



FEATURE ARTICLE

# Effects of blood anticoagulants on stable isotope values of sea turtle blood tissue

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**ABSTRACT:** Collecting tissue samples from sea turtles for stable isotope analysis often occurs at remote field sites. For blood tissue, samples are treated with an anticoagulant that allows for later separation of plasma from cellular components. However, the effect of this technique on stable isotope values of sea turtle blood has not been established. We measured the effects of 3 widely used anticoagulants, acid citrate dextrose (ACD), sodium heparin (SH) and ethylenediaminetetraacetic acid (EDTA), on stable carbon ( $\delta^{13}\text{C}$ ) and stable nitrogen ( $\delta^{15}\text{N}$ ) values in whole blood, red blood cells, and blood plasma of 11 green turtles *Chelonia mydas* captured in San Diego Bay, California, USA. Vials containing each of the 3 blood preservatives as well as a vial containing no additive (i.e. control vial) were filled in random order. Blood in the no-additive vial was immediately separated into fractions (e.g. red blood cells, plasma) via centrifugation, whereas blood collected in the treatment vials was chilled and then centrifuged 48 h after collection. We found that, relative to the controls, ACD-preserved whole blood and blood plasma were  $^{13}\text{C}$  enriched, EDTA-treated red blood cells and plasma were  $^{15}\text{N}$  depleted, and SH-treated whole blood was  $^{15}\text{N}$  enriched. Because SH was the only anticoagulant with no measured effect on blood plasma and red blood cells—the most commonly studied blood fractions for sea turtle stable isotope studies—we recommend its exclusive use as a blood anticoagulant for field studies where prompt centrifugation is not possible.

**KEY WORDS:**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  · Carbon · Cheloniidae · Ectotherm · Isotope enrichment · Nitrogen · Reptilia

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Only 1 of 3 commonly used blood preservatives caused low distortion of stable isotope values in green sea turtles *Chelonia mydas*.

Photo: Michael Jensen

## INTRODUCTION

Over the past few decades, questions about the nutritional ecology of many marine vertebrates have been addressed using stable isotope analysis (SIA; Godley et al. 1998, Kurle 2002, Estrada et al. 2003, Ruiz-Cooley et al. 2004). Because the isotopic composition of a consumer's body tissue is related to that of its diet, nutritional questions can be addressed by SIA of various types of tissues (DeNiro & Epstein 1978, 1981, Hobson & Clark 1992, Michener & Schell 1994). Since isotopic turnover rates (i.e. residence times) vary among tissue types (Seminoff et al. 2007, Reich et al. 2008), analysis of different tissues may

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also yield insights into foraging behavior over different time ranges. For example, in a study of isotopic turnover in hatchling loggerhead sea turtles *Caretta caretta*, Reich et al. (2008) found that red blood cells (RBCs) had the slowest turnover rate of all the blood fractions (76.9 d for  $\delta^{13}\text{C}$ , 71.4 d for  $\delta^{15}\text{N}$ ), while blood plasma (PLA) had the fastest rate (20.0 d for  $\delta^{13}\text{C}$ , 18.5 d for  $\delta^{15}\text{N}$ ). Moreover, for sea turtles, blood tissue is often analysed because it involves a relatively non-invasive sampling procedure (Wallace et al. 2006, 2009, Caut et al. 2008, Dodge et al. 2011).

Despite the advantages of using blood tissue in SIA, one of the primary limitations of its use in sea turtle studies is the rapid coagulation that occurs in turtle blood (Soslau et al. 2004). This rapid coagulation is due in part to thrombocytes and the lack of release of an ADP molecule, a unique hemostatic developmental characteristic found in sea turtles (but not in mammals) (Dessauer 1970, Soslau et al. 2005). Because of this rapid coagulation, SIA of sea turtle blood requires the near-immediate centrifugation of blood after collection, a capability often hampered by the logistically challenging field conditions under which studies of marine organisms are often conducted.

To address this challenge, blood preservatives are commonly used to prevent the coagulation cascade, thus allowing for centrifugation at a later stage. Common preservatives used for blood collection include acid citrate dextrose (ACD), sodium heparin (SH), and ethylenediaminetetraacetic acid (EDTA) (Fig. 1). Whereas the anticoagulative properties of ACD are derived from the process of chelation i.e. the binding of  $\text{Ca}^{+2}$  to retard glycolysis (Rand et al. 1996), SH causes the production of antithrombin III, which inactivates thrombin, thus preventing clotting of blood (Shuman & Majerus 1976). EDTA acts as an efficient chelator through binding calcium and magnesium ions to prevent the coagulation cascade (Rand et al. 1996). All 3 anticoagulants have commonly been used in studies of sea turtles and other wildlife (Laborde et al. 1995, Mohri & Rezapoor 2009), as well as in investigations involving human blood tissue (Cohen et al. 1965, Shuman & Majerus 1976, Rand et al. 1996).

Understanding the effects of preservation on the isotopic composition of animal body tissues is critical for accurately applying SIA. Many studies have examined the effects of preservatives on various soft tissues such as skin and muscle of marine organisms (Kaehler & Pakhomov 2001, Sarakinos et al. 2002, Barrow et al. 2008) and studies have examined the effects of anticoagulants on stable isotope values of blood tissue of birds and mammals (Hobson et al.

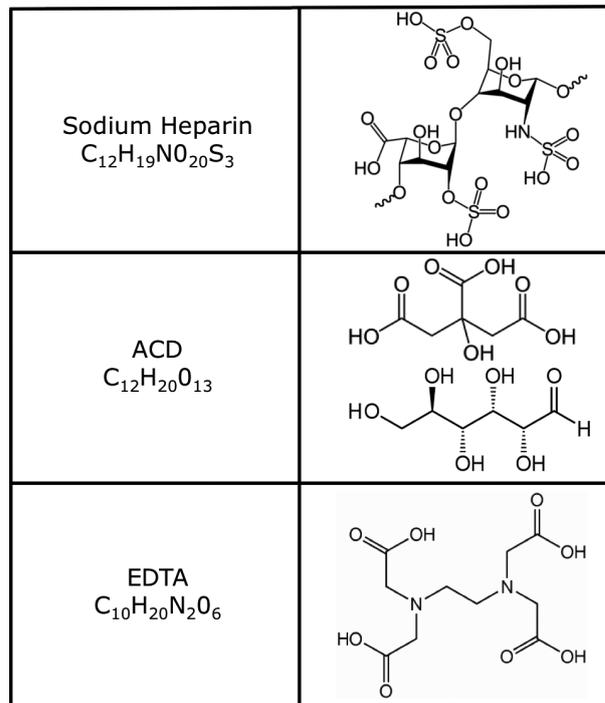


Fig. 1. Structural depiction of anticoagulants acid citrate dextrose (ACD), ethylenediaminetetraacetic acid (EDTA) and sodium heparin (SH). H, N, O, S indicate hydrogen, nitrogen, oxygen and sulfur atoms, respectively. Note that saturated carbon atoms are not depicted in the structures, but are implied at each bonding point in the carbon (hexagonal) ring and at each linear branch point

1997, Bugoni et al. 2008, Kraft et al. 2008). However, to date there have been no studies on the effects of anticoagulants on stable isotope values of sea turtle blood. Considering the unique characteristics of blood from reptiles compared to that of birds and mammals (see above), establishing the efficacy of blood preservation for SIA in sea turtles is a research priority.

In the present study, we measured the effects of 3 commonly used blood preservatives (ACD, EDTA, SH) on stable carbon ( $\delta^{13}\text{C}$ ) and stable nitrogen ( $\delta^{15}\text{N}$ ) values in whole blood (WHB), RBCs, and PLA of green turtles *Chelonia mydas*. We hypothesized that because nitrogen is uncommon or absent in the molecular structures of the anticoagulants tested in this study (Fig. 1),  $\delta^{15}\text{N}$  values of blood fractions would be minimally altered by our preservative treatments. In contrast, the abundance of carbon in ACD, EDTA and SH molecules suggests that blood  $\delta^{13}\text{C}$  values would be more likely to be affected by the introduction of these anticoagulants. The objective of our study was to gain a further understanding of anticoagulant effects on blood tissue, and thus determine the optimal blood anticoagulant for use in sea turtle SIAs.

## MATERIALS AND METHODS

### Turtle capture and tissue collection

Green turtles were captured in San Diego Bay (California, USA) from April 2004 to March 2005 using tangle nets (length = 100 m, depth = 4 m, mesh size = 40 cm stretched). A total of 11 green turtles were captured, with 1 turtle sampled 3 times and another sampled twice (Table 1). Straight carapace length (notch to tip) ranged from 53.1 to 105.5 cm and body weight from 21.8 to 200.0 kg (Table 1).

We collected 28 to 32 cm<sup>3</sup> of blood from each turtle via the dorsal cervical sinus (Owens & Ruiz 1980), in portions of 7 to 8 cm<sup>3</sup> in 4 different vacutainers. These included a control sample that was collected with no preservative (i.e. no-additive or control) and 3 different 'treatment' samples collected in vials containing SH; EDTA and ACD. The order of vial use during blood collection was random.

For control samples collected in 'no-additive' vials, approximately one half of each blood sample was immediately separated into PLA and cellular components by centrifugation (30 × *g* for 5 min), and the remaining half was kept as WHB. All control samples were then chilled until return from the field (<8 h after sampling), at which time they were refrigerated (5°C) for 48 h until analysis. For the treatment portion of this study, vials of non-centrifuged, 'preserved' WHB were chilled upon collection, and transferred to refrigeration upon returning from the field (<8 h after sampling). After 48 h, one half of each sample was centrifuged and separated into blood fractions, while the other half was maintained as WHB for analysis.

Table 1. *Chelonia mydas*. Straight carapace length (SCL), mass and capture date (mm/dd/yy) of green turtles used in this study from San Diego Bay, CA; nd: no data

Turtle ID	Date of capture	SCL (cm)	Mass (kg)
4546	04/21/04	91.9	123.0
7220	11/30/04 <sup>a</sup>	105.5	200.0
8356	04/19/05	92.8	nd
8360	01/13/05	93.6	nd
8367	03/02/05	89.2	nd
9248	02/15/05	65.5	35.5
12114	11/30/04	62.8	38.0
13581	11/18/04	67.4	144.0
13582	02/15/05	54.9	25.0
13583	01/13/05 <sup>b</sup>	53.1	21.8
13584	11/30/04	95.9	105.0

<sup>a</sup>Turtle 7220 was also sampled on 03/16/05; this isotope result is not included in the final analysis  
<sup>b</sup>Turtle 13583 was also sampled on 03/16/05 and 04/19/05; these isotope results are not included in the final analysis

### Stable isotope analysis

Blood samples (WHB, RBC, PLA) were dried at 60°C for 24 h and then powdered with a mortar and pestle. Approximately 0.60 mg were loaded into sterilized tin capsules and analyzed by a continuous-flow isotope-ratio mass spectrometer in the Stable Isotope Laboratory at Scripps Institution of Oceanography, La Jolla, California. We used a Costech ECS 4010 elemental combustion system interfaced via a ConFlo III device (Finnigan MAT) to a Deltaplus gas isotope-ratio mass spectrometer (Finnigan MAT). Sample stable isotope ratios relative to the isotope standard are expressed in the following conventional delta ( $\delta$ ) notation in parts per thousand (‰):

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

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the corresponding ratios of heavy to light isotopes (<sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N) in the sample and standard, respectively.  $R_{\text{standard}}$  for <sup>13</sup>C was Baker Acetanilide (C<sub>8</sub>H<sub>9</sub>NO;  $\delta^{13}\text{C} = -10.4$ ) calibrated monthly against the Peedee Belemnite limestone formation international standard;  $R_{\text{standard}}$  for <sup>15</sup>N was IAEA N1 Ammonium Sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>;  $\delta^{15}\text{N} = +0.4$ ) calibrated against atmospheric N<sub>2</sub> and United States Geological Survey (USGS) nitrogen standards. All analytical runs included samples of standard materials inserted every 6 to 7 samples to calibrate the system and compensate for any drift over time. Hundreds of replicate assays of standard materials indicated measurement errors of 0.05 and 0.09‰ for carbon and nitrogen, respectively. In addition to stable isotope ratios, we measured %C and %N for each sample. Samples were combusted in pure oxygen in the elemental analyzer. Resultant CO<sub>2</sub> and N<sub>2</sub> gases were passed through a series of thermal conductivity detectors and element traps to determine percent compositions. Acetanilide standards (10.36% N, 71.09% C) were used for calibration.

### Statistical analysis

Stable isotope ratios were compared among the 3 treatments (ACD, SH, EDTA) and the control, which did not include any anticoagulants. For each element ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and tissue (WHB, RBC, and PLA) combination, the difference ( $D$ ) between treatments and control for each individual was computed ( $D_{i,j}$ , for  $i$ th element and  $j$ th tissue). This is an array of  $n$  by 3, where  $n$  is the number of individuals with no missing value in the control sample. For example, for the

$\delta^{13}\text{C}$  and WHB combination of the  $k$ th individual with ACD,  $D_{ij,k} = \delta^{13}\text{C}_{\text{WHB,ACD},(k)} - \delta^{13}\text{C}_{\text{ctrl,ACD},(k)}$ . If the anticoagulant had effects on  $\delta^{13}\text{C}$  in the WHB, the mean of the differences would be different from zero. To estimate the mean differences, we used the following Bayesian approach. The differences of the  $i$ th element and  $j$ th tissue were modeled with multivariate normal distributions with means ( $\mu$ ) and a covariance matrix ( $\Sigma$ ), or  $D_{ij} \sim N_3(\mu_{ij}, \Sigma_{ij})$  where  $N_3(\mu, \Sigma)$  indicates a tri-variable normal distribution with the mean vector  $\mu$  and covariance matrix  $\Sigma$ . We used diffuse independent prior distributions ( $N(0, \sigma^2)$ ,  $\sigma^2 = 1000$ ) for each treatment mean  $\mu_{ij}$ . For the covariance matrix, we used an inverse-Wishart distribution (Gelman et al. 2003, p. 483). The joint posterior distributions were computed using Markov chain Monte Carlo using 4 independent chains, 200 000 'burn-in' steps to stabilize the chains, and 100 000 sampling steps, of which every 10 samples were selected to reduce the sample autocorrelations. Resulting 40 000 samples were used to make inference about the parameters of interest ( $\mu_{ij}$ ). Computations were done using Matbugs (<http://code.google.com/p/matbugs/>) and OpenBugs (Lunn et al. 2009). We report median values with 95 % posterior intervals unless otherwise noted. Statistical significance was determined at an approximate probability that the mean difference is greater than zero,  $P(D > 0)$ . If  $P(D > 0)$  was very small or large, the difference was considered statistically significant. Statistical significance was determined by the probability that the mean difference is greater than zero ( $P(D > 0)$ ). If there was no difference, approximately 50 % of  $D$  would be greater than zero and 50 % would be less than zero, i.e.,  $P(D > 0) \cong 0.5$ . On the other hand, if there was a difference,  $P(D > 0)$  would be large ( $D$  is mostly positive) or small ( $D$  is mostly negative). Using the conventional value, we use 0.05 as our cut off value. In other words,  $P(D > 0) < 0.05$  or  $P(D > 0) > 0.95$  was considered statistically significant.

For the 2 turtles sampled more than once (turtles 7220 and 13583, Table 1), we evaluated if the selection of a particular sample from the sampling sequence had any effect on results by comparing the results of all possible combinations of these samples. We found that the sample selection did not affect the results (results not shown). For clarity, we thus show the results from only the first sampling occasion for each of these 2 turtles.

## RESULTS AND DISCUSSION

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were determined for each blood fraction and preservative combination (Table 2). We found that all 3 anticoagulants had at least some effect on stable isotope values of green turtle blood tissue. Whereas ACD-preserved WHB and PLA were  $^{13}\text{C}$  enriched relative to the controls, EDTA-treated RBCs and PLA were  $^{15}\text{N}$  depleted and SH-treated WHB was  $^{15}\text{N}$  enriched relative to the controls. The significant treatment effects for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  may have resulted for at least one of 2 reasons; either the anticoagulants themselves were isotopically distinct from green turtle blood, thus causing a shift in the blood-anticoagulant mixtures' isotope values, or the introduction of anticoagulants altered the inherent chemical properties of the blood in some way that led to a shift in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

Considering the minute amount of anticoagulant ( $< 0.1 \text{ cm}^3$ ) that was introduced to the WHB in each vial (ca.  $8 \text{ cm}^3$ ), the stable isotope values of these preservatives would have had to be substantially different from that of the blood so as to cause a significant difference in stable isotope values. If the stable isotope values of the anticoagulants (ACD, EDTA, SH) were responsible for the shift in isotopic values of the blood samples, then perhaps lesser treatment effects would be apparent if the same study were conducted on a green turtle population with greater similarity between blood and anticoagulant  $\delta^{13}\text{C}$  and/or  $\delta^{15}\text{N}$  values. However, considering that EDTA

Table 2. *Chelonia mydas*. Mean  $\pm$  SD %C,  $\delta^{13}\text{C}$ , %N,  $\delta^{15}\text{N}$  values for each preservative-treated blood fraction including control. ACD: acid citrate dextrose; SH: sodium heparin; EDTA: ethylenediaminetetraacetic acid

Tissue, treatment	%C	$\delta^{13}\text{C}$ (‰)	%N	$\delta^{15}\text{N}$ (‰)
Whole Blood				
Control	46.1 $\pm$ 6.10	-17.1 $\pm$ 0.66	12.6 $\pm$ 1.66	15.5 $\pm$ 1.29
ACD	45.6 $\pm$ 2.23	-16.5 $\pm$ 0.55	12.0 $\pm$ 1.43	15.8 $\pm$ 1.14
SH	47.2 $\pm$ 1.84	-16.8 $\pm$ 0.63	13.4 $\pm$ 0.53	15.7 $\pm$ 1.34
EDTA	44.9 $\pm$ 3.82	-17.4 $\pm$ 1.14	12.5 $\pm$ 1.14	15.4 $\pm$ 1.52
Plasma				
Control	47.7 $\pm$ 7.01	-17.7 $\pm$ 0.85	10.5 $\pm$ 2.05	16.5 $\pm$ 2.05
ACD	43.0 $\pm$ 4.57	-16.5 $\pm$ 1.11	7.8 $\pm$ 2.20	16.4 $\pm$ 0.85
SH	45.5 $\pm$ 4.31	-17.3 $\pm$ 0.99	9.7 $\pm$ 1.40	16.7 $\pm$ 1.15
EDTA	45.7 $\pm$ 4.23	-18.0 $\pm$ 1.25	10.6 $\pm$ 1.57	15.6 $\pm$ 1.76
Red Blood Cells				
Control	47.3 $\pm$ 1.44	-16.7 $\pm$ 0.65	14.0 $\pm$ 0.29	15.4 $\pm$ 1.32
ACD	47.6 $\pm$ 2.93	-16.6 $\pm$ 0.70	13.8 $\pm$ 0.98	15.8 $\pm$ 1.26
SH	47.7 $\pm$ 2.04	-16.7 $\pm$ 0.68	14.2 $\pm$ 0.59	15.8 $\pm$ 1.33
EDTA	46.8 $\pm$ 2.35	-16.9 $\pm$ 1.11	13.7 $\pm$ 1.14	15.2 $\pm$ 1.58

treatment resulted in  $^{15}\text{N}$  depletion while SH treatment caused  $^{15}\text{N}$  enrichment relative to the controls, a shift in baseline  $\delta^{15}\text{N}$  in turtle blood would not have ameliorated the differences in treatment vs. control for all 3 anticoagulants. Nevertheless, for future studies it would be instructive to measure the stable isotope values of each preservative in isolation to better understand the anticoagulant–blood fraction stable isotope dynamic.

Why the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were altered for some blood fractions, but not others is unclear. For example, while ACD-treated PLA and WHB were both significantly  $^{13}\text{C}$  enriched relative to the controls, there was no effect on RBCs (Fig. 2). Perhaps this is because of a relatively high post-centrifugation ACD volume in PLA coupled with the inherent atomic structure of the ACD molecule (Fig. 1). Centrifuga-

tion of blood achieves fractional separation by causing components of blood to separate according to their densities. RBCs compact at the bottom, while less dense platelets and PLA float above. Presumably, preservatives introduced post-collection that did not chelate to the RBC fraction become depredated from the RBC fraction during centrifugation and migrate to the PLA layer, thereby resulting in an elevated concentration of preservative in the PLA relative to RBCs. The elevated ACD levels in PLA may be further exacerbated by this preservative's relatively weak chelation strength (Turgut et al. 2004), causing more ACD to travel to the PLA fraction during centrifugation than other preservatives. These possibilities underscore the importance of achieving a proper blood-to-preservative volumetric ratio during collection to avoid over-saturation of blood samples with preservative, as this may influence blood stable isotope values.

## CONCLUSIONS AND RECOMMENDATIONS

Understanding and applying proper preservation techniques to blood sample collection will provide more reliable results for future stable isotope studies. Here we assessed the effects of 3 commonly applied blood anticoagulants on stable carbon and nitrogen isotopic values in sea turtle blood. We found that WHB and PLA were significantly  $^{13}\text{C}$  enriched by the introduction of ACD. We also learned that  $\delta^{15}\text{N}$  values of RBCs and PLA were influenced by the addition of EDTA and that  $\delta^{15}\text{N}$  in WHB was affected by the presence of SH. Based on these results, it is clear that attention should be given to what types of anticoagulants are used for SIAs of sea turtle blood. Whereas our results indicate that all 3 anticoagulants had at least some effect on stable isotope values, SH was the only treatment to show no impact on PLA or RBCs—the most commonly studied blood fractions for sea turtle stable isotope studies (e.g. Caut et al. 2008, Wallace et al. 2009, Dodge et al. 2011). Indeed it would be fruitful to examine the longer-term (weeks to months) effects of the anticoagulants used in this study, as samples are commonly stored for extended periods prior to SIA. In the meantime, we recommend the exclusive use of SH as a blood anticoagulant for field studies where prompt centrifugation is not possible. We also underscore the need to use appropriately-sized collection vials (or preservative amounts) for the volume of blood being collected from each individual.

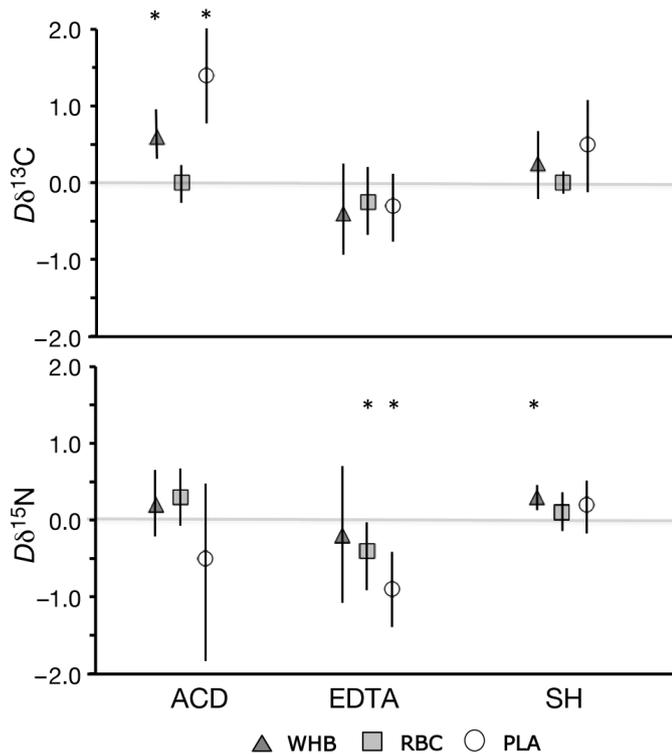


Fig. 2. *Chelonia mydas*. Mean differences ( $D$ ) in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between treated and control samples for all blood fractions and anticoagulants. Error bars indicate the range in which the mean difference is found with probability 0.95. For each combination of blood fraction and anticoagulant, 6 datasets were analyzed for different combinations of 2 repeatedly sampled individuals (one turtle with 3 samples and the other with 2). Horizontal lines: zero. \*Significant difference from control (see text for details). ACD: acid citrate dextrose; SH: sodium heparin; EDTA: ethylenediaminetetraacetic acid; WHB: whole blood; RBC: red blood cells; PLA: blood plasma

**Acknowledgements.** We thank B. Deck, P. Dutton, A. Frey, E. LaCasella, D. Prosperi, S. Roden, L. Saez and G. Serra-Valente for sample collection, data management and laboratory assistance over the years. We thank T. Liebst of Dynergy and E. Maher of the Unified Port of San Diego for site access. B. Wallace provided valuable comments on an earlier version of the manuscript. This study was funded by National Oceanic and Atmospheric Administration (NOAA) and research was authorized under NMFS Research Permit #1227.

#### LITERATURE CITED

- Barrow LM, Bjorndal KA, Reich KJ (2008) Effects of preservation methods on stable isotope signatures. *Physiol Biochem Zool* 81:688–693
- Bugoni L, McGill RAR, Furness RW (2008) Effects of preservation methods on stable isotope signatures in bird tissues. *Rapid Commun Mass Spectrom* 22:2457–2462
- Caut S, Guirlet E, Angulo E, Das K, Girondot M (2008) Stable isotope analysis reveals foraging area dichotomy for Atlantic leatherback turtles. *PLoS ONE* 3
- Cohen P, Cooley MH, Gardner FH (1965) Platelet preservation. Comparison of radioactivity yields of platelet concentrates derived from blood anticoagulated with ACD and EDTA. *N Engl J Med* 273:845–850
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42:495–506
- DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta* 45:341–351
- Dessauer HC (1970) Blood chemistry of reptiles: physiological and evolutionary aspects. Academic Press, London
- Dodge KL, Logan JM, Lutcavage ME (2011) Foraging ecology of leatherback sea turtles in the Western North Atlantic determined through multi-tissue stable isotope analyses. *Mar Biol* 158:2813–2824
- Estrada JA, Rice AN, Lutcavage ME, Skomal GB (2003) Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotope analysis. *J Mar Biol Assoc UK* 83:1347–1350
- Gelman A, Carlin JB, Stern HS, Rubin DB (2003) Bayesian data analysis, 2nd edn. Chapman & Hall/CRC, Boca Raton, FL
- 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
- Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes. I. Turnover of  $^{13}\text{C}$  in tissues. *Condor* 94: 181–188
- Hobson KA, Gibbs HL, Gloutney ML (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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of selected marine organisms. *Mar Ecol Prog Ser* 219:299–304
- Kraft RA, Jahren AH, Saudek CD (2008) Clinical scale investigation of stable isotopes in human blood:  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from 406 patients at the John Hopkins Medical Institutions. *Rapid Commun Mass Spectrom* 22:3683–3692
- Kurle CM (2002) Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. *Can J Zool* 80:902–909
- Laborde CJ, Chapa AM, Burleigh DW, Salgado DJ, Fernandez JM (1995) Effects of processing and storage on the measurement of nitrogenous compounds in ovine blood. *Small Rumin Res* 17:159–166
- Lunn D, Spiegelhalter D, Thomas A, Best N (2009) The BUGS project: evolution, critique, and future directions. *Stat Med* 28:3049–3067
- Michener RH, Schell DM (1994) Stable isotopes as tracers in marine aquatic food webs. In: Latja K, Michener RH (eds) *Stable isotopes in ecology and environmental science*. Blackwell, Oxford, p 138–157
- Mohri M, Rezapoor H (2009) Effects of heparin, citrate and EDTA on plasma biochemistry of sheep: comparison with serum. *Res Vet Sci* 86:111–114
- Owens DW, Ruiz GJ (1980) New methods of obtaining blood and cerebrospinal fluid from marine turtles. *Herpetologica* 36:17–20
- Rand MD, Lock JB, van't Veer C, Gaffney DP, Mann KG (1996) Blood clotting in minimally altered whole blood. *Blood* 88:3432–3445
- Reich KJ, Bjorndal KA, Martinez del Rio C (2008) Effects of growth and tissue type on the kinetics of  $^{13}\text{C}$  and  $^{15}\text{N}$  incorporation in a rapidly growing ectotherm. *Oecologia* 155:651–663
- Ruiz-Cooley RI, Gendron D, Aguiniga S, Mesnick S, Carriquiry JD (2004) Trophic relationships between sperm whales and jumbo squid using stable isotopes of C and N. *Mar Ecol Prog Ser* 277:275–283
- Sarakinos HC, Johnson ML, Vander Zanden JM (2002) A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. *Can J Zool* 80:381–387
- Seminoff JA, Bjorndal KA, Bolten AB (2007) Stable carbon and nitrogen isotope discrimination and turnover in Pond Sliders *Trachemys scripta*: insights for trophic study of freshwater turtles. *Copeia* 534–542
- Shuman MA, Majerus PW (1976) The measurement of thrombin in clotting blood by radioimmunoassay. *J Clin Invest* 58:1249–1258
- Soslau G, Wallace BP, Vincente C, Goldenber SJ and others (2004) Comparison of functional aspects of the coagulation cascade in human and sea turtle plasmas. *Comp Biochem Physiol* 138:399–406
- Soslau G, Prest PJ, Class R, George R, Paladino F, Violetta G (2005) Comparison of sea turtle thrombocyte generation to human platelet aggregation in whole blood. *Comp Biochem Physiol B* 142:353–360
- Turgut C, Pepe KM, Cutright TJ (2004) The effect of EDTA and citric acid on photoremediation of Cd, Cr and Ni from soil using *Helianthus annuus*. *Environ Pollut* 131: 147–154
- Wallace BP, Seminoff JA, Kilham SS, Spotila JR, Dutton PH (2006) Leatherback turtles as oceanographic indicators: stable isotope analysis reveal a trophic dichotomy between ocean basins. *Mar Biol* 149:953–960
- Wallace BP, Avens L, Braun-McNeill J, McClellan CM (2009) The diet composition of immature loggerheads: Insights on trophic niche, growth rates, and fisheries interactions. *J Exp Mar Biol Ecol* 373:50–57