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Stable isotope discrimination ($\delta^{13}C$ and $\delta^{15}N$) between soft tissues of the green sea turtle *Chelonia mydas* and its diet

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ABSTRACT: The foraging ecology and movements of vertebrate species have been increasingly studied via stable isotope analyses of small quantities of body tissues. However, the theoretical and experimental basis of this method remains poorly validated for most taxa despite numerous studies using these techniques in natural systems. In this study, we measured stable carbon and stable nitrogen diet–tissue discrimination (Δ_{dt}) in whole blood, red blood cells, blood plasma, and epidermis of 8 captive green turtles *Chelonia mydas* maintained on a control diet (41% protein, 12% lipids, 4% fiber) for 619 d. During the course of the study, mean straight carapace length increased from 45.2 ± 1.2 to 53.7 ± 2.1 cm, whereas mean body mass increased from 11.7 ± 0.7 to 19.9 ± 2.2 kg. Both diet and tissue isotope values remained constant throughout the study, indicating that diet–tissue equilibrium had been achieved. Whereas $\Delta_{dt}{}^{13}C$ ranged from −1.11‰ (red blood cells) to +0.17‰ (epidermis), $\Delta_{dt}{}^{15}N$ ranged from +0.22‰ (red blood cells) to +2.92‰ (blood plasma). These results contrast with the widely accepted discrimination factors of 0 to 1‰ for $\delta^{13}C$ and 3 to 5‰ for $\delta^{15}N$. Comprising the initial enrichment factors available for green turtles, these data will be useful in future interpretations of field isotopic values for this species.

KEY WORDS: Carbon · Chelonidae · Ectotherm · Fractionation · Isotope enrichment · Nitrogen · Reptilia

INTRODUCTION

Analysis of stable carbon and stable nitrogen isotopes has become a common tool for clarifying questions about the nutritional ecology of a variety of marine vertebrates, including cetaceans (Ruiz-Cooley et al. 2004), pinnipeds (Kurle 2002), seabirds (Hobson 1993), sea turtles (Godley et al. 1998), sharks (Estrada et al. 2003) and teleosts (Thomas & Cahoon 1993). Such inferences are possible because the isotope compositions of consumer body tissues are ultimately derived from those in the diet (DeNiro & Epstein 1978, 1981, Hobson & Clark 1992b, Michener & Schell 1994). Stable isotope analysis is particularly useful when an organism's diet is difficult to establish with conventional techniques (e.g. esophageal lavage, fecal analysis). By providing information on nutrients assimilated over extended periods, stable isotopes are much less affected by short-term temporal change in diet than other methods, which only provide dietary 'snapshots' of recently consumed food items (Peterson & Fry 1987, Hobson et al. 1996). Moreover, because of slow isotopic turnover in tissues of lesser metabolic activity (e.g. epidermis, feathers), highly mobile organisms often retain information about previously occupied habitats, thereby allowing stable isotope analyses to elucidate questions about animal migration (Hatase et al. 2002, Hobson & Barlein 2004, Rubenstein & Hobson 2004).

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Stable carbon (^{13}C:^{12}C; $\delta^{13}C$) and stable nitrogen (^{15}N:^{14}N; $\delta^{15}N$) isotope ratios of animal tissues undergo predictable changes (i.e. discrimination) with each trophic step (DeNiro & Epstein 1978, 1981, Peterson & Fry 1987). The $\delta^{15}N$ of consumer tissues generally is 3 to 5‰ greater than that of their prey, and $\delta^{13}C$ increases by 0 to 1‰ per trophic level (DeNiro & Epstein 1978, 1981, Miniwaga & Wada 1984, Peterson & Fry 1987). The mechanisms for discrimination between stable isotope values in consumer tissues relative to the diet are not thoroughly understood, but are likely to result from a variety of biochemical pathways, such as differential excretion of isotopes in egesta and respired gasses, isotopic fractionation during amino acid amination and transamination, and routing of isotopically distinct dietary fractions to specific tissues or tissue components (Peterson & Fry 1987, Hobson & Clark 1992a, Tieszen & Fagre 1993, Ayliffe et al. 2004). Diet–tissue isotopic discrimination may also be influenced by a consumer's age (Roth & Hobson 2000), nutritional status (Hobson et al. 1993), body temperature (Pinnegar & Polunin 1999), digestive strategy (Macrae & Reeds 1980), and diet quality (Pearson et al. 2003).

Understanding the patterns of stable isotope diet–tissue discrimination in marine species is essential for the correct interpretation of field data. However, despite calls for laboratory experiments to evaluate assumptions about stable isotope ecology (Gannes et al. 1997, Rubenstein & Hobson 2004), such validation studies have been performed on few marine taxa. The variable results found in these studies underscore the need for additional feeding trials under controlled conditions before stable isotope ratios can be reliably used for dietary reconstructions.

There is a paucity of information on diet–tissue discrimination in sea turtles, although stable isotopes have been used to address ecological questions about this marine vertebrate group (Godley et al. 1998, Hatase et al. 2002, Biasatti 2004). To draw meaningful inferences, these researchers used the previously published trophic level enrichment factors from other organisms (see above). Although these values are well substantiated for some endothermic species, the distinct metabolic status and regulatory physiology of the Reptilia brings into question whether or not they are valid for this ectothermic taxon (Bennett & Dawson 1976, Else & Hulbert 1981, Nagy et al. 1999).

Because the use of stable isotope analyses to address ecological questions about reptiles will expand, it is important that the assumptions underlying the use of this technique are validated. In this study, we measured stable carbon and stable nitrogen diet–tissue discrimination in whole blood, red blood cells, blood plasma, and epidermis of the green turtle *Chelonia mydas* maintained in a controlled environment and fed a constant diet for >18 mo. Green turtles occur in tropical and temperate waters worldwide and have been shown to consume a wide variety of sea grass, marine algae, and invertebrates (Bjorndal 1997). Because of its well-studied digestive and behavioral ecology (see Hirth 1997), this species is ideal for establishing initial diet–tissue discrimination values for reptiles. To our knowledge, this is the first controlled study examining isotopic discrimination in green turtles. Consequently, our findings will facilitate accurate interpretations of data generated from future field-based studies.

MATERIALS AND METHODS

Experimental conditions. A group of 8 green turtles from the Cayman Island Turtle Farm was transported to the animal vivaria at the Department of Zoology, University of British Columbia (UBC, Vancouver) in April 2003 and maintained in captivity for 619 d. The turtles ranged between 43.0 and 47.5 cm straight carapace length (SCL, mean = 45.2 ± 1.2 cm) and between 10.8 and 13.0 kg body mass (mean = 11.7 ± 0.7 kg). Each was marked with a unique notch pattern on the posterior marginal scutes for identification. At UBC the study individuals were kept in a sheltered, outdoor, fiberglass saltwater tank (40 000 l, 10 × 4 m, water depth = 1 m). The water temperature was kept at 24°C with a gas water-heater, and salinity was maintained between 2.8 and 3.4 %. In addition to ambient light, the tank was lit for 12 h each day with 20 W full-spectrum grow lights (True Lite; F40 T12/TL).

Feeding experiment. All turtles were maintained on a gelatin amalgamate with fixed portions of a pelleted diet (Aquamax Grower 500 5D05; PMI Nutrition International, LLC; Brentwood, Missouri) with soy- and fishmeal-based protein sources (41% protein, 12% lipids, 4% fiber), vitamins (Reptivite), and calcium (Rep-Cal). To determine the consistency of this diet, we analyzed % C, % N, $\delta^{13}C$ and $\delta^{15}N$ of the diet ca. every 120 d during the study (number of sampling occasions = 5 [A to E]: Table 1). Because of small variability in these measurements among sampling periods (Table 1), we used the overall dietary means ($\delta^{13}C$ and $\delta^{15}N$) to compute discrimination factors for each tissue.

We collected blood and epidermis samples from each turtle. Blood samples (~1.0 ml) were collected with a 21-gauge needle and syringe via the dorsal cervical sinus (Owens & Ruiz 1980) and transferred to a non-heparinized container. Approximately one-half of each blood sample was immediately separated into plasma and cellular components by centrifugation (5000 rpm [= 1118 × *g*] for 5 min). Epidermis samples

Table 1. Percent C and N and $\delta^{13}C$ and $\delta^{15}N$ in diet of *Chelonia mydas* on 5 sampling occasions (A to E), at ~120 d intervals during the course of this study. Boldface indicates values used to compute diet discrimination factors (Δ_{dt}). n = no. of samples taken on each sampling occasion

Sampling occasion	Carbon %C	Carbon $\delta^{13}C$	Nitrogen %N	Nitrogen $\delta^{15}N$
Diet with lipids				
A (n = 5)	53.32 ± 2.77	−18.97 ± 0.19	8.36 ± 0.58	+6.18 ± 0.14
B (n = 6)	47.12 ± 0.70	−18.92 ± 0.19	6.98 ± 0.20	+6.31 ±0.17
C (n = 6)	46.26 ± 1.40	−19.27 ± 0.39	6.98 ± 0.28	+6.23 ± 0.11
D (n = 6)	52.37 ± 8.46	−18.92 ± 0.21	8.54 ± 1.43	+6.38 ± 0.11
E (n = 6)	44.32 ± 1.44	−19.02 ± 0.38	6.06 ± 0.43	+6.10 ± 0.19
Mean	48.52 ± 1.85	**−19.03 ± 0.12**	7.35 ± 0.35	**+6.24 ± 0.06**
Diet without lipids				
B (n = 6)	44.07 ± 1.95	−18.67 ± 0.20	8.07 ± 0.34	+6.16 ± 0.06
E (n = 6)	40.87 ± 1.44	−18.62 ± 0.11	7.69 ± 0.11	+6.26 ± 0.09
Mean	42.47 ± 1.16	**−18.64 ± 0.13**	7.88 ± 0.19	**+6.21 ± 0.05**

(0.10 to 0.25 g wet mass) were collected with a 2 mm biopsy punch from the dorsal surface of the neck. Tissue samples were collected on 2 occasions; the first sample on 19 April 2004 (Day 371) and the second on 13 December 2004 (Day 619).

Sample preparation and analysis. Blood samples (whole blood, red blood cells, blood plasma) were dried at 60°C for 24 h and then powdered with a mortar and pestle. Epidermis samples were rinsed with distilled water, dried at 60°C for 48 h and then ground with a razor. Lipids were removed from epidermis samples and a portion of each diet sample using a Soxhlet apparatus with a 1:1 solvent mixture of petroleum ether and ethyl ether for at least two 10 h cycles. Samples were then dried at 60°C for 24 h to remove any residual solvent. Approximately 0.60 mg of diet and tissue samples 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 Oceanograpy, La Jolla, California. We used a Costech ECS 4010 elemental combustion system interfaced via a ConFlo III device (Finnigan MAT) with 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 (δ) notation in parts per thousand (‰): $\delta = [(R_{sample}/R_{standard}) - 1] (1000)$ where 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 standard, respectively. $R_{standard}$ for ^{13}C was Baker acetanilide (C_8H_9NO; $\delta^{13}C$ = −10.4‰) calibrated monthly against the Peedee Belemnite (PDB) limestone formation international standard; $R_{standard}$ for ^{15}N was IAEA N1 ammonium sulfate, $(NH_4)_2SO_4$; $\delta^{15}N$ = +0.4‰, calibrated against atmospheric N_2 and US Geological Survey 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 0.095‰ for carbon and nitrogen, respectively. In addition to stable isotope ratios, we measured %C and %N for each diet and tissue sample. Samples were combusted in pure oxygen in the elemental analyzer. Resultant CO_2 and N_2 gasses were passed through a series of thermal conductivity detectors and element traps to determine percent compositions. Acetanilide standards (71.09% C, 10.36% N) were used for calibration.

We first evaluated if the stable isotope values for green turtle tissues were at equilibrium between Days 371 and 619 by comparing stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and elemental compositions (% C and % N) between the 2 time periods for each tissue. We used a mixed-effects model in which individuals were treated as random factors whereas time and tissues were treated as fixed effects. We concluded that tissues were at isotopic equilibrium with the diet if the time effects on $\delta^{13}C$ and $\delta^{15}N$ were not statistically significant. Because the validity of significance testing has been questioned by ecologists and statisticians (e.g. Cohen 1994, Johnson 1999), we also present raw data graphically. For tissues that were in equilibrium with the diet, diet–tissue discrimination (Δ_{dt}; Cerling & Harris 1999) was calculated as $\Delta_{dt} = \delta_{tissue} - \delta_{diet}$, where δ_{tissue} represents the mean stable isotope ratio ($\delta^{13}C$ or $\delta^{15}N$) among all turtles for both time periods [$(\delta_{tissue,\ Day\ 371} + \delta_{tissue,\ Day\ 619})/2$] and δ_{diet} represents the overall mean stable isotope ratio for diet. For the epidermis, from which we removed the lipids, we determined Δ_{dt} using $\delta_{diet\ without\ lipids}$, whereas for all other tissues we determined Δ_{dt} using $\delta_{diet\ with\ lipids}$ (Table 1). All statistical analyses were carried out with R (available at: www.r-project.org). Data are presented as means ± SE unless otherwise noted.

RESULTS

We observed feeding by all turtles throughout the study. Between the 2 tissue sampling periods (Days 371 and 619), all 8 individuals increased in SCL (Day 371: SCL = 47.3 ± 2.3 cm, range = 44.5 to 50.5 cm; Day 619: SCL = 53.7 ± 2.1 cm, range = 50.0 to 57.0 cm; 1-sample *t*-test, $t = -5.19$, $p < 0.01$) and mass (Day 371:

15.6 ± 1.7 kg, range = 13.5 to 16.1 kg; Day 619: 19.9 ± 2.2 kg, range = 17.5 to 23.0 kg; 1-sample *t*-test, $t = 9.59$, $p < 0.01$).

Whereas %C ranged from 35.6 ± 2.6 (epidermis, Day 619) to 52.5 ± 0.9 (red blood cells, Day 371), %N ranged from 10.9 ± 0.5 (blood plasma, Day 619) to 15.9 ± 0.4 (red blood cells, Day 371; Table 2). %C and %N values were similar between the 2 sampling occasions for all tissues except the epidermis, for which %C and %N decreased between Days 371 and 619 (Table 2; $p = 0.01$ and $p = 0.02$ for time effects for %C and %N, respectively).

Scatterplots indicated that $\delta^{13}C$ and $\delta^{15}N$ remained virtually constant from Days 371 to 619 (Fig. 1). The mixed-effects models indicated that $\delta^{13}C$ and $\delta^{15}N$ differed significantly among tissues ($F = 440.5$, $p < 0.001$; $F = 455.23$, $p < 0.001$, respectively). However, there were no significant time effects among tissues for $\delta^{13}C$ ($F = 0.9$, $p = 0.36$) or $\delta^{15}N$ ($F = 2.08$, $p = 0.15$). Consequently, we concluded that the carbon and nitrogen isotope ratios in the tissues examined were in equilibrium with the diet from Days 371 to 619.

Whereas the red blood cells, whole blood, and blood plasma were depleted in ^{13}C relative to the diet, the epidermis was enriched in ^{13}C. Mean $\Delta_{dt}{}^{13}C$ ranged from −1.11 ± 0.05‰ (red blood cells) to +0.17 ± 0.03‰ (epidermis), with individual tissue $\Delta_{dt}{}^{13}C$ values

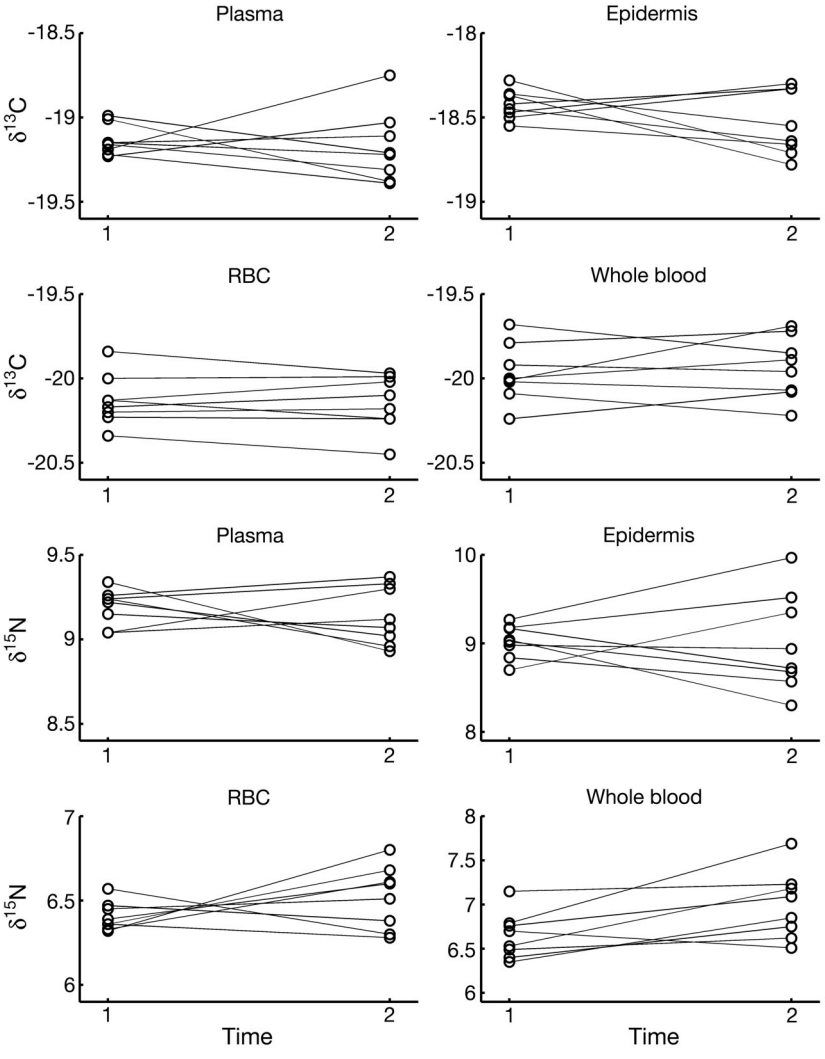

Fig. 1. *Chelonia mydas*. Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) on Days 371 (Time 1) and 619 (Time 2) measured for whole blood, red blood cells (RBC), blood plasma and epidermis (n = 8 turtles)

Table 2. *Chelonia mydas*. Mean sample composition and stable isotope signatures of tissues from captive turtles on Days 371 (n = 8 turtles) and 619 (n = 8 turtles). $\Delta_{dt} = \delta_{tissue} - \delta_{diet}$, where δ_{tissue} = mean stable isotope ratio ($\delta^{13}C$ or $\delta^{15}N$) among all turtles on both occasions and δ_{diet} = overall mean stable isotope ratio for diet. See Table 1 for dietary isotope values

Tissue	Carbon			Nitrogen		
	Day 371	Day 619	Δ_{dt}	Day 371	Day 619	Δ_{dt}
Composition (%C, %N)						
Whole blood	49.19 ± 4.23	47.47 ± 2.29		14.59 ± 1.33	14.36 ± 0.12	
Red blood cells	52.47 ± 0.93	50.84 ± 0.47		15.86 ± 0.35	14.94 ± 0.09	
Blood plasma	45.87 ± 1.47	44.95 ± 2.24		11.55 ± 0.37	10.91 ± 0.48	
Epidermis	41.21 ± 0.49	35.55 ± 2.64		13.18 ± 0.10	11.20 ± 0.51	
Stable isotope values ($\delta^{13}C$, $\delta^{15}N$; ‰)						
Whole blood	−19.97 ± 0.06	−19.94 ± 0.07	−0.92 ± 0.06	+6.64 ± 0.09	+6.99 ± 0.14	+0.57 ± 0.09
Red blood cells	−20.13 ± 0.05	−20.15 ± 0.06	−1.11 ± 0.05	+6.36 ± 0.03	+6.52 ± 0.07	+0.22 ± 0.03
Blood plasma	−19.14 ± 0.03	−19.18 ± 0.08	−0.12 ± 0.03	+9.19 ± 0.04	+9.14 ± 0.06	+2.92 ± 0.03
Epidermis	−18.43 ± 0.03	−18.54 ± 0.07	+0.17 ± 0.03	+9.03 ± 0.07	+9.00 ± 0.20	+2.80 ± 0.11

ranked as epidermis > blood plasma > whole blood > red blood cells (Fig. 2). All tissues were ^{15}N-enriched compared to the diet. The mean $\Delta_{dt}{}^{15}N$ ranged from +0.22 ± 0.03‰ (red blood cells) to +2.92 ± 0.03‰ (blood plasma), with individual tissue $\Delta_{dt}{}^{15}N$ values ranked as blood plasma ≥ epidermis > whole blood > red blood cells (Fig. 2).

DISCUSSION

Validation of underlying assumptions of stable isotope analyses is necessary when using this technique to study sea turtles. Until now, however, patterns of isotope discrimination have been largely unsubstantiated in this group. Our results provide critical information for stable isotope studies on green turtles. Based on the consistency in isotopic tissue values between Days 371 and 619 and the significant increases in body size of the 8 turtles used, we believe that the stable isotope values of their tissues were derived from the experimental diet and did not reflect their previous diet at the turtle farm. The 619 d period of the study was >1 order of magnitude longer than previously established $\delta^{13}C$ and $\delta^{15}N$ half-lives established to date (e.g. Hobson & Clark 1992a, Pearson et al. 2003, Hobson & Barlein 2004), further suggesting that the tissues examined in this study had equilibrated with the experimental diet. This apparent diet–tissue equilibrium, coupled with the low variability among the food and tissue samples, indicated that the enrichment factors derived herein are valid, and thus can be used for dietary reconstructions of green turtles in the wild. Further, the consistency in tissue-specific Δ_{dt} factors among the turtles studied indicated that nondestructive tissue sampling (i.e. blood and epidermis) can provide useful material for stable isotope analyses.

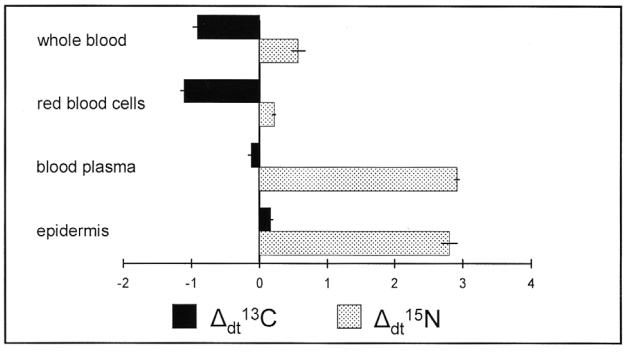

Fig. 2. *Chelonia mydas*. Stable carbon and stable nitrogen diet–tissue discrimination factors for soft tissues. Data are means ± SD. Actual discrimination factors for whole blood, red blood cells, blood plasma, and epidermis were –0.92, –1.11, –0.12 and +0.17, respectively, for ^{13}C, and +0.57, +0.22, +2.92, and +2.80, respectively, for ^{15}N

This bodes well for future isotopic examinations of threatened and endangered sea turtles, since blood and epidermis are commonly included in the sampling protocols of monitoring and stranding programs (e.g. Fair & Hansen 1998). The use of stable isotopes will also enable us to address ecological questions about green turtles whose tissues are stored in tissue archives (e.g. National Sea Turtle Tissue Archive, Southwest Fisheries Science Center, La Jolla, California).

The present study is the first to examine diet–tissue discrimination in green turtles, but it is not the first isotopic examination of the species. Whereas green turtles in this study had a mean $\delta^{13}C$ ranging from –20.15 to –18.43‰, and a mean $\delta^{15}N$ ranging from +6.52 to +9.03‰, values from other green turtle studies were markedly different. For example, in the seminal isotopic study of multiple sea turtle species, Godley et al. (1998) examined bone collagen, nest contents, and carapace material of Mediterranean green turtles and reported $\delta^{13}C$ values of –25.7 to –7.7‰, and $\delta^{15}N$ values of +2.2 to +17.3‰. For 8 green turtles from Tortuguero, Costa Rica, Biasatti (2004) found mean $\delta^{13}C$ values of bone carbonate ranging from –0.5 to +1.5‰, while the $\delta^{13}C$ of the tendon from a single turtle was –7.5‰. Keegan & DeNiro (1988) reported $\delta^{13}C$ and $\delta^{15}N$ values for bone collagen of 1 prehistoric green turtle specimen from Antigua as –3.8 and +5.1‰, respectively. However, the fact that different tissues were examined in each of these studies, coupled with the probable variability among the respective diets for each study group, suggests that any comparisons should be made with caution. It has been well established that diet–tissue discrimination varies among tissues within individuals of a species (e.g. Tieszen et al. 1983, Roth & Hobson 2000, Podlesak et al. 2005), and diet type has also been shown to affect discrimination of specific tissues (e.g. McCutchan et al. 2003, Pearson et al. 2003).

Of the 4 tissues analyzed, only the values for the epidermis were similar to the range of expected discrimination values for both carbon (0 to 1‰; DeNiro & Epstein 1978, Miniwaga & Wada 1984, Peterson & Fry 1987) and nitrogen (3 to 5‰; DeNiro & Epstein 1981, Miniwaga & Wada 1984, Peterson & Fry 1987). Whole blood and red blood cells were more depleted in ^{13}C (–0.92 and –1.11‰, respectively) and only slightly enriched in ^{15}N (+0.57 and +0.22‰, respectively). Because whole blood is composed primarily of red blood cells and plasma, its $\delta^{13}C$ and $\delta^{15}N$ signatures should be derived from these constituents, which is consistent with the intermediate values for $\Delta_{dt}{}^{13}C$ and $\Delta_{dt}{}^{15}N$ of whole blood (Fig. 1). The greater ^{13}C depletion in the red blood cells may therefore be largely responsible for the ^{13}C depletion in the whole blood.

Few studies have addressed Δ_{dt} in blood fractions, but the $\Delta_{dt}{}^{13}C$ and $\Delta_{dt}{}^{15}N$ of red blood cells for green turtles (–1.11 and +0.22‰, respectively) were lower than those established for red foxes *Vulpes vulpes*, harbor seals *Phoca vitulina*, gray seals *Halichoerus grypus*, and harp seals *P. groenlancis* (+0.6 to +1.5‰ for C; +1.7 to +2.6‰ for N; Roth & Hobson 2000, Lesage et al. 2002). The slight depletion of ^{13}C in plasma (–0.12‰) fell within the range for blood plasma $\Delta_{dt}{}^{13}C$ values (–1.5 to +0.8‰) for 2 bird species (yellow-rumped warbler *Dendroica coronata*; Pearson et al. 2003; American crow *Corvus brachyrhynchos*, Hobson et al. 1993), red fox (Roth & Hobson 2000) and 3 species of phocid seals (Lesage et al. 2002). The ^{15}N-enrichment of green turtle plasma was similar to the $\Delta_{dt}{}^{15}N$ range (+2.5 to +4.3‰) found among yellow-rumped warblers and the 4 mammal species (Hobson et al. 1993, Roth & Hobson 2000, Lesage et al. 2002, Pearson et al. 2003).

Perhaps metabolic and regulatory effects stemming from ectothermy contribute to the disparate values found in green turtles versus the aforementioned endothermic species. As suggested by McCutchan et al. (2003), higher respiratory quotients in endotherms versus ectotherms should contribute to higher $\Delta_{dt}{}^{13}C$ values within the latter group. Indeed, a number of studies on ectothermic vertebrates have found carbon discrimination values higher than the generally accepted 0 to 1‰ (DeNiro & Epstein 1978, Peterson & Fry 1987). Whereas Hesslein et al. (1993) reported a $\Delta_{dt}{}^{13}C$ of +2.0‰ in muscle of growing broad whitefish *Coregonus nasus*, Pinnegar & Polunin (1999) found $\Delta_{dt}{}^{13}C$ values of +2.2 to +3.4‰ in the soft tissues of the rainbow trout *Oncorhynchus mykiss*. McCutchan et al. (2003) similarly found $\Delta_{dt}{}^{13}C$ values of +1.9 and +3.3‰ for rainbow trout and the brook trout *Salvelinus fontinalis*. However, these results contrast with our $\Delta_{dt}{}^{13}C$ values for green turtles (–1.11 to +0.17‰), suggesting that for carbon there is no consistent discrimination dichotomy between endotherms and ectotherms. Interestingly, $\Delta_{dt}{}^{15}N$ appears to be more consistent among the 2 groups: the results of our study and those of Hesslein et al. (1993; $\Delta_{dt}{}^{15}N$ in broad whitefish, +3‰), Pinnegar & Polunin (1999; $\Delta_{dt}{}^{15}N$ of rainbow trout, +2.3 to +5.0‰), and McCutchan et al. (2003; $\Delta_{dt}{}^{15}N$ of rainbow trout and brook trout, +3.2 and +3.8‰, respectively) show a greater similarity with the overall 3 to 5‰ increase per trophic level (DeNiro & Epstein 1981, Miniwaga & Wada 1984, Peterson & Fry 1987). Despite these trends, the current understanding of the mechanisms that regulate Δ_{dt} is insufficient to generalize about the effects of endothermy versus ectothermy on discrimination. Nonetheless, the consistencies in $\Delta_{dt}{}^{15}N$ among green turtles, fishes, birds, and mammals suggest that the underlying physiological pathways responsible for discrimination may be consistent among diverse vertebrate taxa, regardless of thermoregulatory physiology.

The depletion of ^{13}C in the whole blood and red blood cells is intriguing. Perhaps the presence of excessive lipids in the blood (a tissue from which we did not extract lipids) played a role, since lipids are depleted in ^{13}C (DeNiro & Epstein 1977). Blood plasma can potentially contain a large proportion of lipids (Lehninger 1982), which may decrease the $\delta^{13}C$ value for the whole blood. Further, if blood serves as a reservoir for lipid accumulation, higher concentrations of lipid in the blood relative to the diet might result, causing a negative $\Delta_{dt}{}^{13}C$. Although this should not account for the low $\delta^{13}C$ value of red blood cells, a blood fraction with low lipid levels, the extended longevity of nucleated red blood cells in turtles (ca. 200 d; Kirkland & Altland 1955) may have contributed to the ^{13}C depletion. Differential lipid loads may affect the $\delta^{15}N$ values of whole blood, red blood cells and blood plasma, but is unlikely to play a major role. The ambiguity in the potential effects of lipids on our results underscores the importance of removing lipids from all diets and tissues prior to stable isotope analyses.

The observed $\Delta_{dt}{}^{13}C$ and $\Delta_{dt}{}^{15}N$ disparities among the tissues were probably caused by differences in the proportional composition of protein, carbohydrates and lipids among the tissues, as well as compositional variation in each of these organic compounds among the tissues. Such differences can result from selective routing of exogenous resources during tissue maintenance and construction, and from differential mobilization of endogenous resources (e.g. bicarbonate from bone) into tissues or tissue components (Peterson & Fry 1987, Tieszen & Fagre 1993). The 2 distinct protein sources in the captive diet (soy and fish meal) suggested the possibility of differential routing of dietary nutrients. In addition, loss of heavier or lighter isotopes via digestion and cellular respiration may have played a role (Tieszen & Fagre 1993, Gannes et al. 1997, Ayliffe et al. 2004). For example, depending on the isotopic routing of nutrients, respired carbon may have a higher ^{13}C content than the diet if it results from utilization of carbohydrates (i.e. enriched in ^{13}C), while it may be lower than the diet if it results from the utilization of lipids (i.e. depleted in ^{13}C; Klein Breteler et al. 2002). However, we were unable to ascertain the relative contributions of these factors to our results because of the unknown isotopic differences among dietary proteins, lipids and body-tissue components. Elucidating the relationships between an animal's diet and the fate of assimilated dietary components, synthesized components and endogenous nutrients is important to fully determine the biochemical mechanisms of discrimination (Gannes et al. 1997).

Our interpretations of carbon and nitrogen discrimination would benefit greatly from a better understanding of animal nutritional ecology. Nevertheless, this study has been successful in establishing initial isotopic discrimination data for green turtles. It should be noted, however, that the Δ_{dt} values found herein may be valid only for juvenile turtles maintained on a similar experimental diet. Whereas growing animals may have different levels of discrimination for proteinaceous tissues such as red blood cells and epidermis (McCutchan et al. 2003), different diets have been shown to affect discrimination values among organisms (e.g. Pearson et al. 2003). Moreover, while all turtles in this study were in excellent health, their tissues might not discriminate to the same degree as tissues from adult turtles or turtles in poor health. For example, fibropapillomatosis, a tumor-bearing disease afflicting many populations of green turtles worldwide (e.g. Work et al. 2004), may compromise food intake in turtles possessing oral, ocular or intestinal tumors. As shown by Hobson et al. (1993) for avian species, nutrient depletion may result in elevated levels of ^{15}N in the body. Based on these considerations, we encourage additional studies of green turtles reared in different settings to elucidate the effects of dietary intake and health status. Inquiries into the stable isotope diet–tissue discrimination in additional reptile species should also be undertaken to provide a better foundation for interpreting results of future isotopic studies on this diverse taxon.

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