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ARTICLE

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Establishing the limitations on using archived marine mammal samples for stable isotope analysis: an examination of differing preservation methods on tissues of harbor porpoise (*Phocoena phocoena*) and gray seal (*Halichoerus grypus*)

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Abstract

The use of biological samples from museum and/or archive collections is common in stable isotope research, particularly for marine mammals. Yet, the temporal stability of isotopic values across various tissue types and the influence of different preservatives on these values are not fully understood, posing significant challenges for accurate data interpretation. Here we examine the impact of three different tissue preservation methods (DMSO, ethanol, freezing), on seven different tissues (blubber, heart, kidney, lung, liver, muscle, and skin) from both a harbor porpoise (*Phocoena phocoena*) and a gray seal (*Halichoerus grypus*) for stable isotope analysis in a 1-year period. Our results demonstrate that storage in DMSO generates greater temporal variability in $\delta^{13}C$ and $\delta^{15}N$ for all tissue types, particularly in the first six months of storage. Furthermore, tissues stored in DMSO often exhibited lower δ13C and δ15N values compared to those stored frozen or in ethanol. This finding highlights a significant issue for studies utilizing tissues stored in DMSO, regardless of the storage duration. These results underscore the critical need for careful consideration of preservation methods in studies

involving stored tissues, providing valuable insights for experimental design and management of tissue archives.

KEYWORDS
archive, DMSO, ethanol, marine mammal, stable isotopes, tissue preservation

1 | INTRODUCTION

A thorough understanding of the impacts of preservation method on biological samples is fundamental in ensuring correct interpretation of downstream analyses. Many studies that examine marine mammals at the population level make use of archived samples or those derived from museum collections (Drago et al., 2009; Parsons et al., 2002; Rosenbaum et al., 2000; Wandeler et al., 2007; Zenteno et al., 2015). Consequently, researchers often have no control over the preservatives or storage temperatures of the samples they use. Furthermore, even when preservation control is possible, studies frequently require extended periods of sample storage, such as throughout a sampling season, with analysis commencing only after its completion. Therefore, understanding the effects of preservation methods is crucial for all stable isotope studies of marine mammals. For those readers unfamiliar with stable isotope analysis (SIA) and its utilization within the field of marine mammal science, we recommend the review by Teixeira et al. (2022) as an excellent primer on SIA applications, underpinning theory and conventions on notation.

Increasingly, marine mammal studies, particularly those concerning evolutionary biology, often seek a more holistic ecological understanding by combining genetic approaches and SIA to investigate potential environmental and/or trophic enrichment factors that might be influencing population structure (Brotons et al., 2019; Genoves et al., 2020). This presents a potential conflict between the preservation method that best supports the highest DNA extraction yield (commonly reported as DMSO; Dahn et al. 2022; Kilpatrick, 2002), and the method that has the least impact on stable isotope ratios. Although commonly used, ethanol as a tissue preservative for genetic use is likely less effective due to the rapid coagulation of proteins, driven by the high concentration of ethanol, that prevents its further penetration into the tissue (Oosting et al., 2020). Freezing and lyophilization of samples can be suitable for both genetic and stable isotope analyses but have significant implications for long-term storage, both requiring careful environmental control (low temperature and low humidity, respectively) to prevent sample damage. The requirement for extended storage periods and the diverse potential applications can prompt considerations about the optimal preservation methods for marine mammal tissue samples, as well as the most suitable tissue types to be used.

Isotopic turnover rates vary across different tissues (Tieszen et al., 1983); for instance, muscle often exhibits longer isotopic half-lives compared to internal organs (Vander Zanden et al., 2015). Researchers frequently aim to investigate long-term dietary preferences in their target species, leading them to choose muscle tissue for stable isotope studies over lung or liver tissue. However, studies utilizing archived tissues from museum repositories may necessitate using whatever tissues are available, rather than those ideally preferred. Hence, this study examines a broad variety of potentially available tissue types.

Numerous studies have investigated the impacts of preservation method on SIA (Bosley & Wainright, 1999; Hobson et al., 1997; Javornik et al., 2019; Kiszka et al., 2014; Sarakinos et al., 2002). However, many of these studies have focused narrowly on specific tissue types or storage methods, often overlooking potential temporal variability in isotopic values during storage (see Teixeira et al., 2022 for a review). Several methods of preservation have been examined, including ethanol (Javornik et al., 2019), formalin (Hobson et al., 1997), freezing (Hidalgo-Reza et al., 2019), DMSO (Lesage et al., 2010; Newsome et al., 2018), and lyophilization (Bosley & Wainwright, 1999). There has been some consideration for temporal variability (Burrows et al., 2014; Rennie et al., 2012), but the tissue type examined is often limited to skin (Newsome et al., 2018) or muscle (Yurkowski et al., 2017) since these

tissue types are most often collected through biopsy sampling (Aubail et al., 2013; Noren & Mocklin, 2012; Parsons et al., 2003). However, museum and tissue bank archives may contain many different tissue types and it is from these archives that population level studies often draw necessary samples.

On May 9, 2017, the Cetacean Strandings Investigation Programme (CSIP) conducted a demonstrative necropsy of a harbor porpoise (*Phocoena phocoena*) and a gray seal (*Halichoerus grypus*) for marine biology undergraduate students at Newcastle University, UK. We utilized this opportunity to obtain samples of multiple tissue types found in tissue archives, from both a cetacean and a pinniped species. We took samples from multiple tissue types available in tissue archives of both cetaceans and pinnipeds. Our study aimed to assess the effects of three common preservation methods (DMSO, ethanol, freezing at −20°C) over an extended storage period on various marine mammal tissues (blubber, heart, kidney, lung, liver, muscle, and skin). Our findings are essential for guiding future marine mammal biology investigations using biopsied samples and archived tissues.

2 | MATERIALS AND METHODS

2.1 | Sample collection

We collected the various tissue samples from both necropsied animals. We examined three storage preservation methods: (1) submersion in 90% ethanol, (2) submersion in salt-saturated dimethyl sulfoxide (DMSO), and (3) freezing at −20°C with no preservative solution. We collected a single individual sample for later isotopic analysis at each of the later analysis periods (months 0, 2, 4, 6, 8, 10, and 12), with an additional sample collected as a spare, giving 8 samples of each tissue type for each species. Samples were analyzed on the same calendar date each month.

Both the *P. phocoena* and *H. grypus* individuals were found dead and stranded in good body condition on the Northumberland coast, with the cause of death unknown. We obtained multiple tissue samples from both necropsied animals, which were immediately frozen following collection. Prior to attending the necropsy, we prelabeled and filled 1.5 ml Eppendorf tubes with 1 ml of preservation fluid (90% ethanol or DMSO) where applicable. Duplicated for the two study species, this method generated a sample suite of 336 samples. During the necropsy, after examination of each tissue type was completed by the UK Cetacean Strandings Investigation Programme (CSIP), a large section was made available for subsampling. From this larger section of tissue, we collected samples of size 1 × 0.5 × 0.5 cm using scalpel, forceps, and dissection scissors. We then transported samples in cold storage back to Durham University where they were all stored at −20°C.

For each analysis period, we removed the appropriate samples from frozen storage and prepared them for stable isotope analysis. To understand the impacts of lipid extraction we divided each sample into thirds, with one third archived for potential future use, the second third used for standard analysis and the final third subject to lipid extraction. We defrosted and transferred the standard isotope analysis sample to a clean prelabeled 1.5 ml Eppendorf tube where we finely diced it using bow scissors. 1,000 µl of deionized H_2O was added to the Eppendorf tube which we then placed in a foam float in an ultrasound bath and sonicated for 15 min. Following this, we centrifuged the sample at 3,000 rpm for 10 min after which we carefully removed the H_2O and placed the sample open in a drying oven heated to 45°C until the sample was fully desiccated.

2.2 | Lipid extraction

We performed lipid extractions using a dichloromethane:methanol protocol (Svensson et al., 2014), modified to exclude the final N_2 drying step. Samples to be lipid extracted prior to stable isotope analysis were defrosted and transferred to a clean prelabled 1.5 ml Eppendorf tube. The same methodology was applied to all tissue types. Dichloromethane:methanol (1,000 µl of 2:1) was added and the Eppendorf placed in a foam float and ultrasonicated

for 15 min. The sample was then centrifuged at 3,000 rpm for 10 min before the dichloromethane:methanol mix was removed. These steps were repeated twice more so that the sample had been sonicated in new 1,000 µl of dichloromethane:methanol mix three times. Upon removal of the final mix the sample was placed in a drying oven set at 45°C until it was fully desiccated.

2.3 | Sample analysis

Once the samples were fully desiccated, they were ground in an Eppendorf using a stainless-steel micro-pestle to produce a fine powder. We then loaded tin capsules with 0.3–0.6 mg of tissue powder to be analyzed. Carbon and nitrogen isotope analysis was performed in the Stable Isotope Biogeochemistry Laboratory (SIBL) at Durham University using an ECS 4010 elemental analyzer (Costech, Valencia, CA) coupled with a Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Scientific, Bremen, Germany). Stable isotope ratios are reported in standard delta (δ) notation in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (AIR) for carbon and nitrogen respectively. Five select in-house standards (Urea [powder], Collagen [powder], Alpha cellulose [fibers], Calcium carbonate [powder], and Spirulina [powder]), that are stringently calibrated against the following international standards (e.g., USGS40, USGS24, IAEA-600, IAEA-CH-3, IAEA-CH-7, IAEA-N-1, IAEA-N-2) were analyzed after every eight samples to monitor accuracy.

2.4 | Data analysis

All statistical analyses were conducted using Minitab 14. Analyses were identically replicated for both study species. Temporal variation in data was calculated across combined tissue data sets and for all preservative methodologies with the 12-month experimental period being split into 0–6- and 6–12-month windows. Homogeneity of variance was tested between these two windows using Levene's test. These data sets were then additionally tested for data normality (Anderson-Darling) prior to testing for significant differences using the appropriate parametric or nonparametric test.

To illustrate the extensive variance seen in all samples stored in DMSO over the 12-month experimental period relative to those stored either frozen or in ethanol, a combined tissue data set for DMSO was tested for homogeneity of variance against a super-combined tissue data set including samples stored either frozen or in ethanol using Levene's test. These data sets were then also additionally tested for data normality (Anderson-Darling) prior to testing for significant differences using the appropriate parametric or nonparametric test.

3 | RESULTS

Initial examination showed similar patterns of preservative impacts for both raw and lipid extracted data. Thus, in the interests of brevity and with the focus remaining on impact of preservation, all analyses were performed on lipid extracted data only. All data (raw and lipid-extracted), including %N and %C for calculation of C:N ratios, are available in Table S1 and Table S2 in Appendix S1.

3.1 | Phocoena phocoena

$\delta^{13}C$ values in tissues from *P. phocoena* that were stored in ethanol or were frozen remained relatively stable throughout the experimental period (Figure 1) with equal variance (Figure 2a) and no significant difference between

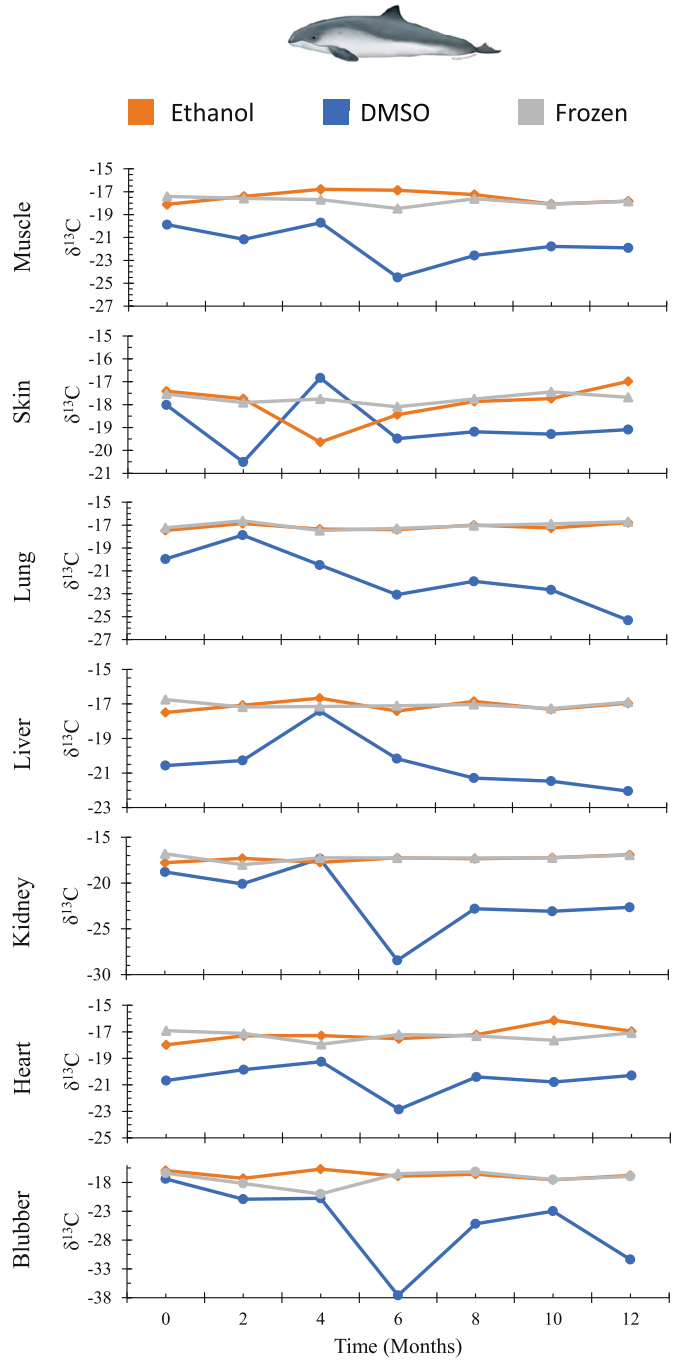

FIGURE 1 Temporal variation in $\delta^{13}C$ for tissues sampled ($n = 7$) from *Phocoena phocoena*. Each line represents a different preservation method, showing ethanol (orange diamonds), DMSO (blue circles) and frozen (gray triangles). Tissues were sampled every two months over a 12-month period.

coefficients of variation (CV) of values recorded in the first 6-month period compared to the second 6-month experimental period (Wilcoxon signed-rank, $p = .63$). However, when *P. phocoena* samples were stored in DMSO variability in $\delta^{13}C$ values was significantly higher across all tissue types in the first 6-month experimental period

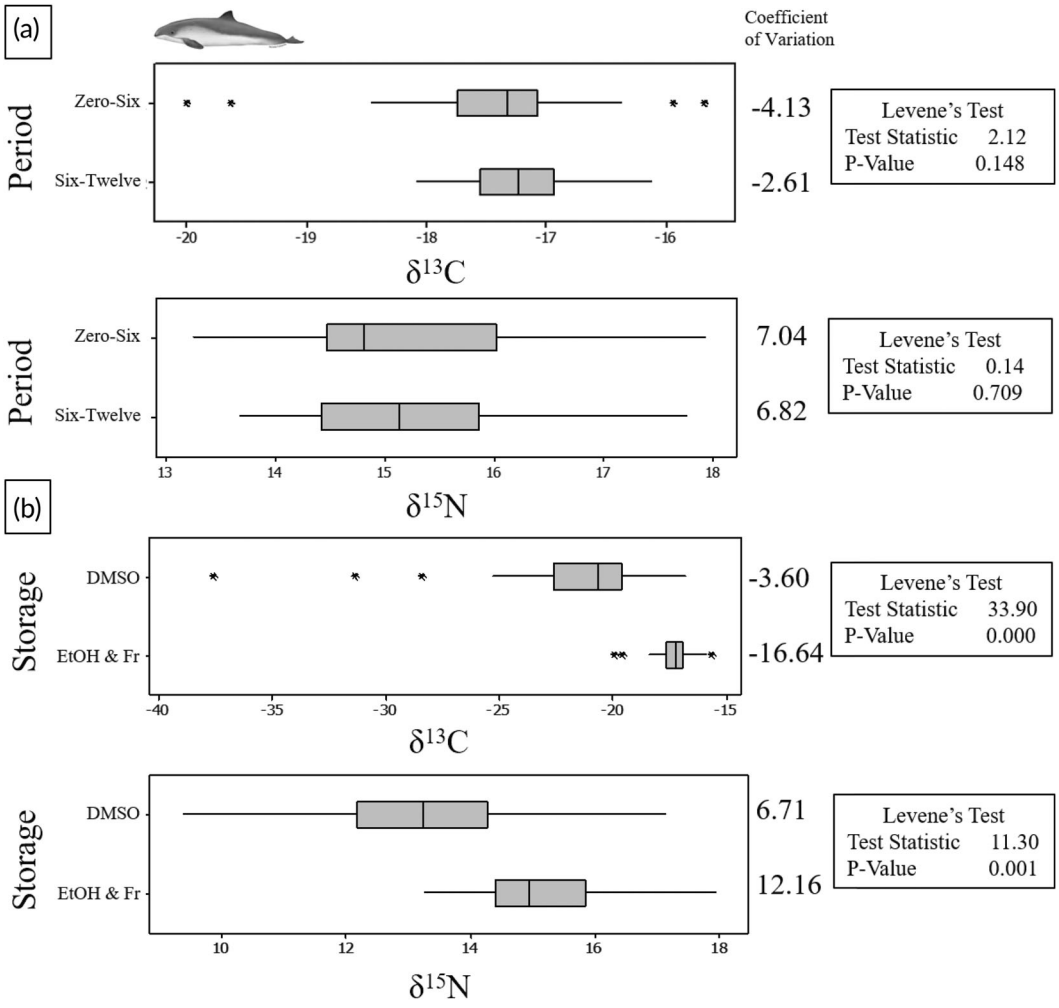

FIGURE 2 (a) Comparison of variation in $\delta^{13}C$ and $\delta^{15}N$, averaged across tissue types, in the first 6 months to the second 6 months of the experimental period for tissues from *Phocoena phocoena*. (b) Variation in $\delta^{13}C$ and $\delta^{15}N$, averaged across tissue types, for those tissues sampled from *Phocoena phocoena* stored in DMSO compared to Frozen/Ethanol storage.

(mean $CV = -15.13$) than in the second 6-month experimental period (mean $CV = -.38$, Wilcoxon signed-rank, $p = .018$), suggesting an initial period of sample instability as clearly apparent in Figure 1. Tissue storage in DMSO produced far higher variability in measured $\delta^{13}C$ values for all tissue types across the experimental period than those stored frozen or in ethanol (Figure 2b). Furthermore, samples preserved using DMSO produced significantly lower $\delta^{13}C$ values across all tissue types (Mann–Whitney U, $p < .005$). Blubber $\delta^{13}C$ values were lower than all other tissue types but retained a similar pattern of storage-influenced variation across the experiment. However, the largest range (and lowest value) in $\delta^{13}C$ for DMSO stored tissue was exhibited by blubber (−20.2‰), followed by kidney (−11.1‰). Interestingly, tissues stored in DMSO demonstrated a drop in $\delta^{13}C$ values after four months (Mann–Whitney U, $p < .005$).

Examination of $\delta^{15}N$ for *P. phocoena* (Figure 3) revealed a similar pattern of results whereby DMSO stored tissues exhibited the greatest variation over the experimental period (Figure 2b). Both ethanol and frozen stored samples recorded greater levels of variation in $\delta^{15}N$ than for $\delta^{13}C$. However, despite the overall greater levels of

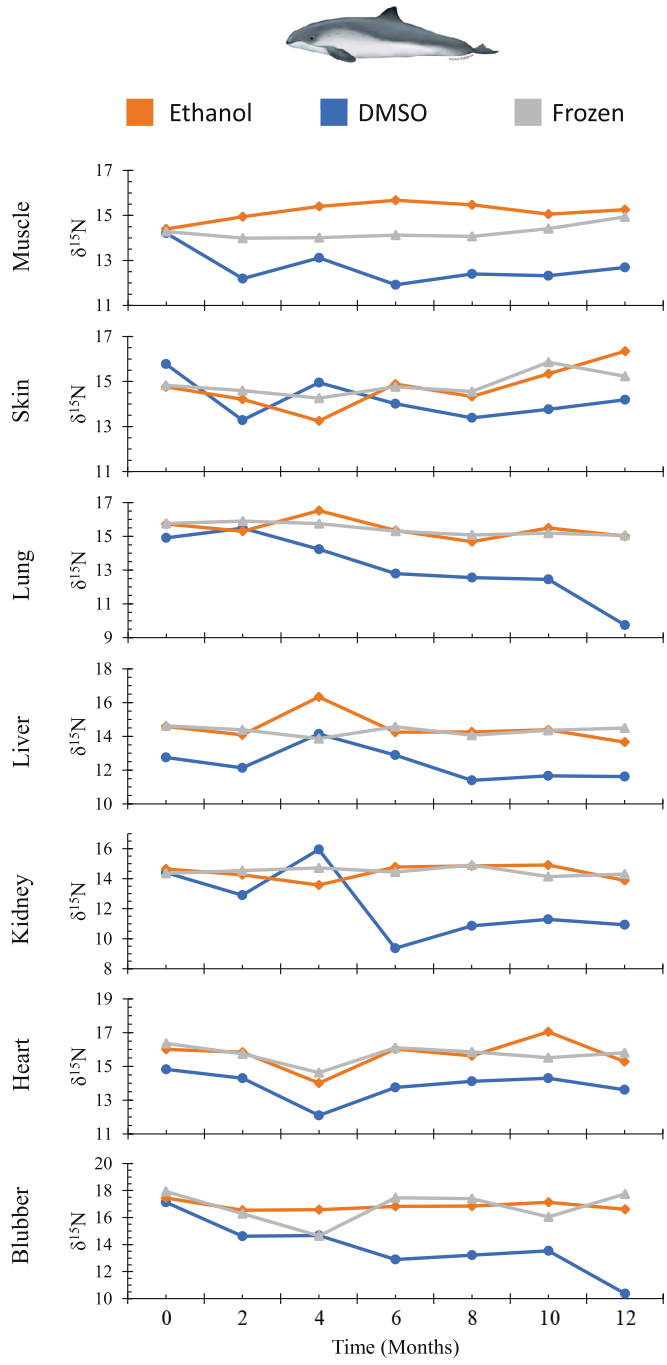

FIGURE 3 Temporal variation in $\delta^{15}N$ for tissues sampled ($n = 7$) from *Phocoena phocoena*. Each line represents a different preservation method, showing ethanol (orange diamonds), DMSO (blue circles) and frozen (gray triangles). Tissues were sampled every two months over a 12-month period.

variation for $\delta^{15}N$ values there was no significant change in *CV* across samples over the course of the experiment whether they were stored frozen or in ethanol (paired sample *t* test, $p = .604$) or in DMSO (Wilcoxon signed-rank, $p = .63$). Tissues preserved in DMSO recorded significantly lower $\delta^{15}N$ values than those which were frozen or

stored in ethanol across the experimental period (Mann–Whitney U, $p < .005$). Kidney and blubber stored in DMSO recorded the greatest range of $\delta^{15}N$ values across the experiment (6.8‰ and 6.6‰, respectively) and blubber tissue stored either frozen or in ethanol produced higher $\delta^{15}N$ values than other tissue types.

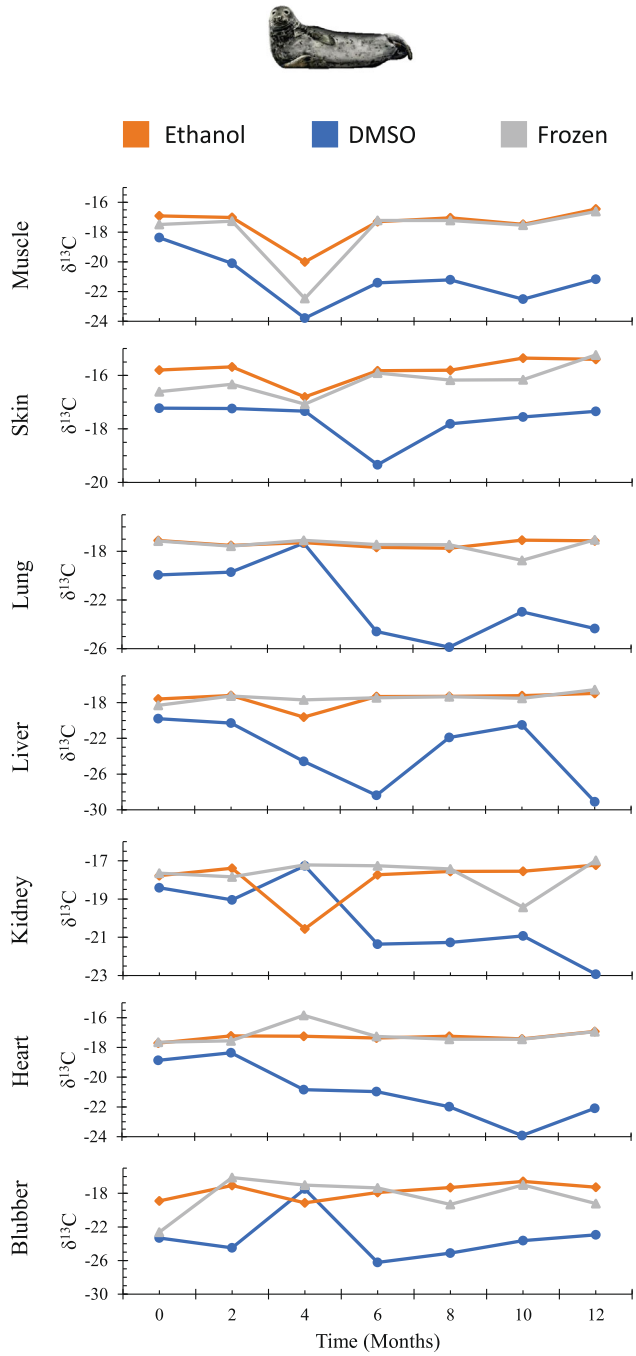

FIGURE 4 Temporal variation in $\delta^{13}C$ for tissues sampled ($n = 7$) from *Halichoerus grypus*. Each line represents a different preservation method, showing ethanol (orange diamonds), DMSO (blue circles) and frozen (gray triangles). Tissues were sampled every two months over a 12-month period.

3.2 | Halichoerus grypus

$\delta^{13}C$ data from *H. grypus* revealed a similar pattern to that reported for *P. phocoena* with those tissues stored in ethanol or frozen remaining relatively stable throughout the experimental period (Figure 4) and exhibiting equal variance (Figure 5a). Tissues stored in ethanol or frozen showed no significant difference between coefficients of variation (*CV*) of $\delta^{13}C$ values recorded in the first 6-month period compared to the second six-month experimental period (paired sample *t* test, $p = .267$). Similar to *P. phocoena*, samples that were stored in DMSO showed significantly higher variability in $\delta^{13}C$ values across all tissue types in the first 6-month experimental period (mean $CV = -11.63$) than in the second 6-month period (mean $CV = -6.40$) (Wilcoxon signed-rank, $p = .018$). This initial period of sample instability can be seen in Figures 4 and 5a. Preservation of tissues in DMSO produced $\delta^{13}C$ values that were both more variable across the experimental period (Figure 5b), as well as being significantly lower across

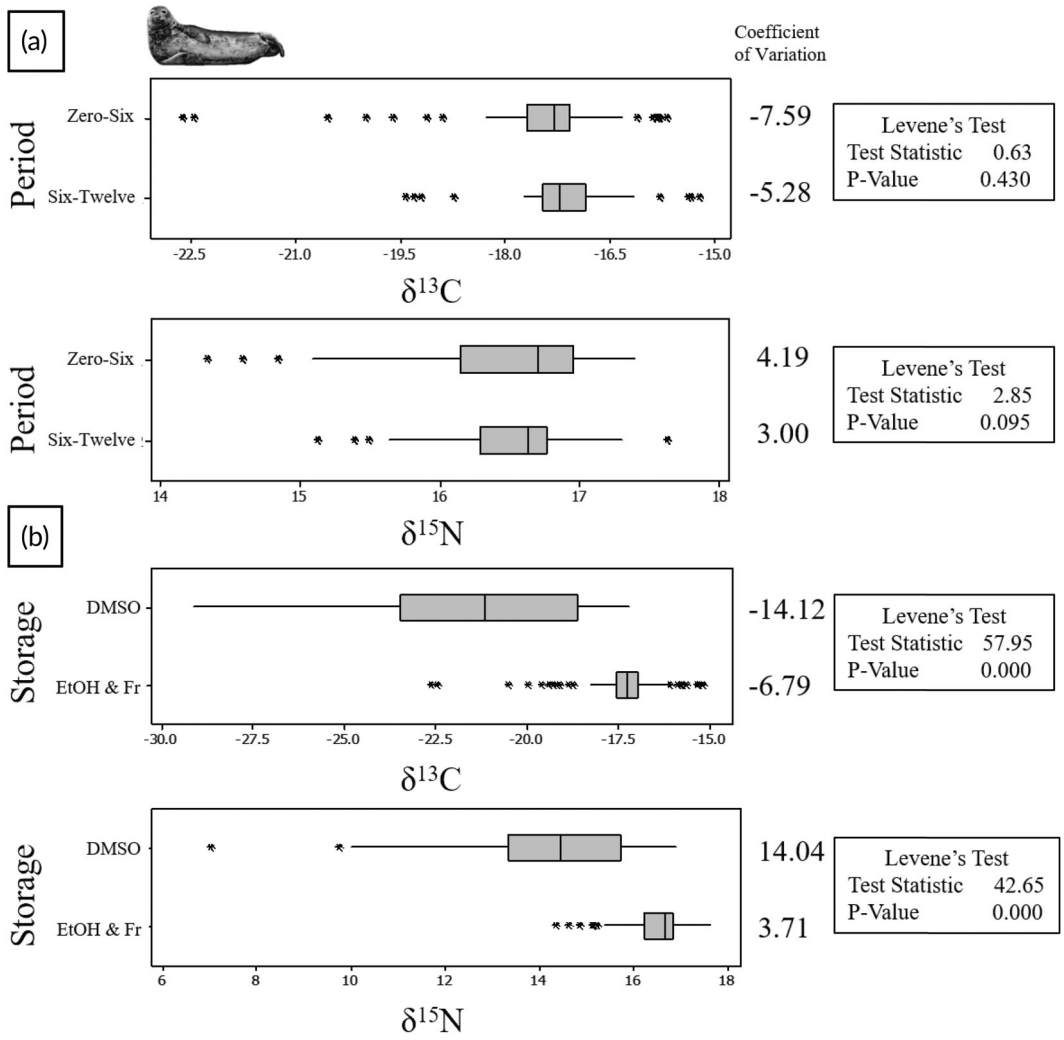

FIGURE 5 (a) Comparison of variation in $\delta^{13}C$ and $\delta^{15}N$, averaged across tissue types, in the first six months to the second six months of the experimental period for tissues *Halichoerus grypus*. (b) Variation in $\delta^{13}C$ and $\delta^{15}N$, averaged across tissue types, for those tissues sampled from *Halichoerus grypus* stored in DMSO compared to Frozen/Ethanol storage.

all tissue types (Mann–Whitney U, $p < .005$). Interestingly some tissue types (muscle, lung, kidney and heart) showed drops in $\delta^{13}C$ after 2–4 months. Several DMSO preserved tissues displayed large ranges in $\delta^{13}C$ values across the experimental period including lung (8.5‰), liver (9.3‰), and kidney (5.7‰).

Preservation of *H. grypus* tissues through freezing or ethanol produced $\delta^{15}N$ values that were stable throughout the experimental period with no significant change in *CV* across all tissue types (Wilcoxon signed-rank, $p = .128$; Figure 6). As in *P. phocoena*, tissues preserved in DMSO did not show significantly higher variability in $\delta^{15}N$ in the first 6-month period than in the second six-month experimental period (Wilcoxon signed-rank, $p = .612$). DMSO preserved tissues produced $\delta^{15}N$ values that were significantly lower over the experimental period than those preserved through freezing or ethanol (Mann Whitney U, $p < .000$; Figure 5b). Similarly to *P. phocoena*, DMSO preserved tissue $\delta^{15}N$ values dropped after 4 months of storage for several tissues including heart, kidney, and lung. Liver showed the greatest range of DMSO preserved $\delta^{15}N$ tissues (8.6‰).

4 | DISCUSSION

This study demonstrates that the preservation method used for archiving tissue samples of marine mammal species can significantly alter stable isotope values. Our study addresses the gap regarding temporal variation in isotopic values, which is often overlooked in many studies. While previous research has reported on the effects of storage methods on δ13C and δ15N (e.g., Arrington & Winemiller, 2002; Burrows et al., 2014; Hobson et al., 1997; Javornik et al., 2019), no prior study has comprehensively investigated temporal variation in isotopic values over a full calendar year or across such a diverse array of tissue types as presented here.

4.1 | Temporal variation under ethanol or freezing preservation

Preservation of marine mammal tissues, either by freezing or storage in ethanol, showed no difference in δ13C or δ15N values over a 12-month period. Similar findings have been previously demonstrated across various species, including cetaceans (Kizska et al., 2014), sharks (Olin et al., 2014), and birds (Bugoni et al., 2008). The consistency of these preservation methods in producing stable isotope data is beneficial for field biologists, particularly those facing limited access to freezing facilities for newly acquired samples. The variation in $\delta^{13}C$ and $\delta^{15}N$ between tissue types observed in this work is expected given the differential turnover and incorporation rates of these tissues as recorded in previous studies (e.g., Bond et al., 2016; Hobson & Clark, 1992; Phillips & Eldridge, 2006; Vander Zanden et al., 2015). The similar values for $\delta^{13}C$ and $\delta^{15}N$ observed between species for each tissue type over the experimental period reflect the similar trophic position of *P. phocoena* and *H. grypus* (Das et al., 2003), with prey species such as Atlantic herring (*Clupea harengus*) being major dietary component for both species (Andreasen et al., 2017; Lundström et al., 2010).

4.2 | Impact of DMSO on temporal variation

Storage in DMSO resulted in lower δ15N and δ13C values across all tissues in both species. However, similar to findings in other studies (Hobson et al., 1997; Marcoux et al., 2007; Todd et al., 1997), this did not lead to significant changes in temporal variation from the first 6 months of the experiment to the second 6 months. DMSO contains no nitrogen, so nitrogen uptake is not possible from the preservative. Hence, any variation in $\delta^{15}N$ is likely due to hydrolysis and leaching of the stored tissue, as suggested previously in ethanol preservation (Sarakinos et al., 2002). Newsome et al. (2018) conducted a study on the impact of DMSO preservation on delphinid skin samples for stable isotope analysis over a multiyear period, where they observed a decrease in δ13C values.

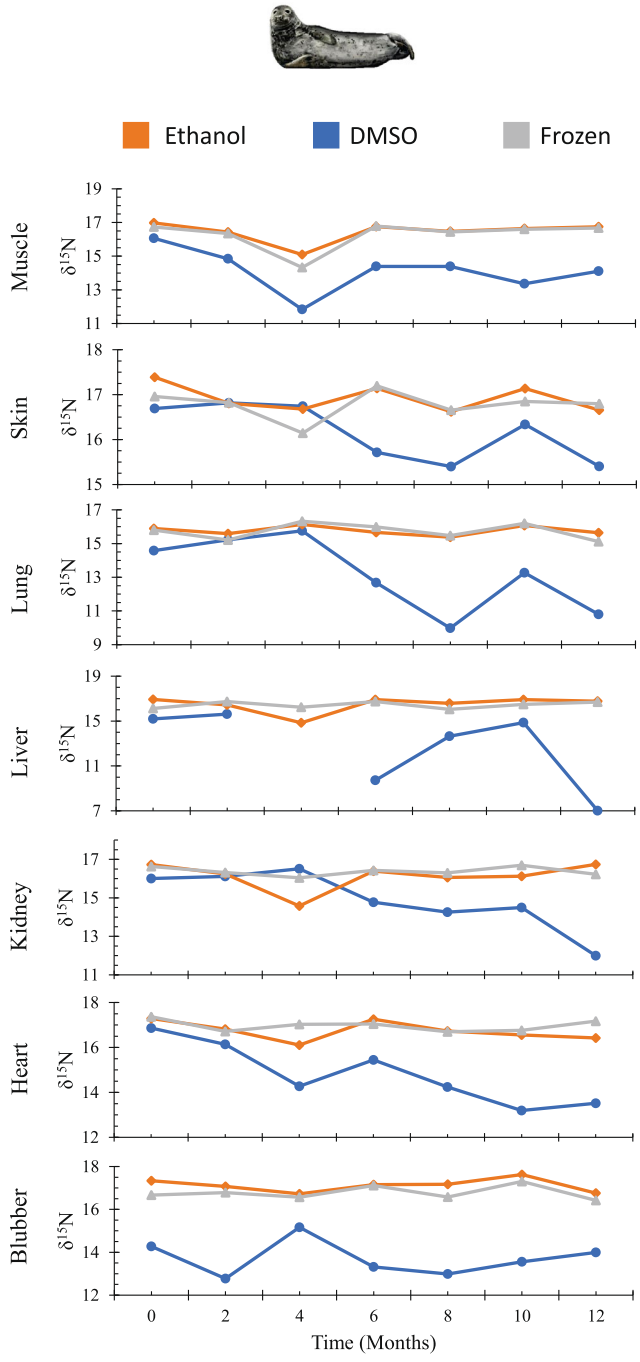

FIGURE 6 Temporal variation in $\delta^{15}N$ for tissues sampled ($n = 7$) from *Halichoerus grypus*. Each line represents a different preservation method, showing ethanol (orange diamonds), DMSO (blue circles) and frozen (gray triangles). Tissues were sampled every two months over a 12-month period.

However, temporal resolution in their experiment did not allow pinpointing the exact timing of the $\delta^{13}C$ decrease. Our study indicates that the greatest deviation from original $\delta^{13}C$ values, as determined from frozen and/or ethanol-preserved tissues, occurred after a 4-month period of storage in DMSO. This finding differs from our

initial expectation of an immediate impact post preservation. However, our expectation that the impact would be consistent across all tissue types was confirmed. It is important to note here that Newsome et al. (2018) compared skin tissues across delphinid species whereas this our study also includes tissues from a pinniped as well. Newsome et al. (2018) demonstrated that isotopic values could be restored to pre-DMSO storage levels through application of a lipid extraction protocol, a finding supported by Lesage et al. (2010). The lipid extraction protocol utilized by Lesage et al. (2010) and Newsome et al. (2018) included an extended period (~12–24 hr) of sonification in solvent solution and future studies should look to include this step. Care should be taken, however, to ensure that the impact of lipid extraction on stable isotope value is fully understood before utilizing data from such studies as the effect is known to vary by species and tissue type (Yukowski et al., 2015). Lipid extraction is known to create unpredictable changes in $\delta^{15}N$, and it is accepted that ascertaining species and tissue specific impacts through duplicate analysis is the most appropriate pathway to robust data (Cloyed et al., 2020; Ryan et al., 2012; Yurkowski et al., 2015).

Due to the challenges marine mammal researchers often face in obtaining tissue samples and the potential information gained from multiple analytical approaches, marine mammal tissue samples are inherently valuable and frequently utilized for multiple studies (e.g., Alter et al., 2012). However, different preservation methods may favor certain analytical approaches over others. For example, preservation of cetacean tissues in DMSO has been demonstrated to yield more consistent amplifications of extracted DNA compared to tissues stored in ethanol (Robertson et al., 2013). Therefore, based on our findings, the optimal strategy would be to split newly obtained samples and store them using multiple preservation methods for archiving purposes—this approach enables future researchers to request specifically preserved material best suited for their particular aims.

4.3 | Conclusions

This study examined three different preservation methods routinely used in marine mammal research on seven different tissue types for their effect on values of $\delta^{13}C$ and $\delta^{15}N$ over a twelve-month period. Our data demonstrated that storage of tissues in DMSO tends to produce greater variability for both $\delta^{13}C$ and $\delta^{15}N$ for all tissue types examined across the experimental period. In addition, we showed that DMSO storage led to lower recorded values of $\delta^{13}C$ and $\delta^{15}N$ (an effect not corrected by our lipid-extraction protocols) than other preservation methods. These results should be considered carefully by practitioners at the experimental design stage and by tissue archive managers.

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AUTHOR CONTRIBUTIONS

Daniel Martin Moore: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; visualization; writing – original draft; writing – review and editing. **Emily Cunningham:** Conceptualization; investigation; methodology; writing – review and editing. **Kayla Crowder:** Investigation; methodology; writing – review and editing. **Darren Grocke:** Conceptualization; data curation; funding acquisition; investigation; methodology; resources; supervision; writing – original draft; writing – review and editing.

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REFERENCES

Alter, S. E., Newsome, S. D., & Palumbi, S. R. (2012). Pre-whaling genetic diversity and population ecology in eastern Pacific gray whales: insights from ancient DNA and stable isotopes. *PLoS ONE*, *7*(5), Article e35039. https://doi.org/10.1371/journal.pone.0035039

Andreasen, H., Ross, S.D., Siebert, U., Andersen, N. G., Ronnenberg, K. and Gilles, A. (2017). Diet composition and food consumption rate of harbor porpoises (*Phocoena phocoena*) in the western Baltic Sea. *Marine Mammal Science*, *33*(4), 1053–1079. https://doi.org/10.1111/mms.12421

Arrington, D. A., & Winemiller, K. O. (2002). Preservation effects on stable isotope analysis of fish muscle. *Transactions of the American Fisheries Society*, *131*(2), 337–342. https://doi.org/10.1577/1548-8659(2002)131<0337:PEOSIA>2.0.CO;2

Aubail, A., Méndez-Fernandez, P., Bustamante, P., Churlaud, C., Ferreira, M., Vingada, J. V., & Caurant, F. (2013). Use of skin and blubber tissues of small cetaceans to assess the trace element content of internal organs. *Marine Pollution Bulletin*, *76*(1–2), 158–169. https://doi.org/10.1016/j.marpolbul.2013.09.008

Bond, A. L., Jardine, T. D., & Hobson, K. A. (2016). Multi tissue stable isotope analyses can identify dietary specialization. *Methods in Ecology and Evolution*, *7*(12), 1428–1437. https://doi.org/10.1111/2041-210X.12620

Bosley, K. L., & Wainright, S. C. (1999). Effects of preservatives and acidification on the stable isotope ratios (^{15}N:^{14}N, ^{13}C:^{12}C) of two species of marine animals. *Canadian Journal of Fisheries and Aquatic Sciences*, *56*(11), 2181–2185. https://doi.org/10.1139/f99-153

Brotons, J. M., Islas-Villanueva, V., Alomar, C., Tor, A., Fernández, R., & Deudero, S. (2019). Genetics and stable isotopes reveal non-obvious population structure of bottlenose dolphins (*Tursiops truncatus*) around the Balearic Islands. *Hydrobiologia*, *842*, 233–247. https://doi.org/10.1007/s10750-019-04038-7

Bugoni, L., McGill, R. A., & Furness, R. W. (2008). Effects of preservation methods on stable isotope signatures in bird tissues. *Rapid Communications in Mass Spectrometry*, *22*(16), 2457–2462. https://doi.org/10.1002/rcm.3633

Burrows, D. G., Reichert, W. L., & Bradley Hanson, M. (2014). Effects of decomposition and storage conditions on the $\delta^{13}C$ and $\delta^{15}N$ isotope values of killer whale (*Orcinus orca*) skin and blubber tissues. *Marine Mammal Science*, *30*(2), 747–762. https://doi.org/10.1111/mms.12076

Cloyed, C. S., DaCosta, K. P., Hodanbosi, M. R., & Carmichael, R. H. (2020). The effects of lipid extraction on δ13C and δ15N values and use of lipid correction models across tissues, taxa and trophic groups. *Methods in Ecology and Evolution*, *11*(6), 751–762. https://doi.org/10.1111/2041-210X.13386

Dahn, H. A., Mountcastle, J., Balacco, J., Winkler, S., Bista, I., Schmitt, A. D., Pettersson, O. V., Formenti, G., Oliver, K., Smith, M. & Tan, W. (2022). Benchmarking ultra-high molecular weight DNA preservation methods for long-read and long-range sequencing. *GigaScience*, *11*, giac068. https://doi.org/10.1093/gigascience/giac068

Das, K., Lepoint, G., Leroy, Y., & Bouquegneau, J. M. (2003). Marine mammals from the southern North Sea: feeding ecology data from $\delta^{13}C$ and $\delta^{15}N$ measurements. *Marine Ecology Progress Series*, *263*, 287–298. https://doi.org/10.3354/meps263287

Drago, M., Crespo, E. A., Aguilar, A., Cardona, L., García, N., Dans, S. L., & Goodall, N. (2009). Historic diet change of the South American sea lion in Patagonia as revealed by isotopic analysis. *Marine Ecology Progress Series*, *384*, 273–286. https://doi.org/10.3354/meps08017

Genoves, R. C., Fruet, P. F., Botta, S., Beheregaray, L. B., Möller, L. M., & Secchi, E. R. (2020). Fine-scale genetic structure in Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) is associated with social structure and feeding ecology. *Marine Biology*, *167*, Article 34. https://doi.org/10.1007/s00227-019-3638-6

Hidalgo Reza, M., Elorriaga Verplancken, F. R., Aguíñiga García, S., & Urbán R, J. (2019). Impact of freezing and ethanol preservation techniques on the stable isotope analysis of humpback whale (*Megaptera novaeangliae*) skin. *Rapid Communications in Mass Spectrometry*, *33*(8), 789–794. https://doi.org/10.1002/rcm.8392

Hobson, K. A., & Clark, R. G. (1992). Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. *The Condor*, *94*(1), 189–197. https://doi.org/10.2307/1368808

Hobson, K. A., Gloutney, M. L., & Gibbs, H. L. (1997). Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. *Canadian Journal of Zoology*, *75*(10), 1720–1723. https://doi.org/10.1139/z97-799

Javornik, J., Hopkins, J. B., III, Zavadlav, S., Levanič, T., Lojen, S., Polak, T., & Jerina, K. (2019). Effects of ethanol storage and lipids on stable isotope values in a large mammalian omnivore. *Journal of Mammalogy*, *100*(1), 150–157. https://doi.org/10.1093/jmammal/gyy187

Kilpatrick, C. W. (2002). Noncryogenic preservation of mammalian tissues for DNA extraction: an assessment of storage methods. *Biochemical Genetics*, *40*, 53–62. https://doi.org/10.1023/A:1014541222816

Kiszka, J., Lesage, V., & Ridoux, V. (2014). Effect of ethanol preservation on stable carbon and nitrogen isotope values in cetacean epidermis: Implication for using archived biopsy samples. *Marine Mammal Science*, *30*(2), 788–795. https://doi.org/10.1111/mms.12058

Lesage, V., Morin, Y., Rioux, È., Pomerleau, C., Ferguson, S. H., & Pelletier, É. (2010). Stable isotopes and trace elements as indicators of diet and habitat use in cetaceans: predicting errors related to preservation, lipid extraction, and lipid normalization. *Marine Ecology Progress Series*, *419*, 249–265. https://doi.org/10.3354/meps08825

Lundström, K., Hjerne, O., Lunneryd, S.G. and Karlsson, O. (2010). Understanding the diet composition of marine mammals: grey seals (*Halichoerus grypus*) in the Baltic Sea. *ICES Journal of Marine Science*, *67*(6), 1230–1239. https://doi.org/10.1093/icesjms/fsq022

Marcoux, M., Whitehead, H., & Rendell, L. (2007). Sperm whale feeding variation by location, year, social group and clan: evidence from stable isotopes. *Marine Ecology Progress Series*, *333*, 309–314. https://doi.org/10.3354/meps333309

Newsome, S. D., Chivers, S. J., & Berman Kowalewski, M. (2018). The influence of lipid extraction and long term DMSO preservation on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values in cetacean skin. *Marine Mammal Science*, *34*(2), 277–293. https://doi.org/10.1111/mms.12454

Noren, D. P., & Mocklin, J. A. (2012). Review of cetacean biopsy techniques: Factors contributing to successful sample collection and physiological and behavioral impacts. *Marine Mammal Science*, *28*(1), 154–199. https://doi.org/10.1111/j.1748-7692.2011.00469.x

Olin, J. A., Poulakis, G. R., Stevens, P. W., DeAngelo, J. A., & Fisk, A. T. (2014). Preservation effects on stable isotope values of archived elasmobranch fin tissue: comparisons between frozen and ethanol-stored samples. *Transactions of the American Fisheries Society*, *143*(6), 1569–1576. https://doi.org/10.1080/00028487.2014.954055

Oosting, T., Hilario, E., Wellenreuther, M. and Ritchie, P.A. (2020). DNA degradation in fish: Practical solutions and guidelines to improve DNA preservation for genomic research. *Ecology and Evolution*, *10*(16), 8643–8651. https://doi.org/10.1002/ece3.6558

Parsons, K. M., Durban, J. W., & Claridge, D. E. (2003). Comparing two alternative methods for sampling small cetaceans for molecular analysis. *Marine Mammal Science*, *19*(1), 224–231. https://doi.org/10.1111/j.1748-7692.2003.tb01104.x

Parsons, K. M., Noble, L. R., Reid, R. J., & Thompson, P. M. (2002). Mitochondrial genetic diversity and population structuring of UK bottlenose dolphins (*Tursiops truncatus*): is the NE Scotland population demographically and geographically isolated? *Biological Conservation*, *108*(2), 175–182. https://doi.org/10.1016/S0006-3207(02)00103-9

Phillips, D. L., & Eldridge, P.M. (2006). Estimating the timing of diet shifts using stable isotopes. *Oecologia*, *147*, 195–203. https://doi.org/10.1007/s00442-005-0292-0

Rennie, M. D., Ozersky, T., & Evans, D. O. (2012). Effects of formalin preservation on invertebrate stable isotope values over decadal time scales. *Canadian Journal of Zoology*, *90*(11), 1320–1327. https://doi.org/10.1139/z2012-101

Robertson, K. M., Minich, J., Bowman, A. J., & Morin, P. A. (2013). A thin soup: extraction and amplification of DNA from DMSO and ethanol used as preservative for cetacean tissue samples. *Conservation Genetics Resources*, *5*, 929–933. https://doi.org/10.1007/s12686-013-9934-4

Rosenbaum, H. C., Egan, M. G., Clapham, P. J., Brownell, R. L., Jr., Malik, S., Brown, M. W., White, B. N., Walsh, P., & Desalle, R. (2000). Utility of North Atlantic right whale museum specimens for assessing changes in genetic diversity. *Conservation Biology*, *14*(6), 1837–1842. https://doi.org/10.1111/j.1523-1739.2000.99310.x

Ryan, C., McHugh, B., Trueman, C. N., Harrod, C., Berrow, S. D., & O'Connor, I. (2012). Accounting for the effects of lipids in stable isotope ($\delta^{13}C$ and $\delta^{15}N$ values) analysis of skin and blubber of balaenopterid whales. *Rapid Communications in Mass Spectrometry*, *26*(23), 2745–2754. https://doi.org/10.1002/rcm.6394

Sarakinos, H. C., Johnson, M. L., & Zanden, M. J. V. (2002). A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. *Canadian Journal of Zoology*, *80*(2), 381–387. https://doi.org/10.1139/z02-007

Svensson, E., Freitas, V., Schouten, S., Middelburg, J. J., van der Veer, H. W., & Damsté, J. S. S. (2014). Comparison of the stable carbon and nitrogen isotopic values of gill and white muscle tissue of fish. *Journal of Experimental Marine Biology and Ecology*, *457*, 173–179. https://doi.org/10.1016/j.jembe.2014.04.014

Teixeira, C. R., Troina, G. C., Daura Jorge, F. G., Simões Lopes, P. C., & Botta, S. (2022). A practical guide on stable isotope analysis for cetacean research. *Marine Mammal Science*, *38*(3), 1200–1228. https://doi.org/10.1111/mms.12911

Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., & Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia*, *57*, 32–37. https://doi.org/10.1007/BF00379558

Todd, S., Ostrom, P., Lien, J., & Abrajano, J. (1997). Use of biopsy samples of humpback whale (*Megaptera novaeangliae*) skin for stable isotope ($\delta^{13}C$) determination. *Journal of Northwest Atlantic Fisheries Science*, *22*, 71–76. https://doi.org/10.2960/J.v22.a6

Vander Zanden, M. J., Clayton, M. K., Moody, E. K., Solomon, C.T., & Weidel, B. C. (2015). Stable isotope turnover and half-life in animal tissues: a literature synthesis. *PLoS ONE*, *10*(1), e0116182. https://doi.org/10.1371/journal.pone.0116182

Wandeler, P., Hoeck, P. E., & Keller, L. F. (2007). Back to the future: museum specimens in population genetics. *Trends in Ecology & Evolution*, *22*(12), 634–642. https://doi.org/10.1016/j.tree.2007.08.017

Yurkowski, D. J., Hussey, N. E., Semeniuk, C., Ferguson, S. H., & Fisk, A. T. (2015). Effects of lipid extraction and the utility of lipid normalization models on $\delta^{13}C$ and $\delta^{15}N$ values in Arctic marine mammal tissues. *Polar Biology*, *38*, 131–143. https://doi.org/10.1007/s00300-014-1571-1

Yurkowski, D. J., Hussey, A. J., Hussey, N. E., & Fisk, A. T. (2017). Effects of decomposition on carbon and nitrogen stable isotope values of muscle tissue of varying lipid content from three aquatic vertebrate species. *Rapid Communications in Mass Spectrometry*, *31*(4), 389–395. https://doi.org/10.1002/rcm.7802

Yurkowski, D. J., Hussey, N. E., Semeniuk, C., Ferguson, S. H., & Fisk, A. T. (2015). Effects of lipid extraction and the utility of lipid normalization models on $\delta^{13}C$ and δ 15 N values in Arctic marine mammal tissues. *Polar Biology*, *38*, 131–143. https://doi.org/10.1007/s00300-014-1571-1

Zenteno, L., Crespo, E., Vales, D., Silva, L., Saporiti, F., Oliveira, L. R., Secchi, E. R., Drago, M., Aguilar, A., & Cardona, L. (2015). Dietary consistency of male South American sea lions (*Otaria flavescens*) in southern Brazil during three decades inferred from stable isotope analysis. *Marine Biology*, *162*, 275–289. https://doi.org/10.1007/s00227-014-2597-1

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