Table S1. Details of pilot whale frozen-stored samples used in the study, with samples ordered by year of sampling. "Stranding #" is the stranding event number where the sample originated from. "Mass (Yes or No)" indicates if that stranding was a mass stranding or not (see mass stranding definition in Materials and Methods). Sample ID refers to the sample identification code in the strandings database of the Marine and Freshwater Research Institute (Reykjavík, Iceland). Sex is given as Male (M) or Female (F). Age class is defined based on length and sex and $\delta^{13}C$ values have been corrected for the Suess effect (see Materials and Methods).

*****Pregnant female

Text S1. Effects of ethanol on δ^{13} C and δ^{15} N values

Different forms of preservation such as dimethyl sulfoxide (DMSO) saturated with sodium chloride (NaCl), ethanol, freezing or immediate freeze drying are used to store samples according to research focus (e.g., genetic analyses) or logistical constraints in the field. Preservatives may, however, add biases to the stable isotope measurements obtained and, thus, should be accounted for (Lesage et al. 2010, Kiszka et al. 2014). The effects of storage in ethanol on stable isotope data from different species and tissues are inconsistent (e.g., Sweeting et al. 2004, Kiszka et al. 2014, Hidalgo-Reza et al. 2019), which means that no universal correction can be applied. Instead, there is a need for species- and tissue-specific experiments to investigate potential effects of preservation conditions. To the best of our knowledge, such a comparison has not been done for pilot whale muscle samples.

We compared $\delta^{13}C$ and $\delta^{15}N$ values in muscle samples stored in ethanol at 3-5°C, with samples from the same individuals but stored frozen (-20°C, Table S2). We assumed that frozen storage did not influence stable isotope values for pilot whale muscle, as has been extensively shown in other tissues and taxa (Gloutney & Hobson 1998, Kaehler & Pakhomov 2001, Sweeting et al. 2004, but see Barrow et al. 2008). The lack of sufficient skin samples preserved in ethanol prevented us from testing these effects on skin as well, but since we only used frozen skin samples in this study, this was not relevant.

At the time of collection, duplicate muscle samples were collected from each whale, one stored in ethanol (for genetic analyses) and another stored frozen. A total of 27 paired muscle samples were used to compare δ^{13} C and δ^{15} N values of frozen and ethanol-preserved samples (Table S2). The samples were stored in ethanol or frozen for a period between 4 months and 31 years prior to stable isotope analyses. However, due to a low sample size, it was not possible to quantitatively investigate trends in effects of ethanol over time. We tested whether the *δ*13C and δ^{15} N values followed a normal distribution using the Shapiro-Wilk test and, because the data did not follow a normal distribution (δ^{13} C Shapiro-Wilk: W = 0.84, p = 0.0002; δ^{15} N Shapiro-Wilk: $W = 0.61$, $p \le 0.0001$), we then used paired Wilcoxon rank sum tests to test for differences between pairs of samples. All samples were lipid-extracted and processed as detailed in the Materials and Methods section of the manuscript, and the *δ*13C-Suess corrected values were used for the comparisons.

There was a significant difference in lipid-extracted δ^{13} C values between frozen and ethanol-preserved samples (paired Wilcoxon rank sum test $V = 280$, $p = 0.03$, Figure S1a), with frozen samples having, on average, δ^{13} C values that were 0.29 ‰ higher than those of ethanol-preserved samples, although variation around the mean was large $(SD = 0.53 \text{ %}$, range $=$ -0.52 to 1.74 ‰, Table S2). The direction of differences was not consistent. Most (n = 19) of the 27 frozen samples had δ^{13} C values higher than ethanol-preserved samples, ranging from a difference of 0.00 to 1.74 ‰. In contrast, eight of the 27 frozen samples had *δ*13C values lower than ethanol-preserved samples, ranging from a difference of -0.05 to 0.52 ‰. Given these differences, we removed all ethanol-preserved muscle samples from subsequent analyses.

There was no statistically significant difference in $\delta^{15}N$ values between ethanol-preserved and frozen samples (paired Wilcoxon rank sum test $V = 122$, $p = 0.11$, Figure S1b). The mean difference between frozen and ethanol-preserved samples was -0.05% (SD = 0.30 ‰, range = -0.55 to 1.02 ‰, Table S2). Because the majority (24 out of 27) of samples had been stored for only a few months (4-10 months) in ethanol, it was not possible to quantitatively investigate trends in effects of ethanol storage over time. Nevertheless, samples that had been stored for decades in ethanol were not always those showing the highest differences in *δ*¹³ C or *δ*15N values compared to frozen samples (Table S2), suggesting that the duration of preservation in ethanol is unlikely to affect δ^{13} C and δ^{15} N values.

Figure S1. Variation in pilot whale muscle $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values with preservation method ($n = 27$). Violin plots show the kernel density distribution as well as the boxplot, where the horizontal line represents the median, the box represents interquartiles and whiskers represent values within 1.5 times the interquartile range from the boxes. Outliers are plotted as single points.

Ethanol preservation effects on δ^{13} C values are not consistent across tissue types and cetacean species. In common dolphin (*Delphinus delphis*) skin, for example, ethanol preservation led to slight depletion of δ^{13} C values (Kiszka et al. 2014), while in humpback whales *(Megaptera novaeangliae)*, the opposite effect was observed *(Hidalgo-Reza et al.*) 2019). Our results show a slight decrease in δ^{13} C in ethanol preserved muscle, compared to frozen muscle, in agreement with the findings of Kiszka et al. (2014) in skin of common dolphins. In our study, the mean difference of 0.29 ‰ was higher than the analytical error of 0.1 ‰ and therefore we removed any ethanol-preserved samples from our study. This mean difference is slightly lower than that found by Kiszka et al. (2014) for common dolphin skin, but similarly with much variation around the mean. Caution in using ethanol-preserved samples to investigate carbon stable isotopes has been highlighted in other taxa (Kaehler & Pakhomov 2001, Sweeting et al. 2004), while other studies did not find any effects (Gloutney & Hobson 1998, Barrow et al. 2008). The variability across studies highlights that tissue- and taxa-specific tests of preservation effects are needed before using ethanol preserved samples to determine *δ*13C values.

With regards to δ^{15} N values, preservation in ethanol appeared to bear no effects on the lipid-extracted skin of either common dolphins or humpback whales (Kiszka et al. 2014, Hidalgo-Reza et al. 2019). Our study shows that, for lipid-extracted muscle from pilot whales, ethanol preservation also does not affect $\delta^{15}N$ values significantly. Indeed, the mean difference in δ^{15} N values between frozen and ethanol-preserved samples was -0.05 ‰, below the 0.15 ‰ analytical error for $\delta^{15}N$ values. This mean difference is the same as found by Kiszka et al. (2014) for common dolphin skin. Such agreement between different studies suggests that any potential variability in $\delta^{15}N$ values introduced by ethanol preservation of skin and muscle is negligible or non-existent. Nevertheless, effects of ethanol are potentially confounded with possible effects of the lipid-extraction process that was undertaken in all these studies. Studies on other taxa also show little or no effect of ethanol preservation on $\delta^{15}N$ values, compared to those of δ^{13} C values (Kaehler & Pakhomov 2001, Sweeting et al. 2004). Further studies on

other cetacean species and tissues using non-lipid extracted samples, would be welcome to confirm the apparent generalised lack of effects of ethanol preservation on $\delta^{15}N$ values.

Kiszka et al. (2014) reported that the time that samples were stored in ethanol did not change the effects of ethanol on $\delta^{13}C$ or $\delta^{15}N$ skin values. Tests in other taxa suggest the same (e.g., Sweeting et al. 2004). Hidalgo-Reza et al. (2019), however, did find that humpback whale skin samples preserved for longer periods of time showed significant differences in *δ*¹³ C values compared to those preserved for shorter periods of time, but cautioned that the number of samples that had been subjected to short-term storage in their study was small. Our small dataset did not permit a full investigation into long-term ethanol storage effects, but suggests that the duration of preservation of the timeframes we studied (between 4 months and 31 years) did not affect pilot whale muscle *δ*¹³ C or *δ*15N values. There is a need for appropriate replicate experiments on different cetacean tissues to be conducted in the future to confirm such assumed lack of effects of preservation duration, particularly when stable isotope analyses make use of archived collections.

Table S2. Carbon and nitrogen stable isotope values of ethanol-preserved vs. frozen pilot whale, lipid-extracted muscle samples. All δ^{13} C values have been corrected for the Suess effect. Sex is given as Male (M) or Female (F). Age class is defined based on length and sex and $\delta^{13}C$ values have been corrected for the Suess effect (see Materials and Methods).

Text S2. Comparison of δ^{13} C and δ^{15} N values in cetacean skin and muscle

The timeframe over which an isotope is integrated into an animal's tissue depends on the isotopic turnover rate of that tissue (Newsome et al. 2010). In addition, for any given tissue the turnover rate may vary depending on factors that include the stable isotope of focus, the species, region, life stage and the environmental conditions in which individuals are sampled (Newsome et al. 2010, Busquets-Vass et al. 2017, Wild et al. 2018). While there is potential variation in the specific stable isotope turnover rates between marine mammal species and even individuals within a species, both skin and muscle are generally assumed to provide dietary information spanning the past several weeks to months prior to sampling (e.g., bottlenose dolphin, *Tursiops truncatus*, skin: mean half-life turnover rate of approximately 24 days for *δ*13C and 48 days for *δ*15N values, Giménez et al. 2016; blue whale, *Balaenoptera musculus*, skin: mean full isotopic turnover of 163 days for $\delta^{15}N$ values). Tissues can be obtained either from stranded cetaceans (all tissues) or from free-ranging individuals through biopsy sampling (skin and blubber only).

Because skin and muscle may have different turnover rates, these tissues can potentially integrate the diet of an individual over different time frames. Thus, it is necessary to test for differences between tissues before pooling samples together. To investigate differences in *δ*13C and δ^{15} N values between tissues (skin vs. muscle) we used samples of both tissues collected from the same individual that had been kept frozen since the time of collection (Table S3). We tested whether the δ^{13} C and δ^{15} N values followed a normal distribution using the Shapiro-Wilk test. To test for differences between pairs of samples, we used a paired Wilcoxon rank sum test for δ^{13} C values because the data was nearly not normal for δ^{13} C values (Shapiro-Wilk: W = 0.95, $p = 0.05$) and a paired t-test for $\delta^{15}N$ values because the data followed a normal distribution for $\delta^{15}N$ values (Shapiro-Wilk: W = 0.97, p = 0.20). All samples were lipidextracted and processed as detailed in the Materials and Methods section of the manuscript, and the δ^{13} C-Suess corrected values were used for the comparisons.

A total of 25 paired frozen-preserved samples were used to compare the $\delta^{13}C$ and $\delta^{15}N$ values of skin and muscle tissues. There was no statistically significant difference in *δ*¹³ C values between skin and muscle samples (paired Wilcoxon rank sum test $V = 227$, $p = 0.09$, Figure S2a). The mean difference in δ^{13} C values between skin and muscle samples was 0.13 $\%$ (SD = 0.31 $\%$, range = -0.38 to 0.77 $\%$, Table S3). There was also no statistically significant difference in $\delta^{15}N$ values between skin and muscle samples (paired t-test t = 0.90, df = 24, p = 0.38, Figure S2b). The mean difference in $\delta^{15}N$ between skin and muscle samples was 0.05 ‰ $(SD = 0.29 \text{ %}$, range = -0.47 to 0.71 ‰, Table S3). Both the $\delta^{13}C$ and $\delta^{15}N$ mean differences between skin and muscle were lower than the analytical error. Given these results, we pooled skin and muscle tissues in subsequent analyses.

Figure S2. Variation in pilot whale skin and muscle $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values (n = 25). Violin plots show the kernel density distribution as well as the boxplot, where the horizontal line represents the median, the box represents interquartiles and whiskers represent values within 1.5 times the interquartile range from the boxes. Outliers are plotted as single points.

Studies investigating stable isotope differences between skin and muscle in cetacean species do not show an overall consistent trend. For example, Horstmann-Dehn et al. (2012) report differences in bowhead whales (*Balaena mysticetus*), with lipid-extracted skin generally enriched in ¹⁵N compared to lipid-extracted muscle, while ¹³C was depleted in skin compared to muscle. In the same study, ¹⁵N was significantly enriched in the skin compared to muscle of grey whales (*Eschrichtius robustus*), while *δ*¹³ C values did not differ (Horstmann-Dehn et al. 2012). In contrast, in belugas (*Delphinapterus leucas*), no differences were found in either *δ*13C or *δ*15N of lipid-extracted skin, when compared to lipid-extracted muscle (Horstmann-Dehn et al. 2012). Similarly, lipid-extracted skin and muscle of fin whales (*Balaenoptera physalus*) had similar δ^{13} C and δ^{15} N values (Borrell et al. 2012), and δ^{13} C values were not significantly different between non-lipid-extracted skin and muscle in humpback whales (Todd et al. 1997).

For pilot whales, results are also inconsistent between studies. For example, Abend and Smith (1997) reported lower δ^{13} C values and higher δ^{15} N values in non-lipid extracted skin, compared to muscle of pilot whales in the western North Atlantic. Fontaine et al. (2015) report higher *δ*¹³ C and *δ*15N values in lipid-extracted skin, compared to lipid-extracted muscle, of southern long-finned pilot whales (*G. m. edwardii*) stranded in the Kerguelen islands. In this study, we found no statistically significant differences between lipid-extracted skin and muscle δ^{13} C and δ^{15} N values in pilot whales stranded in Icelandic coastal waters.

There could be several reasons for the lack of consistency in published studies, including ontogeny, tissue turnover rate in smaller cetaceans as compared to those of larger body size, interspecific differences in the amino acid composition of skin and muscle or differences in diet. For example, predators that consume a varied diet that includes prey spanning multiple trophic levels may show similar skin and muscle δ^{13} C and δ^{15} N values (Todd et al. 2010, Horstmann-Dehn et al. 2012). The diet of pilot whales in Icelandic waters is largely unknown, and the only information available, based on stomach contents from four whales stranded in 1986, suggests a diet composed of squid (Sigurjónsson et al. 1993). Abend and Smith (1997) attributed differences in $\delta^{13}C$ and $\delta^{15}N$ values of skin and muscle of western North Atlantic pilot whales to temporal variation in diet; they suggested that the skin reflected the recent intake of mackerel, while muscle, which has a slower turnover rate, would reflect an earlier consumption of squid. The similarity in the δ^{13} C and δ^{15} N values of skin and muscle observed

in our study may suggest a relatively stable diet composition. Further information on the diet of pilot whales in Iceland, however, is necessary to confirm this, as well as information on the specific stable isotope turnover rates of these tissues for pilot whales. Future studies comparing different skin layers—that may provide a dietary time series (Busquets-Vass et al. 2017, Wild et al. 2018)—or comparing skin and muscle with tissues that integrate diet over longer time periods, such as bone or teeth, would assist in answering these questions.

Figure S3. Prediction plot for the effects of body length on $\delta^{15}N$ values for male (M) and female (F) pilot whales stranded along the Icelandic coast. Neither Sex nor Body Length were significant predictors of *δ*15N values at the 5% significance level. Coloured circles are observed data points.

Figure S4. Skin and muscle *δ*13C and *δ*¹⁵ N values from stranded pilot whales (*Globicephala melas*, $n = 80$ in Iceland, separated by age class (a) and sex (b). Standard Ellipse Areas corrected for sample size (SEA*c*) are also shown. Note that samples shown are those included in the GLM analyses presented in the main manuscript.

Stranding number

Figure S5. Distribution of the standard ellipse area $(\%^2)$ estimates based on 10^6 resampling runs for pilot whales from different mass strandings. Black dots represent the mode, and the shaded boxes represent the 50, 75 and 95% credible intervals from dark to light grey. Red crosses show the maximum likelihood estimates of the standard ellipse area corrected for sample size (SEA_c) .

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