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# Effect of tissue decomposition on stable isotope signatures of striped dolphins *Stenella coeruleoalba* and loggerhead sea turtles *Caretta caretta*

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ABSTRACT: Marine mammals and turtles are often studied using the carcasses of stranded dead individuals. Since decomposition processes might modify the stable isotope ratios of tissues, the present study tested the effects of decomposition on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios in 2 tissue types of striped dolphin *Stenella coeruleoalba* and loggerhead sea turtle *Caretta caretta*. Decomposing carcasses of 3 dolphins and 3 turtles were sampled for muscle and skin for 62 d. Following lipid extraction, samples were analysed regularly using a continuous flow-isotope ratio mass spectrometer. Samples reached decomposition stage CC4 after 62 d at ambient temperature, but no statistical change in $\delta^{13}C$ or $\delta^{15}N$ was observed over that period for either tissue or species. These results imply that muscle and skin samples from carcasses decomposing out of water at stage CC4 or lower can be used as reliable material for stable isotope analysis in these 2 species, and probably in other marine mammal and marine turtle species. The effects of decomposition on the stability of stable isotope ratios in other tissues, in carcasses at stage CC5, or in carcasses decomposing underwater require further study.

KEY WORDS: Stable isotopes · $\delta^{13}C$ · $\delta^{15}N$ · Decomposition effect · Marine mammal · Sea turtle · Muscle · Skin



## INTRODUCTION

Stable isotope ecology is a branch of chemical ecology that uses stable isotopes as chemical tracers to follow ecological dynamics. The occurrence of isotope fractionation at the atomic level allows different ecological processes to be identified, and presents a reliable tool to evaluate trophic ecology (Fry 2006); consequently, stable isotope analysis (SIA) is widely used in trophic ecology (Hobson & Wassenaar 1999, Newsome et al. 2010). Applications of SIA include determining habitat use (Pinela et al. 2010), migration patterns (Kurle & Worthy 2002, Caut et al. 2008), and sources of nutrients in marine food webs (Wada et al.1987, Fry 1988, Hobson & Clark 1992a,b), as well as identifying trophic relationships (Minagawa & Wada 1984) and dietary sources (Hobson & Clark 1992a,b, Hobson et al. 1996, Caut et al. 2009). Stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) can be used as proxies for diet composition, e.g. $\delta^{15}N$ increases between 2 and 5‰ during trophic transfer (DeNiro & Epstein 1981, Minagawa & Wada 1984, Caut et al. 2009), while $\delta^{13}C$ is a useful indicator of food sources (France 1995, Cardona et al. 2007), even though the increment in $\delta^{13}C$ values is smaller, between 0.5 and 1‰ for each trophic level (Caut et al. 2009).

The use of SIA in trophic ecology has become increasingly popular but has been paralleled by increasing concern about the reliability of the different techniques and processes used in laboratories around the world (Newsome et al. 2010). For instance, there are differences among laboratories in sample preparation (Soreide et al. 2006), preservation methods (Barrow et al. 2008), lipid extraction methods (Logan

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& Lutcavage 2008, Tarroux et al. 2010), and the use of different preservatives (Carabel et al. 2009). Studies on the effects of decomposition on the stability of stable isotope ratios have primarily focused on aquatic plants, with microbial activity being reported to modify $\delta^{13}C$ and $\delta^{15}N$ after death (Fogel et al. 1988, Currin et al. 1995, Caraco et al. 1998, Kuehn & Suberkropp 1998, Machás et al. 2006, Hill & McQuaid 2009). Despite the significance of these findings, no studies have assessed whether similar effects occur in animal tissues, probably because many researchers use fresh samples when they study animals that can be captured and easily sampled. However, research on endangered species, such as marine mammals and sea turtles, often involves using the carcasses of animals involuntarily killed as a result of human interactions or stranded for other reasons (e.g. Godley et al. 1998, Revelles et al. 2007a,b, Arthur et al. 2008, Cardona et al. 2009, Pinela et al. 2010, Fernández et al. 2011, Gómez-Campos et al. 2011). Carcasses may be found in various stages of decomposition (Kuiken & García Hartmann 1993), with tissue degradation potentially altering isotope signatures. If this is the case, previous studies using stranded animals might have reached inaccurate conclusions.

The present study explored the effect of decomposition isotope signatures using skin and muscle samples from striped dolphin *Stenella caeruleoalba* and loggerhead sea turtle *Caretta caretta* carcasses. We identified and quantified the changes in $\delta^{13}C$ and $\delta^{15}N$ during a 2 mo decomposition process, and assessed the samples for any impacts on stable isotope interpretation.

## MATERIALS AND METHODS

We sampled 3 striped dolphin and 3 loggerhead sea turtle carcasses from the western Mediterranean (Spain). The carcasses were collected and supplied to the authors by the Marine Animals Recovery Center (CRAM), an organization officially designated by the Catalonian regional government to collect stranded marine animals, and transported to the Veterinary School in Barcelona (Spain). Complete necropsies were performed by specialized biologists and veterinaries following standardized protocols (Wolke & George 1981, Geraci & Lounsbury 1993). All 6 individuals were very fresh at necropsy (Decomposition Code CC1 from Kuiken & García Hartmann 1993), with no significant pathologies or parasitic infections. A large muscle sample and corresponding skin sample from each dolphin was labeled and frozen at −20°C until the onset of the experiment. In turtles, the whole head and neck were frozen. All the samples were taken from the same areas on individual carcasses. In dolphins, muscle (*longissimus dorsi*) and skin were collected from the dorsal region, between the spiracle and the dorsal fin and skin that are next to the muscle. In turtles, skin and muscle (*longus colli*) samples were taken from the neck.

At the beginning of the experiment, the samples, described above, were left unpreserved outdoors in an experimental field at the University of Barcelona, exposed to rain and direct sunlight. At time intervals of 0, 4, 8, 19, 32, 50 and 62 d after defrosting, two 0.5 g muscle and skin subsamples from each specimen were collected and analyzed. This uneven temporal sampling approach allowed us to cover the major stages reported during carcass decomposition (Kuiken & García Hartmann 1993, Anderson & VanLaerhoven 1996). Decomposition stages were classified according to Kuiken & García Hartmann (1993), in agreement with the standard procedure for assessing the decomposition condition of marine mammal carcasses.

For SIA, each muscle and skin subsample (0.5 g) was dried for 3 d at 70°C and ground with a mortar and pestle. Lipids were removed by rinsing the ground tissue several times with a 2:1 ratio of a chloroform:methanol mixture (Bligh & Dyer 1959). Lipids were extracted because they contain lower levels of $\delta^{13}C$ compared to other molecules. DeNiro & Epstein (1977) found that performing an analysis without lipid extraction may lead to erroneous conclusions, which is especially important to avoid when comparing different tissue results (Newsome et al. 2010). After lipid extraction, the subsamples were dried for 24 h at 70°C. Approximately 0.3 mg of the powdered subsample was weighed in a tin capsule and automatically loaded and combusted at 1000°C. The subsample was then analyzed in a continuous-flow isotope-ratio mass spectrometer (Delta C Finnigan MAT).

The results were expressed in delta (δ) notation, in which the relative variations of stable isotope ratios are expressed as ‰ variations from predefined standards, and calculated as:

$$\delta X = [\ (R_{sample} / R_{standard}) - 1\ ] \times 10^3 \quad (1)$$

where $R_{sample}$ is the ratio of the heavy isotope to the light isotope in the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), and $R_{standard}$ is the ratio of the heavy isotope to the light isotope in the standard ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in ‰.

Standards used were Pee Dee Belemnite (V-PDB) calcium carbonate and atmospheric nitrogen (air) for

carbon and nitrogen, respectively. International secondary isotopic standards with known $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in relation to V-PDB and air, respectively, as given by the IAEA (International Atomic Energy Agency), were used for $\delta^{13}C$ (precision of 0.02‰) and $\delta^{15}N$ (precision of 0.03‰) calibration: namely polyethylene (IAEA $CH_7$, $\delta^{13}C = -32.15$ vs. V-PDB), sucrose (IAEA $CH_6$, $\delta^{13}C = -10.4$ vs. V-PDB), L-glutamic acid ($USGS_{40}$ $\delta^{13}C = -26.58$ vs. V-PDB; $\delta^{15}N = 4.5$ vs. air), acetanilide ($\delta^{13}C = -25.3$ vs. V-PDB; $\delta^{15}N = -8.8$ vs. air), $(NH_4)_2SO_4$ (IAEA $N_1$ $\delta^{15}N = +0.4$ vs. air; IAEA $N_2$ $\delta^{15}N = 20.3$ vs. air), $KNO_3$ ($USGS_{34}$, $\delta^{15}N = -1.8$ vs. air), UCGEMA-F ($\delta^{15}N = 4.15$ vs. air; $\delta^{13}C = -26.58$ vs. V-PDB), and caffeine (IAEA-600 $\delta^{15}N = 1$ vs. air; $\delta^{13}C = -27.7$ vs. V-PDB).

### Statistical analysis

Data were analyzed using a Kolmogorov-Smirnov test to assess normality. The homogeneity of variances between sample groups was tested using the Levene test. Repeated-measures ANOVA of 2 factors (time and species) was conducted separately for isotopes ($\delta^{15}N$ and $\delta^{13}C$) and tissues (skin and muscle). Time and species were considered as within-subject and inter-subject factors, respectively. The same analysis was conducted on % C and % N in order to complement the study.

## RESULTS

Samples exuded a moderate smell of decomposition and exhibited changes in colour and consistency on Days 4 and 8 after defrosting, thus qualifying as decomposition stage CC3. From Days 19 to 62 after defrosting, samples were in an advanced stage of decomposition, with the skin and muscle clearly altered and a clear smell of decomposition, thus qualifying as decomposition stage CC4. Fly larvae were present on the samples from Day 4 and remained throughout the experiment, although were more abundant in the turtle samples.

Results of $\delta^{15}N$ and $\delta^{13}C$ values over the 62 d decomposition period are shown in Table 1 and Fig. 1 and of the %N and %C values in Table 2 and Fig. 2.

The results from repeated-measures ANOVA showed that $\delta^{13}C$, $\delta^{15}N$, %N and %C did not vary over time (within-subject) and that there was no temporal variation in the 2 species (inter-subject) for any 2 tissues considered (Tables 3 & 4). Therefore, we infer that samples at different states of decomposition did not differ in isotopic signatures or in C and N percentages as a consequence of the decomposition process.

## DISCUSSION

Fry (2006) defined SIA as one of the most powerful tools to identify different ecological processes, and as such this technique should be accurately tested to achieve depurated methodologies and avoid erroneous conclusions or misinterpretations. Moreover, an increase in the use of SIA has generated the need for tissue collections and preparation protocols to be standardized to improve the quality and reliability of inter-laboratory comparisons. Accordingly, much scientific literature has been produced to test the reliability of sample manipulation techniques (Hobson et al. 1997, Kaehler & Pakhomov 2001, Kelly et al. 2006, Barrow et al. 2008, Carabel et al. 2009).

Table 1. *Stenella coeruleoalba*, *Caretta caretta*. $\delta^{15}N$ and $\delta^{13}C$ values in muscle and skin during the 62 d decomposition process. –: missing data

| Species Tissue | Time (d) | $\delta^{15}N$ Mean | $\delta^{15}N$ SD | $\delta^{13}C$ Mean | $\delta^{13}C$ SD |
|---|---|---|---|---|---|
| ***S. coeruleoalba*** | | | | | |
| Muscle | 0 | 9.67 | 0.51 | −17.44 | 0.37 |
| | 4 | 9.72 | 0.45 | −17.55 | 0.23 |
| | 8 | 9.76 | 0.48 | −17.43 | 0.21 |
| | 19 | 9.82 | 0.12 | −17.58 | 0.04 |
| | 32 | 9.85 | 0.25 | – | – |
| | 50 | 9.63 | 0.28 | −17.63 | 0.18 |
| | 62 | 9.74 | 0.42 | −17.52 | 0.36 |
| Skin | 0 | 10.96 | 0.57 | −17.09 | 0.40 |
| | 4 | 11.30 | 0.76 | −16.91 | 0.36 |
| | 8 | 11.09 | 0.76 | −16.74 | 0.47 |
| | 19 | 10.61 | 0.82 | −17.29 | 0.61 |
| | 32 | 10.88 | 0.51 | −17.18 | 0.18 |
| | 50 | 11.13 | 0.64 | −16.90 | 0.45 |
| | 62 | 11.16 | 0.61 | −17.04 | 0.37 |
| ***C. caretta*** | | | | | |
| Muscle | 0 | 8.74 | 0.30 | −18.30 | 0.04 |
| | 4 | 8.53 | 1.10 | −18.48 | 0.29 |
| | 8 | 7.99 | 0.42 | – | – |
| | 19 | 8.73 | 0.77 | −17.90 | 0.71 |
| | 32 | 8.57 | 0.08 | −18.21 | 0.80 |
| | 50 | 8.51 | 0.91 | −18.17 | 0.12 |
| | 62 | 8.49 | 0.80 | −17.91 | 0.38 |
| Skin | 0 | 7.88 | 0.42 | −16.12 | 0.05 |
| | 4 | 7.97 | 0.41 | −16.19 | 0.37 |
| | 8 | 7.92 | 0.42 | −16.38 | 0.08 |
| | 19 | 7.93 | 0.42 | −16.16 | 0.38 |
| | 32 | 7.94 | 0.41 | −16.05 | 0.17 |
| | 50 | 8.08 | 0.42 | −16.14 | 0.21 |
| | 62 | 7.64 | 0.22 | −16.05 | 0.87 |

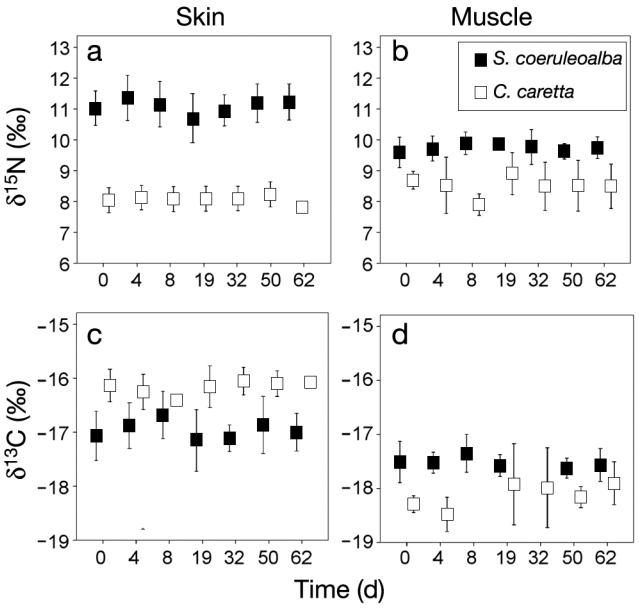


Fig. 1. *Stenella coeruleoalba*, *Caretta caretta*. Mean (±SE) $\delta^{15}N$ in (a) skin and (b) muscle, and $\delta^{13}C$ in (c) skin and (d) muscle of carcass samples left unpreserved outdoors to decompose for 62 d

Table 2. *Stenella coeruleoalba*, *Caretta caretta*. %N and %C values in muscle and skin during the 62 d decomposition process

| Species Tissue | Time (d) | %N Mean | %N SD | %C Mean | %C SD |
|---|---|---|---|---|---|
| ***S. coeruleoalba*** | | | | | |
| Muscle | 0 | 14.40 | 0.34 | 46.35 | 0.91 |
| | 4 | 14.46 | 0.35 | 46.14 | 1.09 |
| | 8 | 14.32 | 0.60 | 46.13 | 1.80 |
| | 19 | 14.47 | 0.46 | 46.63 | 1.17 |
| | 32 | 14.69 | 1.08 | 45.78 | 2.62 |
| | 50 | 14.02 | 1.39 | 44.84 | 4.19 |
| | 62 | 14.73 | 0.38 | 47.12 | 0.63 |
| Skin | 0 | 14.48 | 0.35 | 45.23 | 1.03 |
| | 4 | 14.91 | 0.36 | 45.72 | 0.71 |
| | 8 | 14.74 | 0.36 | 45.19 | 0.98 |
| | 19 | 14.55 | 0.60 | 45.30 | 1.06 |
| | 32 | 14.50 | 0.32 | 44.98 | 0.94 |
| | 50 | 14.41 | 0.37 | 44.87 | 0.81 |
| | 62 | 14.62 | 0.44 | 45.11 | 0.71 |
| ***C. caretta*** | | | | | |
| Muscle | 0 | 14.20 | 0.53 | 45.87 | 1.14 |
| | 4 | 13.28 | 0.98 | 44.12 | 3.69 |
| | 8 | 14.48 | 0.94 | 45.98 | 2.69 |
| | 19 | 13.86 | 1.35 | 42.71 | 4.05 |
| | 32 | 13.04 | 1.42 | 42.58 | 4.16 |
| | 50 | 14.43 | 0.83 | 43.89 | 5.55 |
| | 62 | 13.91 | 0.63 | 44.62 | 1.73 |
| Skin | 0 | 15.20 | 0.56 | 43.50 | 1.24 |
| | 4 | 15.33 | 0.25 | 44.09 | 0.66 |
| | 8 | 14.51 | 0.97 | 41.66 | 2.66 |
| | 19 | 15.03 | 0.70 | 44.40 | 0.80 |
| | 32 | 15.25 | 0.87 | 43.96 | 1.63 |
| | 50 | 15.37 | 0.82 | 44.54 | 2.92 |
| | 62 | 14.89 | 0.68 | 43.19 | 2.62 |

The present study addressed a separate potential source of isotope signature bias by investigating the effect of animal tissue decomposition. It is difficult to obtain tissue samples of marine mammals and marine turtles when alive because of their scarcity and elusiveness; hence, many biochemical analyses are carried out on samples from stranded dead individuals with different preservation conditions. Therefore, it is essential to evaluate how the decomposition process might influence SIA to obtain sound interpretations about ecological processes (Hill & McQuaid 2009). Certainly, a number of carcasses from simultaneously dead turtles and dolphins allowed to decompose on the beach and daily sampling would offer a more realistic experimental approach, but such an experimental design faces a number of logistic challenges.

Firstly, a massive dead stranding would be necessary to assure that all the experimental animals died within a brief time interval and decomposed in a similar environment. Massive strandings of dolphins are relatively common, but turtles seldom strand massively as they are solitary animals. Accordingly, we decided to freeze samples from individual strandings and begin the experiment only when the sample size was large enough to guarantee that all samples could be exposed to the same environmental conditions. Although freezing and thawing cause faster initial water loss and slightly modify the sequence of changes associated with carcass decomposition (Micozzi 1986), freezing does not cause major changes in the composition of bacterial communities (Davis 1976, Micozzi 1986) and it is not thought to affect the stability of stable isotope ratios during the decomposition process. Secondly, daily sampling would have dramatically increased the cost of analysis and it is not cost-effective, because decomposition is a non-linear process and the rate of change slows down through time (Anderson & VanLaerhoven 1996). Accordingly, increasing time between successive sample collections is a more cost-effective approach than regular sampling. Thirdly, leaving the carcasses on the beach was not possible, because Spanish regulations require the immediate removal of stranded carcasses of marine mammals and turtles to protect public health.

The results of the current study revealed no statistically significant differences in $\delta^{13}C$ and $\delta^{15}N$ values of the skin and muscle of either striped dolphins or

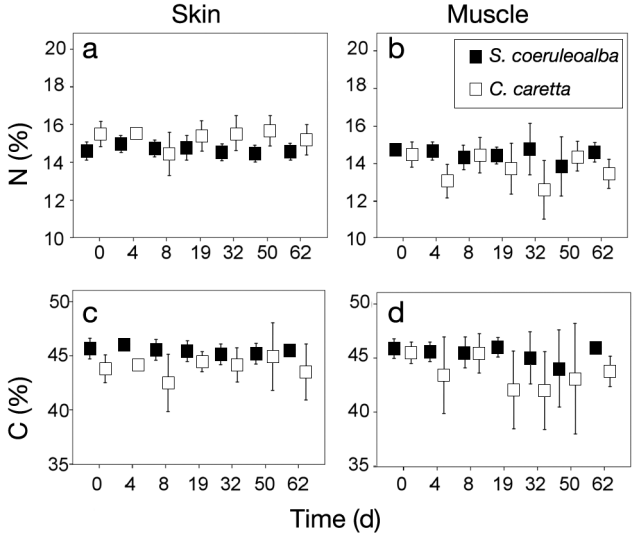


Fig. 2. *Stenella coeruleoalba*, *Caretta caretta*. Mean (±SE) % N in (a) skin and (b) muscle, and %C in (c) skin and (d) muscle of carcass samples left unpreserved outdoors to decompose for 62 d

Table 3. Repeated-measures ANOVA of 1 within-subjects factor: time (0, 4, 8, 19, 32, 50 and 62 d after the start of the decomposition process), and its interaction with inter-subject factors: species (*Stenella coeruleoalba* and *Caretta caretta*) for $\delta^{13}C$ and $\delta^{15}N$

| | Tissue | $\delta^{13}C$ | | $\delta^{15}N$ | |
|---|---|---|---|---|---|
| | | *F* | p | *F* | p |
| Time | Muscle | 0.564 | 0.608 | 0.893 | 0.447 |
| Time × Species | Muscle | 0.676 | 0.550 | 0.936 | 0.488 |
| Time | Skin | 0.365 | 0.713 | 0.859 | 0.439 |
| Time × Species | Skin | 1.486 | 0.281 | 0.856 | 0.440 |

Table 4. Repeated-measures ANOVA of 1 within-subjects factor: time (0, 4, 8, 19, 32, 50 and 62 d after the start of the decomposition process), and its interaction with inter-subject factors: species (*Stenella coeruleoalba* and *Caretta caretta*) for %C and %N

| | Tissue | %C | | %N | |
|---|---|---|---|---|---|
| | | *F* | p | *F* | p |
| Time | Muscle | 0.985 | 0.377 | 0.411 | 0.858 |
| Time × Species | Muscle | 1.580 | 0.277 | 1.603 | 0.229 |
| Time | Skin | 2.004 | 0.105 | 1.150 | 0.365 |
| Time × Species | Skin | 1.000 | 0.231 | 1.459 | 0.234 |

loggerhead turtles across a 62 d period and, hence, confirm that the decomposition process itself is a minor source of variability in the isotope ratios of these 2 species These findings contrast with some of the results reported in the extensive literature about changes in isotopic signature caused by the decomposition of submerged vascular macrophytes, seagrasses, seaweeds, and mangroves. These studies have identified different trends in the temporal variation of $\delta^{13}C$ and $\delta^{15}N$ values over the decomposition process, with significant differences reported among taxonomic groups (Zieman et al. 1984, Benner et al. 1987, Fenton & Ritz 1988, Fellerhoff et al. 2003, Machás et al. 2005, Hill & McQuaid 2009). These differences are caused by variation in the nitrogen content and chemical composition of the cell wall of different taxonomic groups (Zieman et al. 1984, Fenton & Ritz 1988, Hill & McQuaid 2009). In contrast, protein is the major macromolecule of vertebrate soft tissues once fat has been removed, which results in a rather constant C/N ratio in lean tissue (Clawson et al. 1991, Sterner and Elser 2002, Hendrixson et al. 2007) and probably explains why differences are not observed over time between tissues and species.

Although the results reported in the present study suggest that stable isotope ratios of stranded dead dolphins and turtles are suitable for evaluating food sources, habitat use, and trophic relationships, there are a number of limitations, derived from the experimental design. Firstly, the process of decomposition differs in carcasses exposed to air and those totally submerged (Payne & King 1972), so the results reported here cannot be invoked to claim the stability of the stable isotope ratios in carcasses that have remained underwater for a long time. Secondly, the results reported here do not demonstrate the stability of the stable isotope ratios of carcasses reaching decomposition stage CC5, with organs beyond clear recognition or absent (Kuiken & García Hartmann 1993), because the samples used for the present experiment reached only stage CC4. Finally, other internal organs, such as liver or kidneys, may decompose faster than muscle or skin, and, hence, stability of stable isotope ratios cannot be assured until experimentally assessed.

## CONCLUSIONS

To our knowledge, this is the first study verifying that non-submerged marine animal tissue decomposition does not affect stable isotope signatures. Our findings have strong implications for all preceding and future studies, as we did not detect any evidence of change over time in decomposing samples of muscle and skin samples exposed to air and reaching decomposition stage CC4. This study provides strong evidence confirming that the results obtained in pre-

vious studies are statistically comparable despite animal samples being at different states of freshness, and validating their utility in scientific studies. However, further research is needed to confirm that stable isotope ratios in other tissues are not influenced by decomposition and also that they are stable when carcasses decompose under water.

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## LITERATURE CITED

Anderson GS, VanLaerhoven SL (1996) Initial studies on insect succession on carrion in southwestern British Columbia. J Forensic Sci 41:617–625

Arthur KE, Boyle MC, Limpus CJ (2008) Ontogenetic changes in diet and habitat use in green sea turtle (*Chelonia mydas*) life history. Mar Ecol Prog Ser 362:303–311

Barrow LM, Bjorndal KA, Reich KJ (2008) Effects of preservation method on stable carbon and nitrogen isotope values. Physiol Biochem Zool 81:688–693

Benner R, Fogel ML, Sprague EK, Hodson RE (1987) Depletion of $^{13}C$ in lignin and its implications for stable carbon isotope studies. Nature 329:708–710

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917

Carabel S, Verisimo P, Freire J (2009) Effects of preservatives on stable isotope analyses of four marine species. Estuar Coast Shelf Sci 82:348–350

Caraco NF, Lampman G, Cole JJ, Limburg KE, Pace ML, Fischer D (1998) Microbial assimilation of DIN in a nitrogen rich estuary: implications for food quality and isotope studies. Mar Ecol Prog Ser 167:59–71

Cardona L, Revelles M, Sales M, Aguilar A, Borrell A (2007) Meadows of the seagrass *Posidonia oceanica* are a relevant source of organic matter for adjoining ecosystems. Mar Ecol Prog Ser 335:123–131

Cardona L, Aguilar A, Pazos L (2009) Delayed ontogenic dietary shift and high levels of omnivory in green turtles (*Chelonia mydas*) from the NW coast of Africa. Mar Biol 156:1487–1495

Caut S, Fossette S, Guirlet E, Angulo E, Das K, Girondot M, Georges JY (2008) Isotope analysis reveals foraging area dichotomy for Atlantic leatherback turtles. PLoS ONE 3: e1845

Caut S, Angulo E, Courcharmp F (2009) Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46:443–453

Clawson AJ, Garlich JD, Coffey MT, Pond WG (1991) Nutritional, physiological, genetic, sex and age effects on fat-free dry matter composition on the body in avian, fish and mammalian species: a review. J Anim Sci 69:3617–3644

Currin CA, Newell SY, Paerl HW (1995) The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. Mar Ecol Prog Ser 121:99–116

Davis CP (1976) Preservation of gastrointestinal bacteria and their microenvironmental associations in rats by freezing. Appl Environ Microbiol 31:304–312

DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197: 261–263

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

Fellerhoff C, Voss M, Wantzen KM (2003) Stable carbon and nitrogen isotope signatures of decomposing tropical macrophytes. Aquat Ecol 37:361–375

Fenton GE, Ritz DA (1988) Changes in carbon and hydrogen stable isotope ratios of macroalgae and seagrass during decomposition. Estuar Coast Shelf Sci 26:429–436

Fernández R, García-Tiscar S, Santos MB, López A, Martínez-Cedeira JA, Newton J, Pierce GJ (2011) Stable isotope analysis in two sympatric populations of bottlenose dolphins *Tursiops truncatus*: evidence of resource partitioning? Mar Biol 158:1043–1055

Fogel ML, Sprague EK, Gize AP, Frey RW (1988) Diagenesis of organic matter in Georgia salt marshes. Estuar Coast Shelf Sci 28:211–230

France RL (1995) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol Oceanogr 40:1310–1313

Fry B (1988) Food web structure on Georges Bank from stable C, N and S isotopic compositions. Limnol Oceanogr 33:1182–1190

Fry B (2006) Stable isotope ecology. Springer, New York, NY

Geraci JR, Lounsbury VJ (1993) Marine mammals ashore: a field guide to strandings. Texas A&M University Sea Grant College Program Publication TAMU-SG-93-601

Godley BJ, Thompson DR, Waldron S, Furness RW (1998) The trophic status of marine turtles as determined by stable isotope analysis. Mar Ecol Prog Ser 166:277–284

Gómez-Campos E, Borrell A, Aguilar A (2011) Nitrogen and carbon stable isotopes do not reflect nutritional condition in the striped dolphin. Rapid Commun Mass Spectrom 25:1343–1347

Hendrixson HA, Sterner RW, Kay D (2007) Elemental stoichiometry of freshwater fishes in relation to phylogeny, allometry and ecology. J Fish Biol 70:121–140

Hill JM, McQuaid CD (2009) Variability in the fractionation of stable isotopes during degradation of two intertidal red algae. Estuar Coast Shelf Sci 82:397–405

Hobson KA, Clark RG (1992a) Assessing avian diets using stable isotopes 2. Factors influencing diet tissue fractionation. Condor 94:189–197

Hobson KA, Clark RG (1992b) Assessing avian diets using stable isotopes 1. Turnover of $^{13}C$ in tissues. Condor 94: 181–188

Hobson KA, Wassenaar LI (1999) Stable isotope ecology: an introduction. Oecologia 120:312–313

Hobson KA, Schell DM, Renouf D, Noseworthy E (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can J Fish Aquat Sci 53:528–533

Hobson KA, Gloutney ML, Gibbs HL, Hobson KA (1997) Preservation of blood and tissue samples for stable carbon and stable nitrogen isotope analysis. Can J Zool 75:1720–1723

Kaehler S, Pakhomov EA (2001) Effects of storage and preservation on the $\delta^{13}C$ and $\delta^{15}N$ signatures of selected marine organisms. Mar Ecol Prog Ser 219:299–304

Kelly B, Dempson JB, Power M (2006) The effects of preservation on fish tissue stable isotope signatures. J Fish Biol 69:1595–1611

Kuehn KA, Suberkropp K (1998) Decomposition of standing litter of the freshwater emergent macrophyte *Juncus effusus*. Freshw Biol 40:717–727

Kuiken M, García Hartmann M (1993) Proceedings of the first ECS workshop on cetacean pathology: dissection techniques and tissue sampling, Leiden, 13–14 Sep 1991. ECS Special Newsletter, European Cetacean Society

Kurle CM, Worthy GAJ (2002) Stable nitrogen and carbon ratios in multiple tissues of the northern fur seal *Callorhinus ursinus*: implications for dietary and migratory reconstructions. Mar Ecol Prog Ser 236:289–300

Logan JM, Lutcavage ME (2008) A comparison of carbon and nitrogen stable isotope ratios of fish tissues following lipid extractions with non-polar and traditional chloroform/methanol solvent systems. Rapid Commun Mass Spectrom 22:1081–1086

Machás R, Santos R, Peterson B (2006) Elemental and stable isotope composition of *Zostera noltii* (Horneman) leaves during the early phases of decay in a temperate mesotidal lagoon. Estuar Coast Shelf Sci 66:21–29

Micozzi MS (1986) Experimental study of postmortem change under field conditions: effects of freezing, thawing, and mechanical injury. J Forensic Sci 31:953–961

Minagawa M, Wada E (1984) Setwise enrichment of $^{15}N$ along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48:1135–1140

Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mamm Sci 26:509–572

Payne JA, King EW (1972) Insect succession and decomposition of pig carcasses in water. J Georgia Entomol Soc 7:153–162

Pinela AM, Borrell A, Cardona L, Aguilar A (2010) Stable isotope analysis reveals habitat partitioning among marine mammals of the NW African coast and unique trophic niches for two globally threatened species. Mar Ecol Prog Ser 416:295–306

Revelles M, Cardona L, Aguilar A, Borrell A, Fernández G, San Félix M (2007a) Stable C and N isotope concentration in several tissues of the loggerhead sea turtle *Caretta caretta* from the western Mediterranean and dietary implications. Sci Mar 71:87–93

Revelles M, Cardona L, Aguilar A, Fernández G (2007b) The diet of pelagic loggerhead sea turtles (*Caretta caretta*) off the Balearic archipelago (western Mediterranean): relevance of long-line baits. J Mar Biol Assoc UK 87:805–813

Soreide JE, Temalander T, Hop H, Hobson KA, Johansen I (2006) Sample preparation effects on stable C and N isotope values: a comparison of methods in Arctic marine food web studies. Mar Ecol Prog Ser 328:17–28

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, NJ

Tarroux A, Ehrich D, Lecomte N, Jardine TD, Bety J, Berteaux D (2010) Sensitivity of stable isotope mixing models to variation in isotopic ratios: evaluating consequences of lipid extraction. Methods Ecol Evol 1:231–241

Wada E, Terazaki M, Kabya Y, Nemoto T (1987) $^{15}N$ and $^{13}C$ abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. Deep-Sea Res 34:829–841

Wolke RE, George A (1981) Sea turtle necropsy manual. NOAA Tech Mem NMFS-SEFC-24

Zieman JC, Macko SA, Mills AL (1984) Role of seagrasses and mangroves in estuarine food webs: temporal and spatial changes in stable isotope composition and amino acid content during decomposition. Bull Mar Sci 35: 380–392

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