TECHNICAL COMMENT

Effects of Preservation Method on Stable Carbon and Nitrogen Isotope Values

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ABSTRACT

Some methods of tissue preservation have significant effects on values of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), but studies on this topic are scattered in the literature. The goals of this study were to (1) summarize the results from studies of preservation effects in the literature and (2) test the effects of four common preservatives on $\delta^{13}C$ and $\delta^{15}N$ in epidermis tissue of three turtle species. Turtle tissue samples were subjected to up to five time intervals in five methods of preservation: drying at 60°C for 24 h (the control), immersion in a 70% ethanol solution, immersion in a saturated NaCl aqueous solution, freezing at −10°C in a frost-free freezer, and immersion in a dimethyl sulfoxide (DMSO)–ethylenediaminetetraacetic acid buffer. The $\delta^{13}C$ and $\delta^{15}N$ values for tissues preserved in 70% ethanol and NaCl aqueous solution were not significantly different from those of tissues dried at 60°C, but samples preserved in DMSO were significantly different from dried samples. Freezing preservation had a significant effect on $\delta^{13}C$ and $\delta^{15}N$ at 60 d, which may have resulted from the use of a frost-free freezer. The effects of 20 different preservative methods on $\delta^{13}C$ and $\delta^{15}N$ in different tissues are summarized.

Introduction

Stable isotope analysis is increasingly being used by field researchers to answer questions about habitat use, migration patterns, and diets of various organisms (e.g., Braune et al. 2002; Hatase et al. 2002; Kurle and Worthy 2002). Samples collected in the field must often be preserved for varying amounts of time before analyses can be conducted in the laboratory. If the preservation technique alters the isotopic values, improper interpretation of the results will ensue. Similarly, the effects of preservatives are a concern when using archived samples in museum collections (Kiriluk et al. 1997; Hobbie et al. 2001). If preservation significantly affects archived samples, it impacts these repositories' tremendous potential for reconstructing food webs of past ecosystems. However, despite the possible severe effects of preservation, studies on the effects of the type and the duration of preservation on stable isotopes in tissue samples have been limited and are scattered in the literature. The goals of this study were to (1) summarize the results in the literature of studies of preservation effects and (2) test the effects of four common preservatives on $\delta^{13}C$ and $\delta^{15}N$ in epidermis tissue of turtles to validate our studies in these species.

Material and Methods

These studies were conducted in compliance with the Florida Fish and Wildlife Conservation Commission (permit TP016) and the University of Florida Institutional Animal Care and Use Committee (protocol E025). Four sea turtles that were stranded alive on the southeast coast of Florida were necropsied shortly after their death at the University of Florida (UF) School of Veterinary Medicine, and epidermal samples were collected. The two green turtles, *Chelonia mydas*, had a curved carapace length [CCL] of 29.0 cm (*Chelonia* 1) and 44.2 cm (*Chelonia* 2) The two loggerhead turtles, *Caretta caretta*, had a CCL of 68.5 cm (*Caretta* 1) and 58.0 cm (*Caretta* 2). Two red-eared slider turtles, *Trachemys scripta elegans* (*Trachemys* 1, CCL = 18.1 cm; *Trachemys* 2, CCL = 18.8 cm), that were wild-caught adults from Louisiana were killed as controls used in an experiment at the UF School of Veterinary Medicine, and epidermal samples were collected shortly after death. No turtles were killed for this study.

The epidermis was cleaned with alcohol and then washed thoroughly with deionized water to remove the alcohol and any loose particles. The brief exposure to alcohol is unlikely to affect the results. Three epidermal samples were collected for each treatment to be tested. That is, for *Chelonia* 1, 27 samples were collected (Table 1) with 6-mm Miltex biopsy punches. Five preservation methods were used: drying at 60°C for 24 h (the control), immersion in 70% ethanol, immersion in saturated NaCl (sodium chloride) aqueous solution, freezing at

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Table 1: Number of samples in each treatment at each time interval

Treatment, Time (d)	*Chelonia* 1	*Chelonia* 2	*Caretta* 1	*Caretta* 2	*Trachemys* 1	*Trachemys* 2	Total Sample
Dried at 60°C:							
0	3	3	3	3	3	3	18
Ethanol:							
1	3	3	3	3	…	…	12
4	3	3	3	3	3	3	18
15	3	3	3	3	3	3	18
30	3	3	3	3	3	3	18
60	…	3	…	3	…	…	6
Saturated NaCl:							
1	…	3	…	3	…	…	6
4	…	3	…	3	…	…	6
15	…	3	…	3	…	…	6
30	…	3	…	3	…	…	6
60	…	3	…	3	…	…	6
Frozen:							
1	3	3	3	3	…	…	12
4	3	3	3	3	3	3	18
15	3	3	3	3	3	3	18
30	3	3	3	3	3	3	18
60	…	3	…	3	…	…	6
DMSO buffer:							
1	…	3	…	3	…	…	6
4	…	3	…	3	…	…	6
15	…	3	…	3	…	…	6
30	…	3	…	3	…	…	6
60	…	3	…	3	…	…	6

Note. Ellipses indicate no samples were tested. The total number of samples was run for both lipid and lipid extracted samples. See text for description of treatments. DMSO = dimethyl sulfoxide.

−10°C in a frost-free freezer, and immersion in dimethyl sulfoxide (DMSO) buffer (250 mM EDTA [ethylenediaminetetraacetic acid] pH 7.5; 20% DMSO). The samples that were frozen or placed in preservative solutions were held for different time intervals (Table 1). The numbers of samples collected from each turtle for each treatment (preservative × duration) are shown in Table 1. Each sample was placed in a separate vial; that is, three tissue samples collected from one turtle were placed in three vials.

For analysis, samples were removed from each treatment, washed in deionized water, cleaned of connective tissue, diced with a scalpel blade, placed into individual cryovials, and dried at 60°C for 24 h. Lipids were extracted from half of each sample using petroleum ether in a Dionex accelerated solvent extractor (Dodds et al. 2004). Samples, both with and without lipids, were prepared for analysis of stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) by loading 350–550 μg of dried epidermis tissue into tin capsules. These capsules were combusted using a COSTECH ECS 4010 elemental analyzer interfaced via a Finnigan-MAT ConFlow III device to a Finnigan-MAT DeltaPlus XL isotope ratio mass spectrometer. Stable isotope values are expressed in delta (δ) notation, defined as parts per thousand (‰) relative to a standard for carbon and nitrogen, using the following equation:

$$\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1{,}000,$$

where X is ^{13}C or ^{15}N and R_{sample} and R_{standard} are the corresponding ratios of heavy to light isotopes (^{13}C/^{12}C and ^{15}N/^{14}N) in the sample and international standard, respectively. The R_{standard} for ^{13}C was Vienna Pee Dee Belemnite, and for ^{15}N, it was atmospheric N_2. Internal standards were inserted in all runs at regular intervals to calibrate the system and to assess drift over time. Standard deviations of internal standard replicates were 0.11‰ ($N = 88$) and 0.11‰ ($N = 91$) for carbon and nitrogen, respectively.

Data were analyzed according to a randomized complete-block design. Turtles were the random blocks, and the three factors were source (marine or freshwater species), presence or absence of lipids, and treatment (each of 21 combinations of time and preservative). Not all turtles were sampled for all treatments because of different amounts of available epidermis

with homogeneous appearance (Table 1). Replicate samples from each turtle were nested in the model. Dunnett's multiple comparison method was used to determine any significant differences between treatment and the control, and *P* values were calculated with Dunnett's adjustment to control for experiment-wise Type I error (Dunnett 1980). All statistical analyses were performed using SAS software, version 9.1 (SAS Institute, Cary, NC). The α value was 0.05.

Results and Discussion

Lipid extraction did not have a significant effect on the response of δ^{15}N or δ^{13}C to treatment. For δ^{15}N, there was no significant interaction between preservative treatments and presence/absence of lipids ($F_{20,115} = 0.54$, $P = 0.9413$). For δ^{13}C, there was a significant interaction between preservative treatments and the presence/absence of lipids ($F_{20,116} = 2.92$, $P < 0.01$), but inspection of Tukey's pairwise comparisons between all treatment pairs with and without lipids revealed a significant difference only for δ^{13}C for the DMSO treatment on day 4 ($P = 0.0025$). However, the DMSO treatment on day 4 yielded a significant treatment effect for samples with and without lipids. Therefore, lipid extraction did not affect the conclusion, and results were combined for samples with and without lipids.

Results on the main effect of turtle source (marine or freshwater species) were not meaningful because not all treatments were tested for marine and freshwater species (Table 1). Inspection of the data in Tables A1 and B1 in the online edition of *Physiological and Biochemical Zoology*, however, reveals no difference in preservative effects between marine and freshwater turtles.

The effects of preservative type and duration were the same for δ^{13}C and δ^{15}N (Table 2). Isotope values from samples preserved in ethanol or saturated NaCl for all durations were not significantly different from those of the dried controls, but δ^{13}C on day 1 and δ^{15}N on day 15 for NaCl approached significance. Samples frozen through 30 d were not significantly different from those of the dried controls, but samples frozen for 60 d had significant preservation effects. Samples stored in DMSO buffer samples displayed a surprising pattern: samples preserved for 1–30 d had significant preservation effects, but samples preserved for 60 d demonstrated a loss or reversal of the preservative effect for both δ^{13}C and δ^{15}N.

Several studies that evaluated the effect of preservation in 70% ethanol over time also found no significant effect on δ^{13}C and δ^{15}N (Hobson et al. 1997; Gloutney and Hobson 1998; Table C1 in the online edition of *Physiological and Biochemical Zoology*). However, Kaehler and Pakhomov (2001) found a significant effect in three species of invertebrates preserved in 70% ethanol. In addition, studies that examined the effects of ethanol concentrations above 70% found that both δ^{13}C and δ^{15}N could be significantly altered by the ethanol treatment (Ponsard and Amlou 1999; Sarakinos et al. 2002; Tables 3, C1). Therefore, 70% ethanol may not be an appropriate preservative for all tissue types. Concentrations of ethanol above 70% should be tested before being used as a preservative.

Table 2: Effects of 20 preservation treatments on δ^{13}C and δ^{15}N in turtle epidermis samples compared with values from control (dried) treatment

Treatment, Time (d)	δ^{13}C		δ^{15}N	
	N	*P*	*N*	*P*
Ethanol:				
1	12	.8958	12	1.0000
4	18	.6003	18	.9978
15	18	.8680	18	.9230
30	18	.9964	18	.9998
60	6	.7099	6	.9969
Saturated NaCl:				
1	6	.0832	6	.9494
4	6	.9432	6	.9972
15	6	.3784	6	.0834
30	6	.9998	6	.9872
60	6	.5245	6	1.0000
Frozen:				
1	12	1.0000	12	1.0000
4	18	.9997	18	1.0000
15	18	1.0000	18	1.0000
30	18	1.0000	18	.9570
60	6	**<.0001**	6	**.0440**
DMSO buffer:				
1	6	**<.0001**	6	**.0001**
4	6	**<.0001**	6	**<.0001**
15	6	**<.0001**	6	**.0055**
30	6	**<.0001**	6	**.0096**
60	6	.9734	6	1.0000

Note. *N* is number of samples in treatment; $N = 18$ for the dried control in all tests. *P* values were calculated with Dunnett's adjustment to control for experiment-wise Type I error. Samples with and without lipids were combined because lipid extraction did not affect preservation effects. Values in bold differed significantly from dried controls. DMSO = dimethyl sulfoxide.

Although ethanol has been found to be an acceptable preservative for several tissues, it is flammable and difficult to transport because of safety regulations. Preservation in a saturated NaCl aqueous solution or in salt is becoming more popular because of the ease of transport. Preservation in saturated NaCl solution for up to 60 d had no significant effect on δ^{13}C and δ^{15}N compared with the controls in this study. However, the effects of NaCl as a preservative may vary with species and tissue type. Muscle tissues from four fish species preserved in salt had a significant increase in δ^{15}N (Arrington and Winemiller 2002; Tables 3, C1). The characteristics of samples being preserved (e.g., lipid content) should be considered before using saturated salt preservation.

Freezing samples before analysis has always been considered a relatively safe method of preservation, although it is sometimes a difficult method to use in the field. We found that samples preserved frozen at −10°C in a frost-free freezer for

Table 3: Summary of published preservation studies, not including this study

	$\delta^{13}C$			$\delta^{15}N$		
Treatment	Enriched	Depleted	NS	Enriched	Depleted	NS
Oven-dried	0	0	3	0	0	3
Air-dried	0	0	2	0	0	2
Frozen	0	1	10	1	0	9
Shock-frozen	0	0	2	2	0	0
70% ethanol	3	0	6	0	0	11
>70% ethanol	2	2	4	2	1	5
Industrial ethanol	3	0	1	3	0	1
Formalin/ethanol	0	6	0	6	0	0
Formalin	1	16	8	3	6	20
DMSO buffer	0	1	0	0	1	0
NaCl	0	0	4	4	0	0
Aqueous NaCl	0	0	2	1	0	1
ABI buffer	0	2	0	0	2	0
Queen's buffer	0	2	0	0	2	0
Rotting	0	1	0	1	0	0
Ethylene glycol	0	1	0	0	0	1
Petroleum ether	1	0	0	…	…	…
Methanol	1	0	0	1	0	0
Gluteraldehyde	1	0	0	1	0	0
Boiled	0	1	11	1	0	8

Note. Number of tissues in each preservative for which $\delta^{13}C$ and $\delta^{15}N$ were significantly enriched, significantly depleted, or not significantly affected (NS) by the preservative based on comparison with the control for each study. Ellipses indicate no tissue was tested. Totals for $\delta^{13}C$ and $\delta^{15}N$ within each treatment are not always equal because all studies did not evaluate both elements. DMSO = dimethyl sulfoxide. See table C1 in the online edition of the *American Naturalist* for details of these studies.

up to 30 d did not differ significantly from the control; however, samples that were preserved by freezing for up to 60 d were significantly depleted in both $\delta^{13}C$ and $\delta^{15}N$ compared with the control. Many other studies that evaluated the effects of freezing at this temperature and colder temperatures did not find significant changes in $\delta^{13}C$ and $\delta^{15}N$ (Gloutney and Hobson 1998; Kaehler and Pakhomov 2001; Sweeting et al. 2004; Tables 3, C1). However, one study by Feuchtmayr and Grey (2003) found both $\delta^{13}C$ and $\delta^{15}N$ in zooplankton were significantly altered from the control in a freezing treatment. They attributed these differences to the loss of the lighter isotopes of carbon and nitrogen from the mechanical breakdown of cells and via leaching when the samples were thawed or filtered during their preparatory procedure.

DMSO buffer, the fourth preservative, has been commonly used to preserve samples for genetic analyses. These archived samples could be used for studies based on stable isotopes if the preservative has no effect. However, in this study we found that samples preserved in DMSO buffer for 1, 4, 15, and 30 d, but not 60 d, significantly altered $\delta^{13}C$ and $\delta^{15}N$ values compared with those of the controls. Hobson et al. (1997) also found that DMSO buffer significantly affected $\delta^{13}C$ and $\delta^{15}N$, but Todd et al. (1997) found that DMSO alone did not have a significant effect if lipids were extracted from the samples after preservation. Todd et al. (1997) suggested that the EDTA in the buffer solution is responsible for the isotopic alterations. In our study, samples both with and without lipids were found to be significantly different from the control samples. We cannot offer an explanation for the lack of a preservative effect after 60 d in DMSO buffer; further evaluation is needed.

For turtle epidermis, 70% ethanol and saturated NaCl aqueous solution, as well as short-term freezing at −10°C, are suitable methods of preservation for stable carbon and nitrogen isotope analysis. DMSO buffer unpredictably alters $\delta^{13}C$ and $\delta^{15}N$, making results from these preserved samples difficult if not impossible to interpret. The effects of all these preservatives over durations greater than 60 d should be evaluated, and more samples preserved in DMSO buffer should be analyzed to see whether the apparent "recovery" of the isotopic ratios by 60 d is real and lasting. Because the question determines the acceptable level of error, the amount of variation accepted in our analysis may not be sufficient for other studies. Thus, we have provided summarized data for each turtle in Tables A1 and B1 to allow investigators to evaluate the extent of preservative effects and effects of sample size.

Twenty different preservative methods and their effects on different tissue samples from 16 studies are represented in Table C1. However, of these 20 methods, only seven have been ex-

amined in more than one study. All of these seven methods had mixed results concerning their effect on the tissue tested. The samples that were preserved by freezing typically showed no change, but there were instances of effects on both carbon and nitrogen. Shock-frozen samples showed changes only in δ^{15}N. Samples preserved in aqueous NaCl showed either no change or a change in δ^{15}N. Formalin/ethanol and DMSO preservation resulted in significant alterations of both δ^{13}C and δ^{15}N for almost every tissue examined. Formalin affected either δ^{13}C or δ^{15}N in almost every test. The 70%-ethanol solutions that were tested usually showed no effect; however, one study did report a significant change in δ^{13}C in three tissues. Ethanol solutions stronger than 70% demonstrated mixed results. The results summarized in Table C1 are not conclusive but do identify preservatives that should not be used and areas where further research is needed. Given the different effects of preservatives on different species and tissue types, studies should attempt to develop predictable patterns of what preservatives are appropriate for different tissues.

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Appendix A from L. M. Barrow, K. A. Bjorndal, and K. J. Reich, "Effects of Preservation Method on Stable Carbon and Nitrogen Isotope Values"

(Physiol. Biochem. Zool., vol. 81, no. 5, p. 688)

Table A1

Means and standard deviations of $\delta^{13}C$ values from this study

Preservative, Time (d)	*Chelonia* 1		*Chelonia* 2		*Caretta* 1		*Caretta* 2		*Trachemys* 1		*Trachemys* 2	
	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids
Dried at 60°C:												
	−14.74 ± .24	−14.60 ± .21	−15.49 ± .34	−15.40 ± .11	−15.47 ± .22	−15.59 ± .15	−15.03 ± .20	−14.88 ± .04	−19.93 ± .07	−19.92 ± .22	−21.76 ± .09	−22.07 ± .09
70% ethanol:												
1	−14.74 ± .03	−14.66 ± .04	−15.04 ± .27	−14.89 ± .20	−15.28 ± .04	−15.61 ± .26	−14.95 ± .14	−14.90 ± .04				
4	−14.69 ± .06	−14.80 ± .04	−15.19 ± .39	−14.88 ± .05	−15.27 ± .15	−15.47 ± .12	−15.17 ± .13	−14.94 ± .08	−19.74 ± .11	−19.06 ± 1.63	−21.72 ± .03	−21.84 ± .18
15	−14.54 ± .21	−14.59 ± .17	−15.08 ± .38	−14.99 ± .27	−15.59 ± .16	−15.56 ± .12	−14.85 ± .08	−14.80 ± .08	−19.65 ± .12	−19.88 ± .31	−21.59 ± .04	−21.90 ± .05
30	−14.60 ± .11	−15.02 ± .29	−14.76 ± .16	−15.18 ± .30	−15.31 ± .08	−15.76 ± .24	−14.85 ± .03	−15.12 ± .18	−19.68 ± .03	−19.85 ± .15	−21.73 ± .14	−21.88 ± .06
60			−14.80 ± .16	−15.04 ± .37			−14.79 ± .14	−15.00 ± .01				
NaCl saturated aqueous solution:												
1			−14.83 ± .12	−14.74 ± .21			−14.82 ± .03	−14.91 ± .10				
4			−14.90 ± .36	−15.00 ± .57			−14.82 ± .06	−14.96 ± .22				
15			−15.04 ± .23	−14.86 ± .25			−14.82 ± .10	−14.98 ± .31				
30			−14.86 ± .11	−15.02 ± .22			−14.82 ± .05	−14.94 ± .05				
60			−14.71 ± .26	−14.93 ± .08			−14.86 ± .15	−15.00 ± .06				
Frozen −10°C:												
1	−14.63 ± .14	−14.66 ± .09	−15.45 ± .36	−15.08 ± .24	−15.50 ± .08	−15.54 ± .07	−14.95 ± .08	−14.93 ± .16				
4	−14.71 ± .03	−14.77 ± .06	−15.10 ± .10	−15.15 ± .11	−15.46 ± .03	−15.50 ± .15	−14.90 ± .01	−14.81 ± .17	−19.79 ± .09	−19.90 ± .03	−21.88 ± .06	−21.98 ± .11
15	−14.62 ± .19	−14.52 ± .14	−15.30 ± .48	−15.39 ± .38	−15.38 ± .08	−15.38 ± .07	−15.03 ± .17	−14.77 ± .03	−20.05 ± .35	−20.15 ± .47	−21.87 ± .15	−21.89 ± .13
30	−14.76 ± .28	−15.04 ± .37	−15.26 ± .19	−15.63 ± .42	−15.48 ± .10	−15.65 ± .14	−14.89 ± .02	−15.23 ± .16	−19.95 ± .35	−19.92 ± .11	−21.81 ± .13	−21.83 ± .10
60			−15.63 ± .21	−15.61 ± .41			−16.94 ± .14	−16.69 ± .09				
DMSO (250 mM EDTA pH 7.5; 20% DMSO):												
1			−18.01 ± .40	−17.53 ± .44			−17.74 ± .27	−17.27 ± .16				
4			−18.47 ± .42	−17.92 ± .35			−18.69 ± .28	−17.26 ± .28				

Table A1 (*Continued*)

Preservative, Time (d)	*Chelonia* 1		*Chelonia* 2		*Caretta* 1		*Caretta* 2		*Trachemys* 1		*Trachemys* 2	
	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids
15			−19.33 ± .25	−17.82 ± .32			−17.69 ± .38	−17.56 ± .19				
30			−17.27 ± .43	−17.29 ± .09			−16.75 ± .10	−16.49 ± .44				
60			−14.98 ± .21	−15.00 ± .15			−14.89 ± .12	−15.04 ± .11				

Note. DMSO = dimethyl sulfoxide; EDTA = ethylenediaminetetraacetic acid.

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Appendix B from L. M. Barrow, K. A. Bjorndal, and K. J. Reich, "Effects of Preservation Method on Stable Carbon and Nitrogen Isotope Values"

(Physiol. Biochem. Zool., vol. 81, no. 5, p. 688)

Table B1

Means and standard deviations of $\delta^{15}N$ values from this study

Preservative, Time (d)	*Chelonia* 1		*Chelonia* 2		*Caretta* 1		*Caretta* 2		*Trachemys* 1		*Trachemys* 2	
	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids
Dried at 60°C:												
	7.11 ± .25	7.24 ± .62	8.93 ± .07	9.16 ± .13	9.37 ± .37	9.33 ± .47	9.57 ± .17	9.43 ± .11	4.47 ± .73	4.49 ± .37	9.45 ± .10	9.67 ± .05
70% ethanol:												
1	7.47 ± .17	7.49 ± .06	7.92 ± .95	7.69 ± 1.01	9.32 ± .19	9.45 ± .60	9.63 ± .23	9.95 ± .09				
4	7.69 ± .09	7.74 ± .08	8.98 ± .12	9.32 ± .19	9.47 ± .45	9.34 ± .20	9.78 ± .20	9.93 ± .22	4.35 ± .14	4.31 ± .45	9.64 ± .07	9.84 ± .12
15	7.57 ± .24	7.36 ± .25	8.91 ± .09	9.25 ± .15	9.89 ± .56	9.91 ± .29	9.71 ± .29	9.97 ± .04	4.80 ± .60	5.21 ± .51	9.87 ± .16	9.86 ± .15
30	7.71 ± .22	7.70 ± .24	8.31 ± .77	7.87 ± 1.04	9.61 ± .14	9.73 ± .36	10.13 ± .35	9.75 ± .16	4.74 ± .41	4.86 ± .53	9.69 ± .16	9.94 ± .10
60			8.52 ± .57	8.98 ± .40			9.90 ± .20	10.03 ± .32				
NaCl saturated aqueous solution:												
1			7.49 ± 1.49	8.55 ± .92			9.38 ± .24	10.00 ± .65				
4			7.97 ± .63	8.93 ± .21			9.22 ± .57	9.63 ± .14				
15			7.46 ± 1.57	8.45 ± .40			8.96 ± .19	9.67 ± .36				
30			8.86 ± .29	8.83 ± .32			9.90 ± .16	9.97 ± .19				
60			7.97 ± 1.49	8.26 ± 1.30			9.64 ± .32	9.79 ± .12				
Frozen −10°C:												
1	7.26 ± .52	6.54 ± 1.29	8.06 ± 1.23	8.76 ± .63	9.88 ± .24	9.44 ± .31	9.80 ± .02	9.66 ± .12				
4	6.89 ± .38	6.95 ± .26	7.63 ± .74	8.11 ± 1.18	9.39 ± .06	9.24 ± .34	10.12 ± .60	10.28 ± .38	4.43 ± .30	4.40 ± .06	9.59 ± .16	9.84 ± .11
15	6.29 ± .54	6.88 ± .49	8.75 ± .22	8.75 ± .16	9.18 ± .03	9.13 ± .37	9.38 ± .16	9.82 ± .29	5.34 ± .94	5.10 ± 1.12	9.72 ± .11	9.96 ± .07
30	6.20 ± .69	5.81 ± .44	8.03 ± 1.08	7.99 ± 1.03	9.16 ± .02	9.18 ± .08	10.06 ± .31	9.98 ± .21	4.37 ± .29	5.02 ± .69	9.66 ± .11	10.01 ± .16
60			7.67 ± .26	7.19 ± .31			9.02 ± .33	9.23 ± .11				
DMSO (250 mM EDTA pH 7.5; 20% DMSO):												
1			6.91 ± .24	7.72 ± .17			8.04 ± .39	8.66 ± .40				
4			6.33 ± .81	6.28 ± .71			8.51 ± .17	8.80 ± .22				

Table B1 (*Continued*)

Preservative, Time (d)	*Chelonia* 1		*Chelonia* 2		*Caretta* 1		*Caretta* 2		*Trachemys* 1		*Trachemys* 2	
	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids	Lipids	No Lipids
15			6.95 ± .36	7.94 ± .69			8.60 ± .31	8.83 ± .15				
30			6.86 ± .21	7.01 ± .59			9.31 ± .19	9.39 ± .02				
60			7.12 ± 1.35	7.97 ± .92			10.55 ± .22	10.03 ± .21				

Note. DMSO = dimethyl sulfoxide; EDTA = ethylenediaminetetraacetic acid.

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Appendix C from L. M. Barrow, K. A. Bjorndal, and K. J. Reich, "Effects of Preservation Method on Stable Carbon and Nitrogen Isotope Values"

(Physiol. Biochem. Zool., vol. 81, no. 5, p. 688)

Table C1

Summary of results in stable isotope preservation studies (columns 4 and 5 show the difference between treatment and control values; a positive value reflects an enrichment from the control and a negative value reflects a depletion)

Preservative, Tissue	Species	LE	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	*N*	No. Individuals	Preservation Time	$\delta^{13}C$ Significance	$\delta^{15}N$ Significance	Reference
Oven-dried (50°C):										
Muscle	*Argyrosomus hololepidotus*	No	NV	NV	3/interval	1 fillet	1, 4, 12 wk	No	No	Kaehler and Pakhomov 2001; dried at 50°C
Arm	*Octopus vulgaris*	No	NV	NV	3/interval	1 arm	1, 4, 12 wk	No	No	Kaehler and Pakhomov 2001; dried at 50°C
Frond	*Ecklonia radiate*	No	NV	NV	3/interval	1 frond	1, 4, 12 wk	No	No	Kaehler and Pakhomov 2001; dried at 50°C
Air-dried (20°–24°C):										
Blood	*Coturnix coturnix japonica*	No	−.04	0	5 total	25	8 wk	No	No	Hobson et al. 1997; freeze-dried
Blood	*Ovis aries*	No	.02	−.08	5 total	5	8 wk	No	No	Hobson et al. 1997; freeze-dried
Frozen:										
−10°C:										
Epidermis	*Chelonia mydas*	No	.07	−.6	3/interval	2	1, 4, 15, 30 d; 1 individual 60 d	Only 60 d significant	Only 60 d significant	This study; dried at 60°C
Epidermis	*Caretta caretta*	No	−.16	−.03	3/interval	2	1, 4, 15, 30 d; 1 individual 60 d	Only 60 d significant	Only 60 d significant	This study; dried at 60°C
Epidermis	*Trachemys scripta elegans*	No	−.05	.22	3/interval	2	4, 15, 30 d	No	No	This study; dried at 60°C
Epidermis	*C. mydas*	Yes	−.09	−.76	3/interval	2	1, 4, 15, 30 d; 1 individual 60 d	Only 60 d significant	Only 60 d significant	This study; dried at 60°C

Table C1 (*Continued*)

Preservative, Tissue	Species	LE	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	*N*	No. Individuals	Preservation Time	$\delta^{13}C$ Significance	$\delta^{15}N$ Significance	Reference
Epidermis	*C.retta caretta*	Yes	−.15	.17	3/interval	2	1, 4, 15, 30 d; 1 individual 60 d	Only 60 d significant	Only 60 d significant	This study; dried at 60°C
Epidermis	*T. s. elegans*	Yes	.26	−.21	3/interval	2	4, 15, 30 d	No	No	This study; dried at 60°C
Muscle	*Gadus morhua*	No	−.13	.06	3/interval	1 mature female	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	No	No	Sweeting et al. 2004; freeze-dried
Roe	*G. morhua*	No	−.21	.14	3/interval	1 mature female	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	No	No	Sweeting et al. 2004; freeze-dried
Liver	*G. morhua*	No	.16	.17	3/interval	1 mature female	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	No	No	Sweeting et al. 2004; freeze-dried
−18°C:										
Muscle	*A. hololepidotus*	No	NV	NV	3/interval	1 fillet	1, 4, 12 wk	No	No	Kaehler and Pakhomov 2001; dried at 50°C
Arm	*O. vulgaris*	No	NV	NV	3/interval	1 arm	1, 4, 12 wk	No	No	Kaehler and Pakhomov 2001; dried at 50°C
Frond	*E. radiata*	No	NV	NV	3/interval	1 frond	1, 4, 12 wk	No	No	Kaehler and Pakhomov 2001; dried at 50°C
−20°C:										
Entire organisms	*Drosophila melanogaster*	No	.23	−.08	10	4/sample	12 wk	No	No	Ponsard and Amlou 1999; frozen at −80°C, then freeze-dried
Egg, yolk lipids	*Coturnix japonica*	NA	−.02	NA	5	5 eggs	50 d	No	NA	Gloutney and Hobson 1998[a]
Egg, yolk	*C. japonica*	Yes	.08	.05	5	5 eggs	50 d	No	No	Gloutney and Hobson 1998[a]
Egg, albumin	*C. japonica*	Yes	.03	.02	5	5 eggs	50 d	No	No	Gloutney and Hobson 1998[a]
Entire organisms	Bulk zooplankton	No	−.83	.61	5	NA	4 d	Yes	Yes	Feuchtmayr and Grey 2003; dried at 60°C
Shock-frozen (entire organisms):										
Immersed in N_2	Bulk zooplankton	No	.11	1.5	5	NA	4 d	No	Yes	Feuchtmayr and Grey 2003; dried at 60°C
Drowned in liquid N_2	*D. melanogaster*	No	−.34	.07	10	4/sample	12 h	No	Yes	Ponsard and Amlou 1999; frozen at −80°C, then freeze-dried
Ethanol (70%):										
Epidermis	*C. mydas*	No	.29	.1	3/interval	2	1, 4, 15, 30 d; 1 individual 60 d	No	No	This study; dried at 60°C
Epidermis	*C. caretta*	No	.11	.14	3/interval	2	1, 4, 15, 30 d; 1 individual 60 d	No	No	This study; dried at 60°C

Table C1 (*Continued*)

Preservative, Tissue	Species	LE	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	*N*	No. Individuals	Preservation Time	$\delta^{13}C$ Significance	$\delta^{15}N$ Significance	Reference
Epidermis	*T. s. elegans*	No	.16	.22	3/interval	2	4, 15, 30 d	No	No	This study; dried at 60°C
Epidermis	*C. mydas*	Yes	.11	−.04	3/interval	2	1, 4, 15, 30 d; 1 individual 60 d	No	No	This study; dried at 60°C
Epidermis	*C. caretta*	Yes	0	.41	3/interval	2	1, 4, 15, 30 d; 1 individual 60 d	No	No	This study; dried at 60°C
Epidermis	*T. s. elegans*	Yes	.47	−.26	3/interval	2	4, 15, 30 d	No	No	This study; dried at 60°C
Egg, yolk lipids	*C. japonica*	NA	.27	NA	5	5 eggs	50 d	No	NA	Gloutney and Hobson 1998[a]
Egg, yolk	*C. japonica*	Yes	.08	−.1	5	5 eggs	50 d	No	No	Gloutney and Hobson 1998[a]
Egg, albumen	*C. japonica*	Yes	0	−.07	5	5 eggs	50 d	No	No	Gloutney and Hobson 1998[a]
Blood	*C. c. japonica*	No	−.12	−.26	5 total	25	8 wk	No	No	Hobson et al. 1997; freeze-dried
Blood	*O. aries*	No	.02	−.06	5 total	5	8 wk	No	No	Hobson et al. 1997; freeze-dried
Muscle	*C. c. japonica*	No	−.44	.4	5 total	5	8 wk	No	No	Hobson et al. 1997; freeze-dried
Muscle	*A. hololepidotus*	No	.7 to 1.5	NV	3/interval	1	1, 4, 12 wk	Yes	No	Kaehler and Pakhomov 2001; dried at 50°C
Arm	*O. vulgaris*	No	.7 to 1.5	NV	3/interval	1	1, 4, 12 wk	Yes	No	Kaehler and Pakhomov 2001; dried at 50°C
Frond	*E. radiate*	No	.7 to 1.5	NV	3/interval	1	1, 4, 12 wk	Yes	No	Kaehler and Pakhomov 2001; dried at 50°C
Muscle	*Hemibarbus barbus*	No	NA	−.65	NV	NV	9 wk	NA	No	Ogawa et al. 2001; dried at 60°C
Muscle	*Lepomis macrochirus*	No	NA	−.07	NV	NV	9 wk	NA	No	Ogawa et al. 2001; dried at 60°C
Muscle	*Micropterus salmoides salmoides*	No	NA	−.13	NV	NV	9 wk	NA	No	Ogawa et al. 2001; dried at 60°C
Ethanol (75%):										
Muscle	*Catostomus occidentalis*	No	.21	.37	NV	NV	3 d; 3 wk; 3, 6 mo	No	Yes	Sarakinos et al. 2002; frozen at −25°C
Tissue removed from shell	*Corbicula fluminea*	No	2.18	−.39	NV	NV	3 d; 3 wk; 3, 6 mo	Yes	Yes	Sarakinos et al. 2002; frozen at −25°C
Entire organisms	*Hydropsyche* sp.	No	.04	−.21	NV	NV	3 d; 3 wk; 3, 6 mo	No	No	Sarakinos et al. 2002; frozen at −25°C
Ethanol (95%):										
Entire organisms	*D. melanogaster*	No	−1.38	.17	10	4/sample	10 d	Yes	No	Ponsard and Amlou 1999; frozen at −80°C, then freeze-dried

Table C1 (*Continued*)

Preservative, Tissue	Species	LE	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	*N*	No. Individuals	Preservation Time	$\delta^{13}C$ Significance	$\delta^{15}N$ Significance	Reference
Entire organisms	*D. melanogaster*	No	−1.17	.12	10	4/sample	6 wk	Yes	No	Ponsard and Amlou 1999; frozen at −80°C, then freeze-dried
Dorsal muscle	*Salvelinus alpinus*	No	.78	.35	20	20	10 mo	Yes	No	Kelly et al. 2006; dried at 45°C for 48 h
Dorsal muscle	*S. alpinus*	Yes	.2	.06	20	20	10 mo	No	No	Kelly et al. 2006; dried at 45°C for 48 h
Ethanol (96%/total of 30%):										
Entire organisms	Bulk zooplankton	No	.24	.77	5	NA	4 d	No	Yes	Feuchtmayr and Grey 2003; dried at 60°C
Industrial ethanol (95% EtOH, 5% methanol):										
80%:										
Muscle	*G. morhua*	No	.54	1.05	3/interval	1	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	Yes	Yes	Sweeting et al. 2004; freeze-dried
Roe	*G. morhua*	No	.81	.44	3/interval	1	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	No	Yes	Sweeting et al. 2004; freeze-dried
Liver	*G. morhua*	No	1.57	.5	3/interval	1	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	Yes	No	Sweeting et al. 2004; freeze-dried
100%:										
Muscle	*Gadus morhua*	No	.42	.95	3/interval	1	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	Yes	Yes	Sweeting et al. 2004; freeze-dried
Formalin/ethanol (10%/90%):										
Muscle	*Arius felis*	No	−1.12	.62	1	1	2 wk formalin/4 wk EtOH	Yes	Yes	Arrington and Winemiller 2002; frozen
Muscle	*Cynoscion nebulosus*	No	−1.12	.62	1	1	2 wk formalin/4 wk EtOH	Yes	Yes	Arrington and Winemiller 2002; frozen
Muscle	*Dorosoma cepedianum*	No	−1.12	.62	2	2	2 wk formalin/4 wk EtOH	Yes	Yes	Arrington and Winemiller 2002; frozen
Muscle	*Mugil cephalus*	No	−1.12	.62	12	12	2 wk formalin/4 wk EtOH	Yes	Yes	Arrington and Winemiller 2002; frozen
Tail	*Crangon septemspinosa*	No	−2.15	1.1	6	3	2 mo formalin/2 mo EtOH	Yes	Yes	Bosley and Wainright 1999; frozen at −80°C
Muscle	*Pleuronectes americanus*	No	−2.17	1.41	3	3	2 mo formalin/2 mo EtOH	Yes	Yes	Bosley and Wainright 1999; frozen at −80°C
Formalin:										
5%:										
Entire organisms	*Neomysis intermedia*	No	NA	.04	NA	NV	8.5 mo	NA	No	Toda and Wada 1990; fresh[b] dried samples

Table C1 (*Continued*)

Preservative, Tissue	Species	LE	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	*N*	No. Individuals	Preservation Time	$\delta^{13}C$ Significance	$\delta^{15}N$ Significance	Reference
Muscle	*H. barbus*	No	NA	−.03	NV	NV	9, 62, 117 wk	NA	No	Ogawa et al. 2001; dried at 60°C
Muscle	*L. macrochirus*	No	NA	−.07	NV	NV	9, 62, 117 wk	NA	No	Ogawa et al. 2001; dried at 60°C
Muscle	*M. s. salmoides*	No	NA	−.07	NV	NV	9, 62, 117 wk	NA	No	Ogawa et al. 2001; dried at 60°C
Muscle	*Zacco platyput*	No	NA	.38	NV	NV	9, 62, 117 wk	NA	No	Ogawa et al. 2001; dried at 60°C
10%:										
Egg, yolk lipids	*C. japonica*	NA	−.16	NA	5	5 eggs	50 d	No	NA	Gloutney and Hobson 1998[a]
Egg, yolk	*C. japonica*	Yes	−2.44	.03	5	5 eggs	50 d	Yes	No	Gloutney and Hobson 1998[a]
Egg, albumen	*C. japonica*	Yes	−2.39	.24	5	5 eggs	50 d	Yes	No	Gloutney and Hobson 1998[a]
Muscle	*C. occidentalis*	No	−1.33	.16	NV	NV	3 d; 3 wk; 3, 6 mo	Yes	No	Sarakinos et al. 2002; frozen at −25°C
Tissue removed from shell	*C. fluminea*	No	.67	−.48	NV	NV	4 d; 3 wk; 3, 6 mo	No	Yes	Sarakinos et al. 2002; frozen at −25°C
Entire organisms	*Hydropsyche* sp.	No	−.75	−.121	NV	NV	5 d; 3 wk; 3, 6 mo	Yes	No	Sarakinos et al. 2002; frozen at −25°C
Tail	*Crangon septemspinosa*	No	−2.05	.35	6	3	NV	No	No	Bosley and Wainright 1999; frozen at −80°C
Muscle	*Pleuronectes americanus*	No	−.74	1.21	3	3	NV	No	Yes	Bosley and Wainright 1999; frozen at −80°C
Muscle	*C. c. japonica*	No	−1.78	.04	5 total	5	8 wk	Yes	No	Hobson et al. 1997; freeze-dried
Blood	*C. c. japonica*	No	−.94	−.34	5 total	25	8 wk	No	Yes	Hobson et al. 1997; freeze-dried
Blood	*O. aries*	No	−1.32	−.44	5 total	5	8 wk	No	Yes	Hobson et al. 1997; freeze-dried
Dorsal muscle	*S. alpinus*	No	−2.21	.66	20	20	10 mo	Yes	Yes	Kelly et al. 2006; dried at 45°C for 48 h
Dorsal muscle	*S. alpinus*	Yes	−2.78	.03	20	20	10 mo	Yes	No	Kelly et al. 2006; dried at 45°C for 48 h
37% (10%–15% methanol):										
Entire organisms	*D. melanogaster*	No	−2.92	.34	10	4/sample	10 d	Yes	Yes	Ponsard and Amlou 1999; frozen at −80°C, then freeze-dried
Entire organisms	*D. melanogaster*	No	−2.69	.08	10	4/sample	6 wk	Yes	No	Ponsard and Amlou 1999; frozen at −80°C, then freeze-dried
4% borax-buffered formaldehyde:										

Table C1 (*Continued*)

Preservative, Tissue	Species	LE	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	*N*	No. Individuals	Preservation Time	$\delta^{13}C$ Significance	$\delta^{15}N$ Significance	Reference
Bulk samples	*Cercopagis pengoi*	No	−.40	.10	36	NV	All storage periods and years	No	No	Gorokhova et al. 2005; frozen samples at −20°C and untreated samples
4% sodium phosphate:										
Muscle	*G. morhua*	No	−1.96	.89	3/interval	1	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	Yes	Yes	Sweeting et al. 2004; freeze-dried
4%/3 g L^{-1} Na acetate trihydrate:										
Muscle	*G. morhua*	No	−1.49	.71	3/interval	1	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	Yes	Yes	Sweeting et al. 2004; freeze-dried
Roe	*Gadus morhua*	No	−1.22	.28	3/interval	1	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	Yes	No	Sweeting et al. 2004; freeze-dried
Liver	*G. morhua*	No	−1.06	−.14	3/interval	1	1, 8 d; 2 wk; 1, 3, 7, 11, 15, 19, 21 mo	Yes	No	Sweeting et al. 2004; freeze-dried
Hexamine-buffered 4% saline formalin:										
Muscle	*A. hololepidotus*	No	−.6	−.28 to −3	3/interval	1	1, 4, 12 wk	Yes	No	Kaehler and Pakhomov 2001; dried at 50°C
Arm	*O. vulgaris*	No	−.6	−.28 to −3	3/interval	1	1, 4, 12 wk	Yes	No	Kaehler and Pakhomov 2001; dried at 50°C
Frond	*E. radiata*	No	−1.5	−.28 to −3	3/interval	1	1, 4, 12 wk	Yes	No	Kaehler and Pakhomov 2001; dried at 50°C
37% formaldehyde/total 10%:										
Entire organisms	Bulk zooplankton	No	1.09	.8	5	NA	4 d	Yes	Yes	Feuchtmayr and Grey 2003; dried at 60°C
Unbuffered formalin-seawater (3% v/v):										
Entire organisms	Marine zooplankton	No	−2.5	−1	NV	NA	5–8 yr	No	No	Mullin et al. 1984; fresh samples dried at 60°C for several days
DMSO (250 mM EDTA pH 7.5; 20% DMSO):										
Epidermis	*C. mydas*	No	−2.49	−1.18	3/interval	1	1, 4, 15, 30, 60 d	Only 60 d, not significant	Only 60 d, not significant	This study; dried at 60°C
Epidermis	*C. caretta*	No	−1.9	−.47	3/interval	1	1, 4, 15, 30, 60 d	Only 60 d, not significant	Only 60 d, not significant	This study; dried at 60°C
Epidermis	*C. mydas*	Yes	−2.02	−.8	3/interval	1	1, 4, 15, 30, 60 d	Only 60 d, not significant	Only 60 d, not significant	This study; dried at 60°C
Epidermis	*C. caretta*	Yes	−1.49	−.24	3/interval	1	1, 4, 15, 30, 60 d	Only 60 d, not significant	Only 60 d, not significant	This study; dried at 60°C

Table C1 (*Continued*)

Preservative, Tissue	Species	LE	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	*N*	No. Individuals	Preservation Time	$\delta^{13}C$ Significance	$\delta^{15}N$ Significance	Reference
Muscle	*C. c. japonica*	No	−4.74	−.74	5 total	5	8 wk	Yes	Yes	Hobson et al. 1997; freeze-dried
NaCl (>99.1%):										
Muscle	*Arius felis*	No	.13	.72	16	1	6 wk	No	Yes	Arrington and Winemiller 2002; frozen
Muscle	*Cynoscion nebulosus*	No	.13	.72	16	1	6 wk	No	Yes	Arrington and Winemiller 2002; frozen
Muscle	*Dorosoma cepedianum*	No	.13	.72	16	2	6 wk	No	Yes	Arrington and Winemiller 2002; frozen
Muscle	*Mugil cephalus*	No	.13	.72	16	12	6 wk	No	Yes	Arrington and Winemiller 2002; frozen
Aqueous NaCl:										
Epidermis	*C. mydas*	No	.25	−.07	3/interval	1	1, 4, 15, 30, 60 d	No	No	This study; dried at 60°C
Epidermis	*C. caretta*	No	.38	−.25	3/interval	1	1, 4, 15, 30, 60 d	No	No	This study; dried at 60°C
Epidermis	*C. mydas*	Yes	.09	.4	3/interval	1	1, 4, 15, 30, 60 d	No	No	This study; dried at 60°C
Epidermis	*C. caretta*	Yes	.25	.46	3/interval	1	1, 4, 15, 30, 60 d	No	No	This study; dried at 60°C
33 g L^{-1}:										
Entire organisms	*D. melanogaster*	No	−.63	.21	10	4/sample	10 d	No	No	Ponsard and Amlou 1999; frozen at −80°C, then freeze-dried
Seawater/HgCl:										
Muscle	*Pleuronectes americanus*	No	−.62	.69	3	3	NV	No	Yes	Bosley and Wainright 1999; frozen at −80°C
ABI lysis buffer:[c]										
Blood	*C. c. japonica*	No	−18.76	−5.16	5 total	25	8 wk	Yes	Yes	Hobson et al. 1997; freeze-dried
Blood	*O. aries*	No	−17.2	−6.58	5 total	5	8 wk	Yes	Yes	Hobson et al. 1997; freeze-dried
Queen's lysis buffer:										
Blood	*C. c. japonica*	No	−4.56	−1.32	5 total	25	8 wk	Yes	Yes	Hobson et al. 1997; freeze-dried
Blood	*O. aries*	No	−4.36	−1.92	5 total	5	8 wk	Yes	Yes	Hobson et al. 1997; freeze-dried
Rotting:										
10 d in 5 mL of H_2O:										
Entire organisms	*D. melanogaster*	No	−.81	.42	10	4/sample	10 d	Yes	Yes	Ponsard and Amlou 1999; frozen at −80°C, then freeze-dried

Table C1 (*Continued*)

Preservative, Tissue	Species	LE	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	*N*	No. Individuals	Preservation Time	$\delta^{13}C$ Significance	$\delta^{15}N$ Significance	Reference
Museum preservation:										
10% formalin-water, distilled water, 3–5 d; 35% EtOH, 2 wk; 70% EtOH, long term:										
Muscle	*Rhinichthys cataractae*	No	−2	.4	3	3	10 d formalin	NV	NV	Edwards et al. 2002; frozen at −80°C
Muscle	*R. cataractae*	No	−2	.4	3	3	40 d formalin	NV	NV	Edwards et al. 2002; frozen at −80°C
Muscle	*R. cataractae*	No	−2	.4	3	3	70 d formalin	NV	NV	Edwards et al. 2002; frozen at −80°C
Muscle	*R. cataractae*	No	−2	.4	2	3	100 d formalin	NV	NV	Edwards et al. 2002; frozen at −80°C
Muscle	*R. cataractae*	No	−2	.4	2	3	130 d formalin	NV	NV	Edwards et al. 2002; frozen at −80°C
Muscle	*R. cataractae*	No	−2	.4	3	3	160 d formalin	NV	NV	Edwards et al. 2002; frozen at −80°C
Muscle	*R. cataractae*	No	−2	.4	3	3	190 d formalin	NV	NV	Edwards et al. 2002; frozen at −80°C
Muscle	*Percina caprodes*	No	−.8	.5	3	3	12–15 yr	NV	NV	Edwards et al. 2002; frozen at −80°C
Muscle	*Percina roanoka*	No	−.8	.5	3	3	12–15 yr	NV	NV	Edwards et al. 2002; frozen at −80°C
Muscle	*Etheostoma tippecanoe*	No	−.8	.5	3	3	12–15 yr	NV	NV	Edwards et al. 2002; frozen at −80°C
Muscle	*R. cataractae*	No	−1.5	NA	10	10	NV	Yes	NA	Edwards et al. 2002; frozen at −80°C
Ethylene glycol:										
Entire organisms	*D. melanogaster*	No	−1.52	.15	10	4/sample	10 d	Yes	No	Ponsard and Amlou 1999; frozen at −80°C, then freeze-dried
Petroleum ether treated:										
Muscle	*R. cataractae*	Yes	−.058	NA	10	10	4 h	No	NA	Edwards et al. 2002; frozen at −80°C
Methanol (30%):										
Entire organisms	Bulk zooplankton	No	.48	.68	5	NA	4 d	Yes	Yes	Feuchtmayr and Grey 2003; dried at 60°C
Gluteraldehyde (4%):										
Entire organisms	Bulk zooplankton	No	.65	.04	5	NA	4 d	Yes	Yes	Feuchtmayr and Grey 2003; dried at 60°C
Boiled:										
Egg, yolk lipids	*C. japonica*	NA	.14	NA	5	5 eggs	Frozen 50 d	No	NA	Gloutney and Hobson 1998[a]
Egg, yolk	*C. japonica*	Yes	−.03	.22	5	5 eggs	Frozen 50 d	No	No	Gloutney and Hobson 1998[a]

Table C1 (*Continued*)

Preservative, Tissue	Species	LE	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	*N*	No. Individuals	Preservation Time	$\delta^{13}C$ Significance	$\delta^{15}N$ Significance	Reference
Egg, albumin	*C. japonica*	Yes	−.11	.14	5	5 eggs	Frozen 50 d	No	No	Gloutney and Hobson 1998[a]
Egg, yolk lipids	*C. japonica*	NA	.41	NA	5	5 eggs	Frozen 50 d/7 d at room temp	No	No	Gloutney and Hobson 1998[a]
Egg, yolk	*C. japonica*	Yes	.03	.3	5	5 eggs	Frozen 50 d/7 d at room temp	No	No	Gloutney and Hobson 1998[a]
Egg, albumin	*C. japonica*	Yes	−.21	−.2	5	5 eggs	Frozen 50 d/7 d at room temp	No	No	Gloutney and Hobson 1998[a]
Egg, yolk lipids	*C. japonica*	NA	.13	NA	5	5 eggs	Frozen 50 d/7 d at 6°C	No	NA	Gloutney and Hobson 1998[a]
Egg, yolk	*C. japonica*	Yes	−.55	−.36	5	5 eggs	Frozen 50 d/7 d at 6°C	Yes	No	Gloutney and Hobson 1998[a]
Egg, albumin	*C. japonica*	Yes	−.2	.21	5	5 eggs	Frozen 50 d/7 d at 6°C	No	No	Gloutney and Hobson 1998[a]
Egg, yolk lipids	*C. japonica*	NA	.03	NA	5	5 eggs	50 d at 6°C	No	NA	Gloutney and Hobson 1998[a]
Egg, yolk	*C. japonica*	Yes	−.13	.13	5	5 eggs	50 d at 6°C	No	No	Gloutney and Hobson 1998[a]
Egg, albumin	*C. japonica*	Yes	0	.41	5	5 eggs	50 d at 6°C	No	Yes	Gloutney and Hobson 1998[a]

Note. LE = lipid extracted. NV = no value or information is available for that study; NA = does not apply to the study. *N* = within-sample replicates. The reference column includes controls against which preservative treatments were compared. DMSO = dimethyl sulfoxide; EDTA = ethylenediaminetetraacetic acid.

[a] Control separated into egg components, dried at 60°C, preserved in a 2 : 1 chloroform : methanol solution for 50 d, and then dried.

[b] Fresh = control samples were collected at a different time than the experimental samples.

[c] ABI = Applied Biosystems.