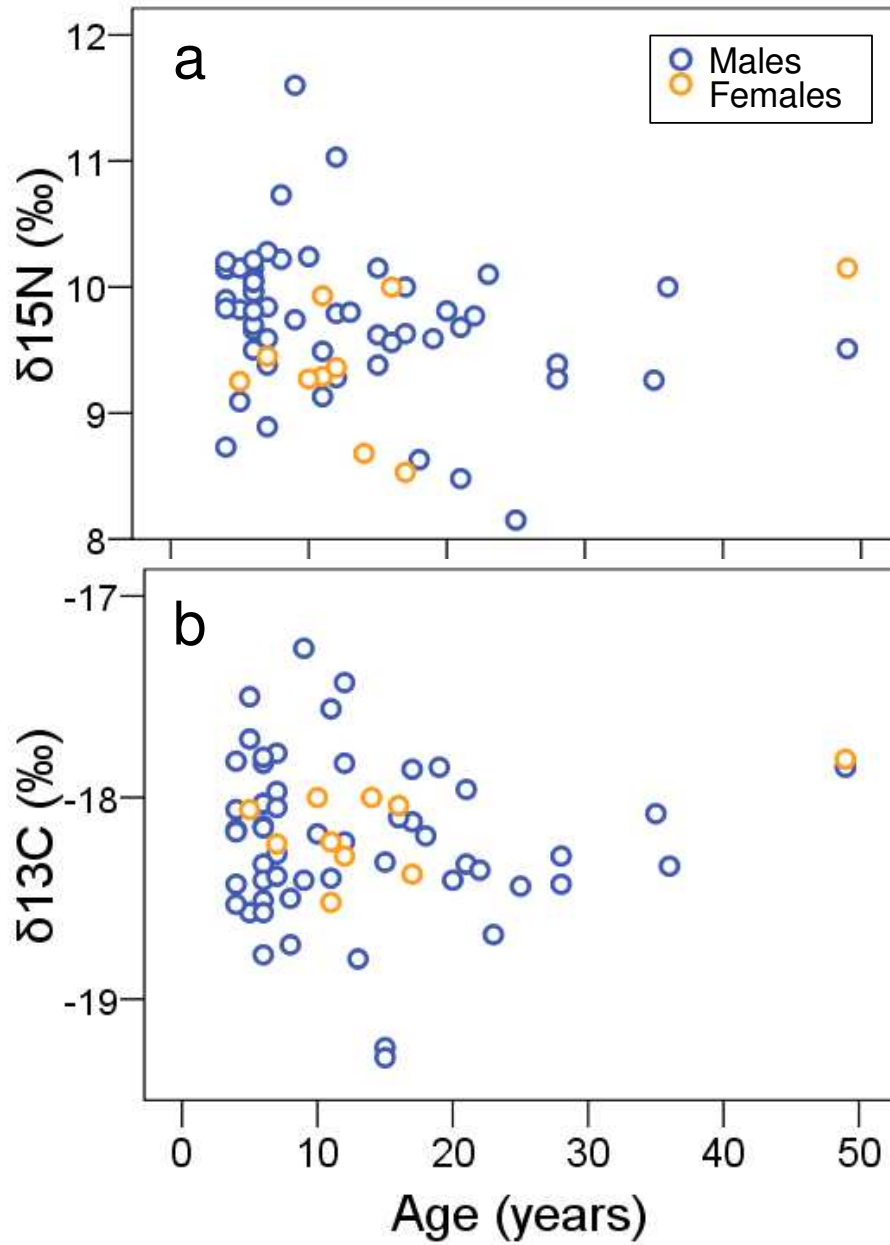


Figure 2

