RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2008; **22**: 2457–2462
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.3633

Effects of preservation methods on stable isotope signatures in bird tissues

Leandro Bugoni[1]*, Rona A. R. McGill[2] and Robert W. Furness[1]

[1]Institute of Biomedical and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, UK
[2]NERC Life Sciences Mass Spectrometry Facility, Scottish Universities Environmental Research Centre, Scottish Enterprise Technology Park, East Kilbride G75 0QF, UK

Received 18 April 2008; Revised 11 June 2008; Accepted 13 June 2008

Increasing use is being made of stable isotopes as indicators of habitat use and trophic ecology of animals. Preservation of tissues can alter stable isotope signatures. We investigated the effects of addition of ethanol and NaCl solution (hereafter 'salt'), and of freezing and drying, on carbon and nitrogen isotopic values in blood of the spectacled petrel *Procellaria conspicillata*, and compared these with those from simultaneously growing feathers. The mean δ^{13}C values of blood preserved in ethanol was significantly higher, and of blood preserved in salt was significantly lower than that of dried or frozen samples. δ^{13}C values in ethanol showed high variation according to brand and batch and could account for the differences found in δ^{13}C ratios in ethanol-preserved blood samples. Mean δ^{13}C and δ^{15}N values in growing feathers were higher than in blood, suggesting tissue-specific fractionation. We conclude that different methods of preserving tissues such as blood may bias stable isotope values, and urge researchers to consider this issue. Air drying is proposed as a practical and unbiased method for blood preservation in field situations where freezing is not a practical option, and a mathematical approach is suggested to permit comparison between studies using different preservation methods or tissues. Copyright © 2008 John Wiley & Sons, Ltd.

Stable isotopes of carbon and nitrogen in bird tissues have been increasingly used in a range of ecological studies.[1–3] Ecological applications of the stable isotope technique include analysis of food-web structure,[4,5] sexual or age-related segregation,[6] contribution of nutrients from different sources such as marine versus freshwater environments,[7] impact of human activities,[8] habitat use and dispersion,[2,9] and latitudinal differences in the distribution of foraging or wintering areas.[10–12] The method is based on the fact that stable isotopes in prey frequently have distinct values, and are transferred from prey to predators in a predictable manner.[1] Unlike traditional methods (e.g. regurgitations, pellets, stomach flushing) that provide a snapshot of the predator's diet, stable isotopes in animal tissues integrate dietary information over space and time, ranging from days to months.[3]

For bird studies, muscle and bone collagen were the preferred tissues for stable isotopes during early development of the techniques,[1] but feather and blood are currently the preferred tissues because they can be sampled non-destructively and the individual can be re-sampled several times.[13] Thus the techniques could also be used for studies involving endangered species where lethal methods would raise ethical questions. Feathers are generally preserved dry, with no addition of preservative at this stage, but prior to isotope analysis are sometimes washed with substances containing carbon such as chloroform, methanol, ether or a mixture of those,[14–16] under the assumption that subsequent air drying removes added solvents. Conversely, a range of methods has been used to preserve wet tissues for stable isotope analysis. Muscle of fish, amphibians and small reptiles for historical collections is usually fixed in formalin and stored in 70% ethanol[17,18] whereas blood is preserved in ethanol, in heparinised solution, frozen or dried. There is no consensus on the best method for preservation, and some studies suggest that preservation methods alter stable isotope ratios whereas other studies failed to detect any difference caused by preservation.[19,20] Despite controversies and a range of studies detecting changes associating with preservation methods, studies measuring stable isotopes in birds frequently continue to make use of preservatives that contain the isotope to be analysed (e.g. ethanol or heparin for analysis of carbon[21,22]). In addition, most studies do not report potential errors due to preservatives, and a few even do not mention the blood preservation method. The assumption that preservation effects are negligible, even when using a fixative which contains carbon, apparently still persists in the ornithology literature,[e.g.11,23–25] probably because it was previously shown that ethanol does not change the carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic signature of preserved blood of captivity Japanese quail *Coturnix japonica* fed on a commercial ration.[26] Studies from other taxa have, however, shown clear effects of preservation

*Correspondence to: L. Bugoni, Institute of Biomedical and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, UK.
E-mail: l.bugoni.1@research.gla.ac.uk
Contract/grant sponsor: NERC-UK; contract/grant number: EK81-07/05.
Contract/grant sponsor: CAPES Foundation; contract/grant number: 2832-04-6.

methods on stable isotope ratios, e.g. in fish tissues,[19,20,27] and this question has yet to be fully addressed in wild birds. Furthermore, values of nitrogen in formalin from three different brands showed different isotopic signatures,[28] but similar comparisons between batches of ethanol have not yet been made, despite there being a clear need for that.[27]

In the present study we investigate the effect of preservation methods on carbon and nitrogen stable isotope values in whole blood of spectacled petrel (*Procellaria conspicillata*) and propose a simple regression approach to correct for the effects of different preservation methods and tissues. Mathematical normalisation of data has been applied to the problem of samples with high and variable lipid content, which considerably affects the $\delta^{13}C$ and $\delta^{15}N$ values,[29,30] or for the effects of preservatives in fish tissues,[20] but the determination of correction factors has not been previously addressed in bird blood where different preservation methods are used. We were particularly interested in methods that could be used in a range of situations and field conditions, which frequently occur in remote study sites, such as onboard fishing vessels at sea or on remote oceanic islands. We also addressed the effects of preservatives that contain carbon (i.e. ethanol) in comparison with a carbon-free preservative (i.e. NaCl), and measured isotope signatures in different brands and batches of ethanol.

EXPERIMENTAL

Study species

The spectacled petrel is a pelagic seabird endemic to the Tristan da Cunha Archipelago in the South Atlantic Ocean.[31] It is a medium-sized petrel (1.3 kg[32]) with at sea distribution mainly between 25 and 40°S,[33] but concentrated in deep and warm waters of the southwestern Atlantic Ocean, off southern Brazil and Uruguay.[34] The species is the most common seabird following pelagic longliners off the Brazilian coast throughout the year[34,35] feeding on baits and offal discharged by vessels, and non-negligible numbers are killed by pelagic longline fisheries[35] and a range of other hook-and-line fisheries.[36]

Sampling methods and preservation

Twenty-one spectacled petrels were sampled over the continental shelf in southern Brazil, in February 2006. Birds scavenging for offal and baits were attracted close to the vessel and captured using a cast net.[37] Birds were individually marked using metal rings to avoid re-sampling the same individual. Needles and syringes were used to collect 1 mL of blood from the tarsal vein of birds. Sub-samples composed of two or three drops of whole blood were stored in different ways for testing for the effects of differences in preservation methods on carbon and nitrogen isotope ratios. Treatments (preservations methods) were: (1) blood in 1.5 mL of absolute Merck® ethanol; (2) blood in 1.5 mL of saturated salt solution ($NaCl/H_2O$); (3) drops of blood placed in a glass microscope slide (previously cleaned of possible surface contaminants using cotton), dried in air, scraped and stored in plastic tubes; and (4) blood frozen in the facilities available onboard the vessel. In addition, five to eight partially grown body feathers, still with blood in the calamus (shaft) were collected, the blood in the calamus removed, and the feathers placed in sealed plastic bags. Growing feathers are tissues with similar age to whole blood, i.e. a few weeks, and assumed to be formed by the same pool of nutrients circulating in the blood. All samples were transported under permit to the University of Glasgow where they were prepared for analysis. During transport, frozen samples were maintained in a Styrofoam box with ice replaced frequently.

Carbon isotope values in the absolute ethanol from two bottles of different batches from Merck® (Darmstadt, Germany), one of which was also used for blood preservation, and from a bottle from Fisher Scientific (Loughborough, UK) were analysed. As the $\delta^{13}C$ values between brands were different and close to values expected for C_3 and C_4 plants (see Results and Discussion), we contacted manufacturers asking for methods of ethanol production, and were informed that both brands and batches were chemically produced in the laboratory, rather than being derived from crops.

Sample analysis

All blood samples were freeze-dried, ground and homogenised. Feathers were washed five times in distilled water, dried in an oven for 2 h under a constant temperature of 70°C and cut with scissors. Fragments of several feathers were used. Samples of approximately 0.65 mg of blood or 0.70 mg of feathers were weighed, placed in tin capsules (4 × 6 mm) and analysed by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a model ECS 4010 elemental analyser (Costech, Milan, Italy) linked to a Delta Plus XP mass spectrometer (ThermoFinnigan, Bremen, Germany). Three laboratory standards were analysed for every ten unknown samples in each analytical sequence, allowing instrument drift to be corrected. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) deviation from the international standards Pee Dee Belemnite limestone (carbon) and atmospheric air (nitrogen), according to the following equation:

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

where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Samples were shuffled in the analytical sequence according to different tissues, individuals, and preservation methods to minimise drift with time.[38] For ethanol samples, an aliquot of 1.5 μL was pipetted into model D4042 smooth wall tin capsules (5 × 2 mm; Elemental Micro Analysis Ltd., Okehampton, UK) and were sealed using small wire cutters (side cutters) and analysed as above.

Data analysis

$\delta^{15}N$ and $\delta^{13}C$ values for each preservation method were compared by one-way analysis of variance (ANOVA) considering individual birds as random effects, for both nitrogen and carbon. $\delta^{13}C$ values were transformed $\log(n \times -1)$, thus accounting for negative values, and aiming to achieve normality (tested by Kolmogorov-Smirnov test) and homoscedasticy of residuals (tested by Levene's test). For post hoc comparisons Tukey's HSD test was used. Correlation coefficients between values found for preservation

DOI: 10.1002/rcm

Table 1. Mean ± one standard deviation (minimum and maximum) of values of carbon and nitrogen stable isotope ratios in blood and growing feathers of spectacled petrel *Procellaria conspicillata* sampled in southern Brazil and preserved using different methods

Sample (sample size)	δ^{13}C (‰)	δ^{15}N (‰)
Blood		
Dried (21)	−17.7 ± 0.7 (−20.5 to −16.7)	14.0 ± 0.8 (12.3 to 15.3)
Frozen (20)	−17.7 ± 0.5 (−18.4 to −16.7)	14.0 ± 0.8 (12.3 to 15.4)
Absolute ethanol (21)	−17.1 ± 0.4 (−17.8 to −16.3)	14.3 ± 0.8 (12.4 to 15.7)
Saturated NaCl solution (18)	−18.4 ± 1.2 (−22.5 to −16.7)	13.6 ± 0.7 (12.2 to 14.8)
Feathers (21)	−16.0 ± 0.6 (−16.9 to −14.8)	15.5 ± 1.0 (13.8 to 17.1)
Absolute ethanol Merck batch 1 (10)	−12.1 ± 0.1 (−12.2 to −12.0)	—
Absolute ethanol Merck batch 2 (5)	−24.3 ± 0.1 (−24.5 to −24.2)	—
Absolute ethanol Fisher (10)	−30.0 ± 0.1 (−30.0 to −29.9)	—

methods were calculated and the Bonferroni correction applied for multiple comparisons. Values were reported as mean ± one standard deviation (SD). The measurement precision of both δ ^{15}N and δ ^{13}C was better than 0.3‰.

RESULTS

Carbon

Mean δ^{13}C in blood varied from −17.1 to −18.4‰ and in growing feathers was −16.0‰ (Table 1, Fig. 1). Random effects were significant ($F_{20,75} = 13.6$, $p < 0.001$), indicating consistent differences among individuals. Different preservation methods affected the carbon signatures in blood ($F_{4,75} = 174.3$, $p < 0.001$, Fig. 1). Carbon isotope ratios were different for all treatments ($p < 0.001$), except between dry and frozen samples ($p = 0.34$). Ethanol-preserved samples were ^{13}C-enriched and simultaneously growing feathers considerably enriched in comparison with dry and frozen blood samples (Fig. 1). In contrast, NaCl-preserved samples were ^{13}C-depleted, and values were more variable than for other preservation methods (see SD values in Table 1), despite non-significant differences in variance of untransformed data between treatments (Levene's test = 0.69, $p = 0.6$).

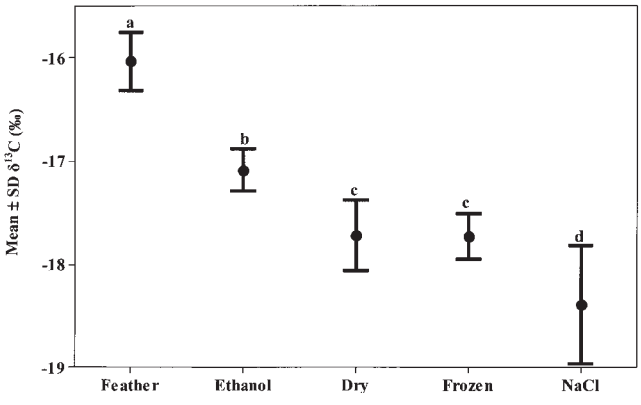

Figure 1. Carbon isotope ratios in blood preserved in different ways and in simultaneously growing feathers of spectacled petrels *Procellaria conspicillata* wintering off Brazil. Different letters indicates significant differences at $p < 0.05$ (Tukey's HSD test).

Carbon isotope ratios in ethanol differed, showing very different values according to manufacturer (Table 1). Merck ethanol used for blood preservation in this study had higher δ^{13}C values (−12.1‰) than the range of individual values found in blood samples (−22.5 to −16.3‰), whereas Fisher ethanol had lower δ^{13}C value (−30.0‰). Another batch of Merck ethanol had intermediate values of −24.3‰.

Correlations in δ^{13}C values in blood preserved using the different methods, and feathers, among individual birds were strong, despite the effect of treatment (Table 2).

Nitrogen

Mean values of δ^{15}N in blood varied from 13.6‰ to 14.0‰ according to different treatments and were significantly more enriched in growing feathers (15.5‰, Table 1, Fig. 2). Variation among treatments was also found in a similar way as found for carbon ($F_{4,75} = 174.3$, $p < 0.001$, Fig. 2). There were also differences between individuals (random effects $F_{20,75} = 13.6$, $p < 0.001$). The main distinction in comparison with carbon was that nitrogen isotope ratios in samples preserved in ethanol were not significantly different from those in dry and frozen samples ($p = 0.11$ and $p = 0.10$, respectively), a predicted result since ethanol does not contain nitrogen. NaCl-preserved samples were ^{15}N-depleted, but values showed similar variability to other treatments (Table 1, Fig. 2).

Correlations in δ^{15}N values in blood preserved using the different methods, and feathers, among individual birds were high, despite the effect of treatment (Table 3).

Mathematical correction

It is shown in the present study (Tables 2 and 3) that there is strong correlation, across individual birds, of isotopic values in different tissues for both δ^{15}N and δ^{13}C. This property allows the data to be mathematically corrected. For example, using the equation:

$$\delta^{13}C_{Frozen} = -7.794 + 0.6176\delta^{13}C_{Feather}$$

obtained from the correlation between δ^{13}C in growing feather and in frozen blood in spectacled petrels, one could remove the effect of different tissues and make values obtained by the analysis of different tissues comparable. Similarly, the effects of preservation methods can be corrected. For instance, to remove the effect caused by

DOI: 10.1002/rcm

RCM

Table 2. Correlation coefficients of carbon isotope ratios across individual birds in blood preserved in different ways and simultaneously growing feathers of spectacled petrels *Procellaria conspicillata*. All values are significant at $p < 0.005$ (Bonferroni correction for multiple comparisons applied)

Treatments (sample size)	Blood dried	Blood frozen	Blood in absolute ethanol	Blood in saturated NaCl solution
Blood frozen (n = 20)	0.66			
Blood in absolute ethanol (n = 21)	0.88	0.75		
Blood in NaCl saturated solution (n = 17)	0.79	0.64[a]	0.85	
Growing feathers (n = 21)	0.71	0.75	0.81	0.69

[a]Non-significant after Bonferroni correction ($p = 0.0056$).

ethanol on $\delta^{13}C$ values in blood, one could use the equation:

$$\delta^{13}C_{Frozen} = -3.395 + 0.8371\delta^{13}C_{Ethanol}$$

which relates $\delta^{13}C$ values in frozen blood with $\delta^{13}C$ values in ethanol-preserved blood.

DISCUSSION

Ecological questions addressed by stable isotope analysis often require detection of changes of less than 2‰ (see[39] and references cited therein). For example, diet-tissue fractionation of $\delta^{13}C$ in trophodynamic studies is assumed to be 0–1‰ per trophic level,[40,41] or smaller, among species in studies comparing several species in a community (e.g.[4] and references cited therein). Thus, variation caused by preservation could influence the interpretation of a biological phenomenon underlying the differences. As we have shown here, differences of this scale could be caused by the preservation method (e.g. $\delta^{13}C$ mean difference in blood preserved in NaCl vs. ethanol = 1.3‰), different tissues being synthesised simultaneously (e.g. mean difference in $\delta^{15}N$ in frozen blood vs. growing feathers = 1.5‰, see also[42]), or using different brands or batches of the same type of preservative (Merck ethanol vs. Fisher ethanol, Table 1). It is also noteworthy that, although we freeze-dried blood samples prior to isotope analysis, the results show clearly

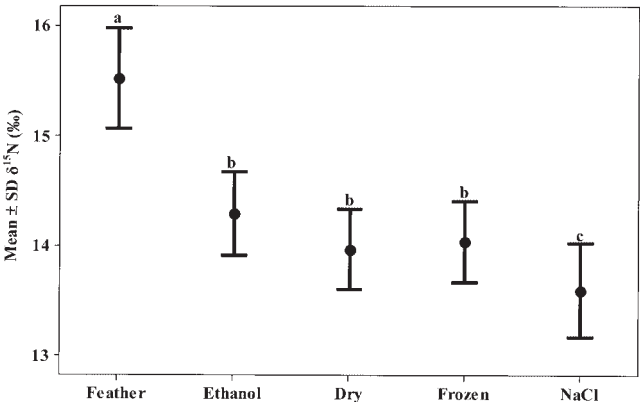

Figure 2. Nitrogen isotope ratios in blood preserved in different ways and in simultaneously growing feathers of spectacled petrels *Procellaria conspicillata* wintering off Brazil. Different letters indicates significant differences at $p < 0.05$ (Tukey's HSD test).

that this procedure did not remove the influence of ethanol on the carbon isotope signal. These examples emphasise the need to be consistent, which means being aware of the isotopes in the preservatives and using the same brand and/or batch of preservative (first mixing several bottles if large volumes are required). Lack of information on preservatives used may preclude accurate comparisons across different studies.

In studies of seabirds using stable isotopes, samples are frequently collected in different islands or colonies and preservation methods can be chosen based on practicality in field situations, sometimes resulting in the preservation of blood in one way and potential prey such as fish, squid, and crustaceans in another. Additional problems may occur using a standard preservative, e.g. ethanol, without taking into account the carbon signature in the preservative. By using ethanol from different crops, such as C_3 and C_4 plants, or different brands, the changes to the stable isotope value of the sample caused by the preservative could be as great as the biological differences under investigation.

A decrease of $\delta^{13}C$ (by 0.7‰) and $\delta^{15}N$ (by 0.6‰) in samples preserved in NaCl solution in comparison with frozen blood, and greater variance in values from samples, parallel results reported for the use of mercury chloride ($HgCl_2$) to preserve fish and crustacean tissues.[43] Mechanical cell destruction and loss of cytosol or metabolic shifts may occur. These effects were suggested as possible causes of decrease in carbon and nitrogen values in bulk frozen macrozoobenthos.[44] From a fieldwork perspective, NaCl is an easy method to use to preserve a range of tissues and is applicable in remote field sites, but it greatly affected results for carbon and nitrogen isotopes and resulted in greater sample variability. In contrast to our results, a previous study[17] suggested that salt is as good as freezing for the preservation of fish muscle for $\delta^{13}C$, but that it significantly altered values of $\delta^{15}N$.

Using ethanol to preserve blood significantly increased $\delta^{13}C$ (0.6‰), but caused a non-significant increase in $\delta^{15}N$ (0.3‰). A similar enrichment in $\delta^{13}C$ of 0.7–1.5‰ was also detected in fish, octopus and kelp,[45] fish muscle and liver,[27] and fish and mollusc muscle.[19] For blood samples preserved in ethanol, mechanical cell destruction could potentially occur, with effects similar to those suggested above for blood preserved in NaCl. This process resulted in lower carbon isotope values in blood samples, but the $\delta^{13}C$ value of the ethanol itself is much higher. Carbon isotope values of blood preserved in ethanol were higher than for the dried and frozen samples, which suggests that

DOI: 10.1002/rcm

Table 3. Correlation coefficients of nitrogen isotope ratios across individual birds in blood preserved in different ways and simultaneously growing feathers of spectacled petrels *Procellaria conspicillata*. All values are significant at $p < 0.005$ (Bonferroni correction for multiple comparisons applied)

Treatments (sample size)	Blood dried	Blood frozen	Blood in absolute ethanol	Blood in saturated NaCl solution
Blood frozen (n = 20)	0.87			
Blood in absolute ethanol (n = 21)	0.90	0.91		
Blood in NaCl saturated solution (n = 17)	0.79	0.96	0.91	
Growing feathers (n = 21)	0.71	0.85	0.82	0.84

some carbon present in the ethanol was incorporated into the sample. It is suggested that ethanol alters the carbon signature by acting as a solvent of compounds which have lower carbon values such as lipids.[27,44] Blood is a lipid-poor tissue, so this is unlikely to be the case in the present study. Moreover, the $\delta^{13}C$ value in ethanol used previously to preserve fish tissues[27] was −28.72‰ and this also resulted in ^{13}C enrichment in samples, although this $\delta^{13}C$ value in ethanol was lower than $\delta^{13}C$ values in samples. These authors suggested that ethanol could be used in low fat tissues, but our results have shown that ethanol does affect $\delta^{13}C$ values in lipid-poor tissues such as blood.

Drying blood at ambient air temperature is also a valuable method of blood preservation in remote field sites because only two or three drops of blood are enough for stable isotope analysis. These drops can be dried on a clean glass slide, scraped off once dry and stored in a tube. This method was as effective as freezing samples for $\delta^{13}C$ and $\delta^{15}N$ in the current study and dispenses with the need for a power supply for freezing and sample storage.

There is no consensus regarding sample preservation and preparation for stable isotope analysis. Some aspects such as the choice of tissue are hard to standardise because different tissues provide information on diet at different time scales. However, preservation methods could be standardised in order to improve the comparability of data across studies. Differences between feather and blood isotopic signatures are due to different protein turnover rates and metabolic processes;[21,46,47] thus different tissues are not directly comparable.[21] Feathers usually have enriched ^{13}C and ^{15}N values relative to blood,[21] as we found in this study. However, values in different tissues could be arithmetically corrected if their relationships are first determined, as with the equations based on regressions shown in the Results section. Mathematical corrections could also be applied to $\delta^{15}N$ values where shifts are consistent, as demonstrated in this study (Table 3). Moreover, empirical mathematical corrections should be considered on a case-specific basis, because they can vary according to tissue, species, isotope and preservation method, preservative brand and/or batch; or no correlation maybe found,[27] so no correction is possible. Thus, despite the promising nature of the method proposed here for inter-tissue comparison, more detailed studies are required in order to test their validity under different conditions. Understanding the effects of preservatives and fixatives in different tissues, with the simultaneous establishment of corrections factors, could greatly aid the use of isotope ratios in preserved specimens from museums.[17,28] Lipid composition in fish tissues is variable and has been shown to influence isotopic values in preserved samples, which complicates the determination of a non-species-specific correction.[20,48] Despite variations in lipid composition in bird blood according to absorptive,[13] reproductive and nutritional states,[49] large variations in lipid content are not expected and a general correction factor is a further possibility.

Carbon isotope ratios in ethanol measured in the present study were distinguished by a difference of 17.9‰ between two different producers, with values characteristic of C_3 and C_4 plants (Fisher and Merck, respectively) and two different batches from the same manufacturer showed a difference of 12.2‰ (Table 1). However, the manufacturers informed the authors that both were derived in laboratory rather than produced from crops. Thus the measured carbon isotope difference was caused by some chemical procedure or variation in the carbon present in the raw material used. Differences in the signatures of C_3 and C_4 plants have been widely used in ecological studies,[1] as well as for distinguishing ethanol produced from C_3 (e.g. barley, sugar beet) or C_4 plants (e.g. corn, sugar cane).[50] Isotopic differences in ethanol are largely used to determine the authenticity of food, beverages, cosmetics and other products.[50] Because differences in ethanol $\delta^{13}C$ values vary widely according to the crop (from −10.7 to −27.7‰ reported in[50]), or the brand (−12.1 and −30.0‰, this study), ethanol can potentially introduce significant errors in the isotopic values of blood samples. We did not test the effect of ethanol from different manufacturers on blood, but because ^{13}C-enriched ethanol increased the $\delta^{13}C$ value in blood, we can predict that ^{13}C-depleted ethanol will cause a decrease in $\delta^{13}C$ values in bird blood. $\delta^{13}C$ in fish muscle was shown to have isotopic values altered by ^{13}C-depleted formalin used as fixative from three different manufacturers and delta values ranging from −37.8 to −52.5‰.[28] These authors demonstrated that the magnitude of carbon isotopic change in tissues can depend on the isotopic composition in the fixative. To address the problem of variation in seabird studies due to the use of different preservation methods or chemical preservatives (or the same preservative with different isotopic signatures) within prey types or between prey and bird tissues we strongly recommend improved standardisation of sample preparation. In particular the isotope value of the preservation agent should be measured or known, e.g. $\delta^{13}C$ analysis for ethanol was a simple procedure in this study, and ethanol aliquots were run in parallel with tissue samples.[27,50]

DOI: 10.1002/rcm

CONCLUDING REMARKS

In general, different studies agree that the freezing of samples causes little or no effect on organic tissues for stable isotope analysis ([27,29] but see[51]), consistent with our results on spectacled petrel blood. Freezing samples is a good option in some field situations and when a portable freezer or dry ice is available. When possible, air drying could also be a practical, inexpensive and reliable method for blood preservation. Drying could also be feasible in some places by using an oven/grill or similar equipment that does not produce fire and smoke.

From the results presented here, freezing and drying preservation methods are the recommended options for $\delta^{13}C$ and $\delta^{15}N$ studies, when logistics allow the use of these techniques. However, due to the practicality of ethanol as a fixative and preservative, particularly for muscle, their utility in $\delta^{13}C$ studies could be further benefited by mathematical corrections like those suggested here, while its effect on $\delta^{15}N$ appears to be negligible. The development of species-specific correction factors is recommended until the effects of preservation methods across species and tissues are better understood.

Acknowledgements

We thank Drs P. Bize, L. D'Alba and J. Newton for helpful comments and support during lab work and data analysis. Thanks are also due to Captain C. Oliveira and the crew of the fishing vessel 'Ana Amaral I' for logistic support onboard the vessel. Sample analysis was funded by the Natural Environment Research Council (NERC-UK, Grant No. EK81-07/05). L.B. received a CAPES Scholarship. This study was carried out according to permits No. 0128931BR, No. 203/2006, No. 02001.005981/2005, No. 023/2006, No. 040/2006 and No. 1282/1, all granted by the Brazilian Environmental Agency (IBAMA), and International Animal Health Certificate No. 0975-06, issued by the Brazilian Government. The Scottish Executive - Rural Affairs Directorate also kindly provided us the permit POAO 2007/91 to import samples into Scotland. Thanks are due to anonymous referees for helpful comments.

REFERENCES

1. Kelly JF. *Can. J. Zool.* 2000; **78**: 1.
2. Hobson KA. *Auk* 2005; **122**: 1037.
3. Barrett RT, Camphuysen K, Anker-Nilssen T, Chardine JW, Furness RW, Garthe S, Hüppop O, Leopold MF, Montevecchi WA, Veit RR. *ICES J. Mar. Sci.* 2007; **64**: 1675.
4. Forero MG, Bortolotti GR, Hobson KA, Donazar JA, Bertelloti M, Blanco G. *J. Anim. Ecol.* 2004; **73**: 789.
5. Aberle N, Malzahn AM. *Oecologia* 2007; **154**: 291.
6. Forero MG, González-Solís J, Hobson KA, Donázar JA, Bertellotti M, Blanco G, Bortolotti GR. *Mar. Ecol. Prog. Ser.* 2005; **296**: 107.
7. Bearhop S, Thompson DR, Waldron S, Russel IC, Alexander G, Furness RW. *J. Appl. Ecol.* 1999; **36**: 75.
8. Bearhop S, Thompson DR, Phillips RA, Waldron S, Hamer KC, Gray CM, Votier SC, Ross BP, Furness RW. *Condor* 2001; **103**: 802.
9. Bearhop S, Furness RW, Hilton GM, Votier SC, Waldron S. *Funct. Ecol.* 2003; **17**: 270.
10. Quillfeldt P, McGill RAR, Furness RW. *Mar. Ecol. Prog. Ser.* 2005; **295**: 295.
11. Cherel Y, Hobson KA, Guinet C, Vanpe C. *J. Anim. Ecol.* 2007; **76**: 826.
12. Gómez-Díaz E, González-Solís J. *Ecol. Appl.* 2007; **17**: 1484.
13. Bearhop S, Waldron S, Votier SC, Furness RW. *Physiol. Biochem. Zool.* 2002; **75**: 451.
14. Cherel Y, Phillips RA, Hobson KA, McGill R. *Biol. Lett.* 2006; **2**: 301.
15. Hedd A, Montevecchi WA. *Mar. Ecol. Prog. Ser.* 2006; **322**: 291.
16. Becker BH, Newman SH, Inglis S, Beissinger SR. *Condor* 2007; **109**: 451.
17. Arrington BA, Winemiller KO. *Trans. Am. Fish. Soc.* 2002; **131**: 337.
18. Araújo MS, Bolnick DI, Machado G, Giaretta AA, Reis SF. *Oecologia* 2007; **152**: 643.
19. Sarakinos HC, Johnson ML, Zanden MJV. *Can. J. Zool.* 2002; **80**: 381.
20. Kelly B, Dempson JB, Power M. *J. Fish Biol.* 2006; **69**: 1595.
21. Cherel Y, Hobson KA. *Mar. Ecol. Prog. Ser.* 2007; **329**: 281.
22. Williams CT, Buck CL, Sears J, Kitaysky AS. *Oecologia* 2007; **153**: 11.
23. Harding AMA, Hobson KA, Walkusz W, Dmoch K, Karnovsky NJ, Pelt TIV, Lifjeld JT. *Polar Biol.* 2008; **31**: 725.
24. Haramis GM, Link WA, Osenton PC, Carter DB, Weber RG, Clark NA, Teece MA, Mizrahi DS. *J. Avian Biol.* 2007; **38**: 367.
25. Cherel Y. *Mar. Biol.* 2008; **154**: 813.
26. Hobson KA, Gibbs HL, Gloutney ML. *Can. J. Zool.* 1997; **75**: 1720.
27. Sweeting CJ, Polunin NVC, Jennings S. *Rapid Commun. Mass Spectrom.* 2004; **18**: 2587.
28. Edwards MS, Turner TF, Sharp ZD. *Copeia* 2002; **2002**: 1106.
29. Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG. *Oecologia* 2007; **152**: 179.
30. Logan JM, Lutcavage ME. *Rapid Commun. Mass Spectrom.* 2008; **22**: 1081.
31. Ryan PG, Dorse C, Hilton GM. *Biol. Conserv.* 2006; **131**: 575.
32. Ryan PG. *Bird Conserv. Internat.* 1998; **8**: 223.
33. Enticott JW, O'Connell M. *Br. Antarct. Surv. Bull.* 1985; **66**: 83.
34. Neves T, Vooren CM, Bugoni L, Olmos F, Nascimento L. Distribuição e abundância de aves marinhas no sul do Brasil. In *Aves oceânicas da região sudeste-sul do Brasil*, Neves T, Bugoni L, Rossi-Wongtschowski CLB (eds). REVIZEE: São Paulo, 2006; 11.
35. Bugoni L, Mancini PL, Monteiro DS, Nascimento L, Neves TS. *Endang. Species Res.* 2008; in press. DOI: 10.3354/esr00115.
36. Bugoni L, Neves TS, Leite-Jr NO, Carvalho D, Sales G, Furness RW, Stein CE, Peppes FV, Giffoni BB, Monteiro DS. *Fish. Res.* 2008; **90**: 217.
37. Bugoni L, Neves TS, Peppes FV, Furness RW. *J. Field Ornithol.* 2008; **79**: in press.
38. Jardine TD, Cunjak RA. *Oecologia* **144**: 528.
39. Sweeting CJ, Jennings S, Polunin NVC. *Funct. Ecol.* 2005; **19**: 777.
40. Ng JSS, Wai TC, Williams GA. *Mar. Chem.* 2007; **103**: 97.
41. Sweeting CJ, Barry JT, Polunin NVC, Jennings S. *J. Exp. Mar. Biol. Ecol.* 2007; **352**: 165.
42. Cherel Y, Hobson KA, Hassani S. *Physiol. Biochem. Zool.* 2005; **78**: 106.
43. Bosley KL, Wainright SC. *Can. J. Fish. Aquat. Sci.* 1999; **56**: 2181.
44. Dannheim J, Struck U, Brey T. *J. Exp. Mar. Biol. Ecol.* 2007; **351**: 37.
45. Kaehler S, Pakhomov EA. *Mar. Ecol. Prog. Ser.* 2001; **219**: 299.
46. Hobson KA, Clark RG. *Condor* 1992; **94**: 189.
47. Vanderklift MA, Ponsard S. *Oecologia* 2003; **136**: 169.
48. Sweeting CJ, Polunin NVC, Jennings S. *Rapid Commun. Mass Spectrom.* 2006; **20**: 595.
49. Hobson KA, Alisauskas RT, Clark RG. *Condor* 1993; **95**: 388.
50. Ishida-Fujii K, Goto S, Uemura R, Yamada K, Sato M, Yoshida N. *Biosc. Biotechnol. Biochem.* 2005; **69**: 2193.
51. Feuchtmayr H, Grey J. *Rapid Commun. Mass Spectrom.* 2003; **17**: 2605.

DOI: 10.1002/rcm