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RESEARCH ARTICLE

Stable isotope discrimination factors and between-tissue isotope comparisons for bone and skin from captive and wild green sea turtles (*Chelonia mydas*)

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Rationale: The ecological application of stable isotope analysis (SIA) relies on taxa- and tissue-specific stable carbon ($\Delta^{13}C$) and nitrogen ($\Delta^{15}N$) isotope discrimination factors, determined with captive animals reared on known diets for sufficient time to reflect dietary isotope ratios. However, captive studies often prohibit lethal sampling, are difficult with endangered species, and reflect conditions not experienced in the wild.

Methods: We overcame these constraints and determined the $\Delta^{13}C$ and $\Delta^{15}N$ values for skin and cortical bone from green sea turtles (*Chelonia mydas*) that died in captivity and evaluated the utility of a mathematical approach to predict discrimination factors. Using stable carbon ($\delta^{13}C$ values) and nitrogen ($\delta^{15}N$ values) isotope ratios from captive and wild turtles, we established relationships between bone stable isotope (SI) ratios and those from skin, a non-lethally sampled tissue, to facilitate comparisons of SI ratios among studies using multiple tissues.

Results: The mean (±SD) $\Delta^{13}C$ and $\Delta^{15}N$ values (‰) between skin and bone from captive turtles and their diet (non-lipid-extracted) were 2.3 ± 0.3 and 4.1 ± 0.4 and 2.1 ± 0.6 and 5.1 ± 1.1, respectively. The mathematically predicted $\Delta^{13}C$ and $\Delta^{15}N$ values were similar (to within 1‰) to the experimentally derived values. The mean $\delta^{15}N$ values from bone were higher than those from skin for captive (+1.0 ± 0.9‰) and wild (+0.8 ± 1.0‰) turtles; the mean $\delta^{13}C$ values from bone were lower than those from skin for wild turtles (−0.6 ± 0.9‰), but the same as for captive turtles. We used linear regression equations to describe bone vs skin relationships and create bone-to-skin isotope conversion equations.

Conclusions: For sea turtles, we provide the first (a) bone-diet SI discrimination factors, (b) comparison of SI ratios from individual-specific bone and skin, and (c) evaluation of the application of a mathematical approach to predict stable isotope discrimination factors. Our approach opens the door for future studies comparing different tissues, and relating SI ratios of captive to wild animals.

1 | INTRODUCTION

The ratios of stable isotopes (SI) such as carbon ($^{12}C/^{13}C$; $\delta^{13}C$ value) and nitrogen ($^{14}N/^{15}N$; $\delta^{15}N$ value) in consumer tissues can provide valuable insights into the trophic relationships and foraging ecology of populations and communities in a variety of ecosystems (e.g. [1-5]). Stable carbon and nitrogen isotopes fractionate during biological processing (e.g. catabolism, assimilation, and elimination)[6-8] which results in animal tissues having higher delta values than their diet items.[9] These differences between consumer tissues and diet are called trophic discrimination factors,[10] are typically expressed with Δ notation ($\Delta^{15}N$ for nitrogen and $\Delta^{13}C$ for carbon), and are defined as $\Delta X = \delta_{tissue} - \delta_{diet}$.[11,12] Once established, tissue-specific discrimination factors can be used to 'correct' consumer SI ratios in order to make direct comparisons between consumers and their prey for insights into trophic ecology and for the best application of stable isotope mixing models.[13-17] In addition, experimentally derived mathematical conversions between stable isotope ratios from different tissues can (1) facilitate direct comparisons among isotopic ratios of different tissues (e.g. [18-20]) which improves interpretation of SI ratios for trophic and habitat use behavior, and (2) provide an independent mathematical method to estimate, or predict, the expected discrimination factor for a new tissue from a tissue with previously estimated discrimination factors. This last point, validating an approach to predict tissue-specific

discrimination factor values, has important implications for future stable isotope studies, especially those able to compare isotope ratios among previously unsampled tissues collected opportunistically from dead wild animals.

Discrimination factors have been determined for several tissues from most orders, such as birds,[21,22] mammals (e.g. ungulates,[11,23-25] rats,[22,23] felids,[26] manatees[27]), fish (e.g. sharks,[28] blue fin tuna[29]), and reptiles (summarized in Steinitz et al[30]), including sea turtles (e.g. [31-34]). However, very few studies have reported the $\Delta^{13}C$ and $\Delta^{15}N$ values for specific sections of bone material (i.e. cortical bone vs whole homogenized bone). Studies using whole bone, often using archeological or paleontological samples, typically focus on the inorganic component, apatite, and less frequently on the organic matrix, collagen (see [35,36]). For collagen, the commonly assumed $\Delta^{13}C$ value is ~5 ‰,[37-39] yet experimental studies show a range from ~1 to 6 ‰ from a variety of taxa (reviewed in Lee-Thorp et al:[23] mice ~3 to 4 ‰, rats ~3 ‰, ungulates ~5 ‰, and humans ~6 ‰; dugong and manatees ~2 to 4 ‰,[40] pigs ~1 to 3 ‰[41]). Fewer studies have evaluated collagen $\Delta^{15}N$ values, but estimates range from ~2 to 5 ‰ (pigs ~2 ‰;[41] other mammals and birds ~4 to 5 ‰[36]) with many studies simply assuming a value of ~3 ‰ as observed for other tissues or whole organisms when experimentally estimated values are unavailable.[35]

Bones are especially useful for reconstructing long-term diet and habitat use patterns as the long bones (i.e. humerus, femur) in many species grow in layers which record multiple, sequential years of isotopic information[42] and whole bone records information from several years up to the lifetime of an individual (e.g. [5,43-45]). For example, our previous work demonstrated that the annually formed growth layers within the compact portion of sea turtle humerus bones contained the isotopic signatures reflecting diet and location of turtles during the time in which each growth layer was formed, allowing us to recreate foraging dynamics for over 20 years of an individual turtle's life.[5,45]

Although bone has a high potential for recording long-term trophic data, its SI analysis is complicated by its composition.[36,46] The isotope ratios from bone apatite and collagen reflect carbon from the inorganic (the mineral structure) and organic (protein collagen) portions of bone material, respectively, and these two bone components are generally separated prior to analysis. Because apatite and collagen are composed of different materials, the $\delta^{13}C$ values from the two components differ, and the $\delta^{15}N$ values can only be measured in bone collagen because apatite does not contain nitrogen.[36,47] Because $\delta^{15}N$ values are especially useful for informing consumer trophic levels, diet studies utilizing SI ratios of modern animal bones use primarily bone collagen. Practitioners of SI studies using sea turtle bones typically analyze the cortical portion because that is where the growth layers are retained,[5,43-45,48] the carbonate content is low,[47,49] and the composition is almost entirely of collagen.[47,50] To our knowledge, no study has yet assessed the cortical bone-diet discrimination factors of any reptile, including sea turtles. Therefore, establishing the $\Delta^{13}C$ and $\Delta^{15}N$ values for cortical bone would be extremely useful for more accurate interpretation of stable isotope analysis (SIA) of turtle bone.

Despite its utility, studies using SI ratios of bones can be limited because they require samples from dead animals and the determination of SI discrimination factors for bone requires that animals be held on a known diet for at least one year for proper incorporation of dietary isotopes into an annual bone growth layer, which can be logistically difficult. Therefore, acquiring SI data from more easily accessed, non-lethally collected tissues with faster protein and isotope turnover, and mathematically relating those data to SI data from bone material, is useful for comparing and interpreting isotope data from different tissue types collected from the same or similar species.[20] This approach, if validated, could be applied to facilitate the comparison of multiple tissues for any species, not just sea turtles.

Finally, establishing relationships between multiple tissues has another potential advantage. We can use the data relating two tissues, one of which has an established discrimination factor, i.e. sea turtle skin, to then estimate the expected discrimination values of a different tissue, i.e. sea turtle cortical bone, where discrimination factors have not yet been established. This allows for prediction of the expected values for comparison with new experimentally derived discrimination factors when the samples used for the discrimination factor study (i.e. captive diet-controlled animals) are distinct from those used for the tissue-to-tissue comparison (i.e. wild animals on an unknown diet). If validated, this approach would provide a valuable mathematical method by which to check newly estimated discrimination factors, leading to greater confidence in the application of discrimination factors derived from captive animals to diet studies with wild animals, and also provide the opportunity to use tissues that may lack experimentally derived discrimination factors.

We utilized samples from dead, juvenile, captive green turtles (*Chelonia mydas*) raised at SeaWorld San Diego (San Diego, CA, USA) and maintained on a consistent diet for over 2 years to estimate their cortical bone-diet and skin-diet discrimination factors. We then quantified the relationships between the $\delta^{13}C$ and $\delta^{15}N$ values of skin and bone from the captive turtles, as well as wild green turtles, to establish bone-to-skin conversions to facilitate better interpretation of isotope data across tissue types. We also used the bone-to-skin isotopic relationships from the wild turtles as an independent and mathematical approach to estimate the expected bone-diet discrimination factors experimentally derived from the captive turtles. Finally, we tested for the effect of lipid extraction on SI ratios from skin to add to the body of literature assessing its necessity for the correct preparation of sea turtle skin for isotope analysis.

2 | EXPERIMENTAL

2.1 | Sample collection

We collected skin and humerus bone samples in fall 2012 from five dead male juvenile green turtles that had previously been in good health, and had been raised in captivity at SeaWorld San Diego since hatching in October 2009. The turtles ranged in size from 46 to 53 cm in curved carapace length (CCL), and all were 3 years old. We collected skin samples with a 6-mm biopsy punch from the upper shoulder region and manually removed the left humerus bone. SeaWorld staff provided feeding records detailing the consistent diet that all the turtles had consumed for the 2 years before their deaths and sample collection. As this study was opportunistic, we were unable to collect samples from items which the turtles ate while alive,

so we collected samples from diet items fed to their living counterparts and procured after the turtles targeted for this study had died. Diet items collected included fish (capelin *Mallotus villosus* and blue runner *Caranx crysos*), shrimp, market squid (*Doryteuthis opalescens*), and lettuce. The contribution to the diet, by weight, was approximately 43% lettuce, 24% squid, 23% fish, and 10% shrimp (Table 1). We collected samples of each diet item from SeaWorld at three different time periods (fall 2012, spring 2014, and summer 2014) to account for possible source or seasonal variations that could affect stable carbon and nitrogen isotope ratios. We stored all samples at −20°C upon collection and until analysis.

We collected humerus bones and skin in June and July 2012 from 25 wild, dead-stranded turtles during shoreline surveys along a 45-km stretch of Playa San Lázaro on the Pacific coast of the Baja California Peninsula (BCP), Mexico (Figure 1), as part of a larger on-going study. Turtle body size ranged from 42 to 71cm CCL (mean ± SD: 56.8 ± 8.23cm). We collected skin samples as described above and stored them dry in salt until further processing. We manually collected humerus bones, then cleaned, dried, and stored them at room temperature until analysis.

2.2 | Sample processing

For all diet and skin, we homogenized all the samples, split them in half, then freeze-dried them at −50°C for a minimum of 8 h using a lyophilizer (BenchTopK, VisTis; SP Industries, Warminster, PA, USA). We left one half intact and lipid extracted the other half using an accelerated solvent extractor (ASE model 200; Dionex, Thermo Scientific, Milan, Italy) with petroleum ether. We then lyophilized the samples again, as described above, to ensure removal of residual solvent. We analyzed the samples for their stable carbon and nitrogen isotope ratios, as well as their percentage carbon and nitrogen content as described below.

We processed captive and wild turtle bones in the same manner. Sea turtle humerus bones grow continuously, in pace with turtle body size, and the newest growth occurs at the outer edge, while older growth is retained toward the bone interior.[36,43,52] As turtles grow, the innermost (oldest) growth layers are gradually resorbed into the bone interior medullary cavity,[43,52] but the retained cortical bone is inert, and the chemistry measured by SIA reflects diet ingested by the turtle at the time of bone formation.[36,43,47,48] Therefore, we

TABLE 1 Details for applying the concentration-dependent approach for calculation of dietary $\delta^{13}C$ and $\delta^{15}N$ values (‰), corrected for their differential amounts of digestible C and N

	Values from NLE prey					Values from LE prey				
Nitrogen	**Squid**	**Shrimp**	**Fish**	**Lettuce**	**Total**	**Squid**	**Shrimp**	**Fish**	**Lettuce**	**Total**
% diet by weight[a]	24%	10%	23%	43%	100%	24%	10%	23%	43%	100%
Mean $\delta^{15}N$ value	13.5	5.8	12.7	5.0		13.8	5.6	12.5	4.9	
Mean %N	12.0%	13.3%	13.1%	3.6%		13.6%	13.6%	13.9%	3.6%	
Digestible protein[b]	72.6%	87.5%	75.3%	20.5%		72.6%	87.5%	75.3%	20.5%	
Digestible N[c]	8.7%	11.6%	9.9%	0.7%		9.9%	11.9%	10.5%	0.7%	
Digestible matter[d]	90%	90%	90%	67%		90%	90%	90%	67%	
Digestible concentration of N[e]	9.7	12.9	11.0	1.1	34.7	11.0	13.2	11.6	1.1	36.9
% Digestible N from protein[f]	27.9%	37.2%	31.7%	3.2%	100%	29.7%	35.8%	31.5%	3.0%	100%
Weighted mean $\delta^{15}N$ value[g]	3.8	2.2	4.0	0.2	10.1	4.1	2.0	3.9	0.1	10.2
Carbon										
Mean $\delta^{13}C$ value	−18.7	−18.3	−19.1	−28.4		−17.7	−18.1	−17.9	−28.1	
Mean %C	42.3%	43.2%	48.4%	29.7%		43.9%	43.6%	46.3%	36.9%	
Digestible C[h]	38.1%	38.9%	43.5%	19.9%		39.5%	39.2%	41.6%	24.7%	
Digestible concentration of C[i]	42.3	43.2	48.4	29.7	163.7	43.9	43.6	46.3	36.9	170.6
% Digestible C[j]	25.9%	26.4%	29.6%	18.2%	100%	25.7%	25.5%	27.1%	21.6%	100%
Weighted $\delta^{13}C$ value[k]	−4.8	−4.8	−5.6	−5.2	−20.5	−4.6	−4.6	−4.9	−6.1	−20.1

Data are included for lipid-extracted (LE) and intact, non-lipid-extracted (NLE) diet items for the $\delta^{13}C$ values and only NLE diet items for the $\delta^{15}N$ values. See text and Koch and Phillips.[51]

[a]Obtained from feeding records.

[b]DP = (Wet weight/Dry mass)*100% for animal matter or 90% for plants.

[c]DN = DP*%N.

[d]DM is 90% for animal matter, 67% for plants (Amorocho and Reina[56]).

[e]D[N] = (DN/DM)*100.

[f]$\%DN_{protein} = D[N]_{individual\ diet\ component}/\text{sum}\ D[N]_{all\ diet\ components}$.

[g]Weighted $\delta^{15}N = (\delta^{15}N_{diet\ item})*(\%DN_{protein})$.

[h]DC = DM*%C.

[i]D[C] = (DC/DM)*100.

[j]$\%DC = D[C]_{individual\ diet\ component}/\text{sum}\ D[C]_{all\ diet\ components}$.

[k]Weighted $\delta^{13}C = (\delta^{13}C_{diet\ item})*(\%DC)$.

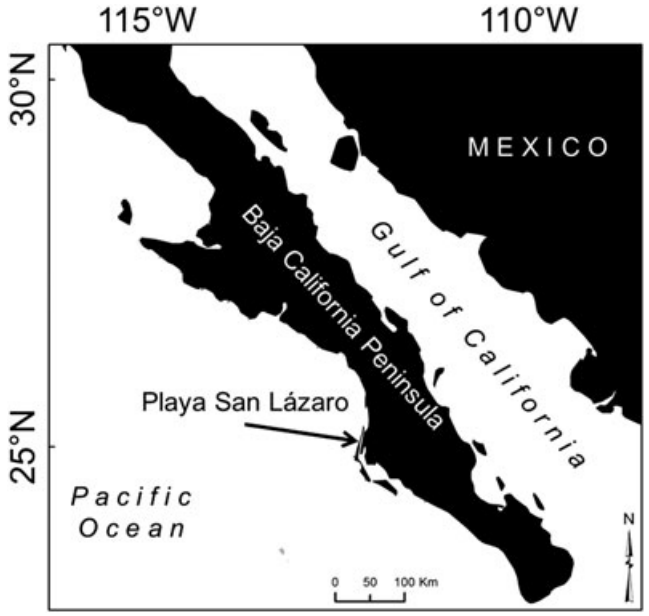

FIGURE 1 Sampling location for wild green turtles at Playa San Lázaro on the Pacific coast of the Baja California Peninsula (BCP), Mexico

sampled from the outer portion of each bone, targeting only the most recent growth, to ensure complete incorporation of the constant diet that the captive turtles had consumed for the 2 years previous to bone collection. We also selected this sampling site because the outer layer most closely corresponds to the diet reflected by skin samples, which is assumed to represent diet consumed ~4 to 6 months previous and up to the time of tissue collection[20,33,53] for both the wild and the captive turtles. To extract the targeted portion of bone for analysis, we cross-sectioned all humerus bones at the site of the deltopectoral insertion scar, a location known to contain the greatest proportion of cortical bone and previously used for skeletochronological and SI studies.[42,52] We collected bone powder from the outermost growth layer using a computer guided micromill as described in Turner et al,[45] and weighed ~1.5 mg of the powder into tin capsules for SI analysis. We did not lipid extract bone samples as cortical bone has low lipid content, as indicated by its low carbon/nitrogen (C:N) ratios[47] and lipid extraction has been deemed unnecessary when C:N ratios are <3.5.[49]

2.3 | Stable isotope and diet statistical analysis

We analyzed all diet items, and captive and wild turtle skin samples for $\delta^{13}C$, $\delta^{15}N$, percentage carbon (%C), and percentage nitrogen (%N) values by combustion in a Carlo Erba NA 1500 CNS elemental analyzer (Thermo Scientific) interfaced via a ConFlo II device (Finnigan MAT, Bremen, Germany) to a Thermo Electron DeltaV Advantage isotope ratio mass spectrometer (Finnigan MAT) in the Stable Isotope Geochemistry Lab at the University of Florida, Gainesville , FL, USA (UF). We similarly analyzed wild turtle bone samples by combustion in a Carlo Erba CE1108 elemental analyzer (Thermo Scientific) interfaced via a ConFlo III device (Finnigan MAT) to a Thermo Electron Delta Plus XP mass spectrometer (Finnigan MAT) at the Department of Earth and Marine Sciences, University of California, Santa Cruz, Santa Cruz, CA, USA (UCSC). We calculated the average precision for all data as the standard deviation (SD) of the $\delta^{13}C$ and $\delta^{15}N$ values from a set of standards (L-glutamic acid at UF, acetanilide at UCSC), and these were 0.07 ‰ for $\delta^{13}C$ values and 0.04 ‰ for $\delta^{15}N$ values at UF, and 0.05 ‰ for $\delta^{13}C$ values and 0.02 ‰ for $\delta^{15}N$ values at UCSC. We used conventional delta (δ) notation in parts per thousand, or permil (‰), to express the SI ratios of the samples relative to the isotope standards:

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

where the ratios of heavy to light isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and standard are represented by R_{sample} and $R_{standard}$, respectively. The standard for $\delta^{13}C$ values was Vienna Pee Dee Belemnite and the standard for $\delta^{15}N$ values was atmospheric N_2.

We referenced the C:N ratios for all samples to assess protein purity and identify samples with potential lipid contamination.[34,46,49] Any bone or non-lipid-extracted (NLE) skin samples with a C:N ratio >3.5 were excluded from final analysis as recommended in the literature.[34,47,49] A C:N ratio >3.5 can indicate higher lipid content,[49] which, for turtle skin, can result from unintentional inclusion of the subcutaneous dermis layer,[34] and, for bone, could be due to contamination from the interior medullary cavity region.[45,47,54]

We used analysis of variance (ANOVA) tests to assess differences in the $\delta^{13}C$ and $\delta^{15}N$ values from prey collected over the three time periods. We found no differences ($p > 0.05$) for both $\delta^{13}C$ ($F_{(2,17)} = 1.41$, $p = 0.25$) and $\delta^{15}N$ ($F_{(2,17)} = 3.57$, $p = 0.076$) values, so we used the mean $\delta^{13}C$ and $\delta^{15}N$ values from the diet items collected over the three time periods for the remainder of the analyses (Table S1, supporting information). We used paired t-tests (significance level at $p < 0.05$) to assess potential differences in SI ratios between paired bone and skin samples, paired LE vs NLE dietary items, and paired LE vs NLE skin samples from turtles. There were no significant differences between the LE and NLE skin SI ratios (see section 3, and Table S2, supporting information) and the $\delta^{15}N$ values from the LE and NLE diet items, so the NLE SI ratios were used in the remaining analyses.[34] However, we used the $\delta^{13}C$ values from both LE and NLE diet samples for discrimination factor calculations, as the consideration of dietary lipid content and its effect on the isotope ratios of consumer tissues can be important for determining the most accurate discrimination factors.[26] Finally, as recommended by Turner Tomaszewicz et al[47] and applied in Ramirez et al,[44] we mathematically corrected the $\delta^{13}C$ values from bone ($\delta^{13}C_{bulk}$ values) to account for the trace presence of carbonate-derived $\delta^{13}C$ values in cortical bone samples, in order to obtain more accurate $\delta^{13}C$ values for collagen ($\delta^{13}C_{corrected}$ values), using the linear equation developed previously[47] for east Pacific green turtles ($\delta^{13}C_{corrected} = 1.2 * \delta^{13}C_{bulk} + 2.1$).

We used the concentration-dependent approach as described by Koch and Phillips[51] to establish the overall $\delta^{13}C$ and $\delta^{15}N$ values from the diet items. We determined the percentage C and N by weight of each diet item in the overall turtle diet based on the feeding records provided from SeaWorld San Diego. We then weighted the percentage that each dietary item contributed to the overall dietary composition based on its concentration of carbon and nitrogen as described by Koch and Phillips.[51] This was important as the C and N contents of the diet items varied widely (e.g. lettuce vs shrimp nitrogen content: 3.6% vs 13.3%) and therefore the contributions of C and N to turtle body tissues for each diet item were not considered equal. We also

took into account the amount of digestible C and N in each diet item as described in Kurle et al,[22] then considered all these factors to calculate the subsequent weighted $\delta^{13}C$ and $\delta^{15}N$ values for each diet item (Table 1).[22,55] We used digestible protein (100% for animal matter and 90% for plant matter) and digestible matter (90% for animal matter and 67% for plant matter) estimates from Amorocho and Reina[56] for green sea turtles. We selected these over other values because those authors[56] focused on the digestion rates of turtles on an omnivorous diet, whereas other studies focused on sea turtles consuming herbivorous (i.e. [57-59]) or pelletized diets (i.e. [60]).

We calculated the overall $\delta^{13}C$ and $\delta^{15}N$ values of the diet as the sum of the products of the diet contributions and weighted $\delta^{13}C$ and $\delta^{15}N$ values. To obtain the overall dietary $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) for each diet item, we calculated the weighted averages of the $\delta^{13}C$ and $\delta^{15}N$ values by summing the products of the isotopic ratios of each diet item and the percentage of the total diet (see also [22,26,51]):

$$\delta^{13}C_{\text{total diet}} = \left(\delta^{13}C_{\text{diet item1}} * \%\ \text{diet item1}\right) + \left(\delta^{13}C_{\text{diet item2}} * \%\ \text{diet item2}\right) + \left(\delta^{13}C_{\text{diet item}i} * \%\ \text{diet item}i\right) + \ldots$$

We used these total diet $\delta^{13}C$ and $\delta^{15}N$ values to calculate the tissue-diet (bone-diet and skin-diet) discrimination factor values for carbon ($\Delta^{13}C$) and nitrogen ($\Delta^{15}N$):

$$\Delta X = \delta_{\text{tissue}} - \delta_{\text{total diet}}$$

We calculated tissue-diet discrimination values ($\Delta^{13}C$ and $\Delta^{15}N$) for each turtle individually and then reported the mean values (± SD).

To assess the tissue-to-tissue relationship between bone (using the corrected $\delta^{13}C$ values from bone; see above) and skin SI ratios of both captive and wild turtle samples, we first compared samples by paired t-tests, excluding the $\delta^{13}C$ values of any samples with a C:N ratio >3.5. Next, we examined and reported the linear regression relationships between the SI ratios from the two tissues for both captive and wild turtle samples as recommended by Vander Zanden et al.[20] Then, using the slopes and y-intercepts of the regression lines, we calculated the tissue-to-tissue conversions for the $\delta^{13}C$ and $\delta^{15}N$ values from the paired bone and skin samples.

It is important to use the data from the linear regression equations to determine tissue-to-tissue isotope conversions if the relationship between the $\delta^{13}C$ (and the $\delta^{15}N$) values from the two tissues of interest (in this case, skin and bone) is not 1:1. A regression line slope that deviates from 1 indicates that multiple factors may be driving the isotope differences between tissues that are potentially not consistent among individuals and so must be accounted for in a manner that goes beyond simply taking the difference between the mean isotope ratios from each tissue in question (see section 4).

Finally, to compare our experimentally derived bone-diet $\Delta^{13}C$ and $\Delta^{15}N$ values for turtles (estimated using captive turtles, see above) with mathematically predicted $\Delta^{13}C$ and $\Delta^{15}N$ values, we used published skin-diet $\Delta^{13}C$ and $\Delta^{15}N$ values and the skin-to-bone isotopic relationships between bone and skin that we established in this investigation. In doing so, we tested the validity of estimating discrimination factors for one tissue (bone) when (a) the discrimination factors for another tissue (skin) are known and (b) the relationships between the SI ratios from the two tissues (bone and skin) are known (i.e. paired samples). Therefore, to estimate an expected range/value for bone-diet $\Delta^{13}C$ and $\Delta^{15}N$ values, we used our skin-to-bone isotope conversion results, together with previously estimated skin-diet $\Delta^{13}C$ and $\Delta^{15}N$ values. Assuming that the paired bone and skin samples represent approximately the same foraging period (see section 2 for sample processing), and therefore a constant diet assimilated into both tissues, the following relationship exists: the bone-diet discrimination factors will be approximately equal to the skin discrimination factors plus the differences in SI ratios that we measured between the paired bone and skin samples. For example:

$$\text{Expected } \Delta_{\text{bone}} = \text{published } \Delta_{\text{skin}} + (\delta_{\text{bone}} - \delta_{\text{skin}}),$$

where the published Δ_{skin} = the $\Delta^{13}C$ or $\Delta^{15}N$ values between skin from captive turtles and their diets established in previous studies[31,34] as well as in the current study, δ_{bone} = the $\delta^{13}C$ or $\delta^{15}N$ values from bone (using the $\delta^{13}C$ values corrected to most accurately reflect those from collagen; see above), and δ_{skin} = the $\delta^{13}C$ or $\delta^{15}N$ values from skin, all calculated in this study. For the δ_{bone} and δ_{skin} values, we used the mean value from the wild turtles (n = 25). In testing this methodology, we hope to validate its use for wide applicability across multiple species.

3 | RESULTS

3.1 | Diet

The overall $\delta^{13}C$ and $\delta^{15}N$ values for the diet items, calculated by incorporating concentration dependence and the % contribution of each item to the overall diet, were −20.5 ‰ and 10.1 ‰, respectively (NLE diet), and −20.1 ‰ and 10.2 ‰, respectively (LE diet) (Table 1; and Table S1, supporting information). Lipid extraction had no effect on the $\delta^{15}N$ values from any of the diet items (paired t-tests, seafood items: p = 0.83, t = 0.2132, df = 50; lettuce p = 0.81, t = 0.2757, df = 2) (Table S1, supporting information). Therefore, we used the $\delta^{15}N$ values from the NLE prey to calculate all the $\Delta^{15}N$ values. The $\delta^{13}C$ values from the LE marine prey items (fish, shrimp, squid) were significantly higher (paired t-tests, p <0.0001, t = −6.3991, df = 50) than those from the NLE marine prey (Table 1; Table S1, supporting information), but there was no difference in the $\delta^{13}C$ values from LE vs NLE lettuce samples (paired t-tests, p = 0.40, t = −1.0737, df = 2) (Table 1; Table S1, supporting information). As there were differences in the LE and NLE $\delta^{13}C$ values, and consideration of lipids from diet can be important when determining the $\Delta^{13}C$ values in predators or omnivores,[3] we included the $\delta^{13}C$ values from both LE and NLE diet items in our analyses.

3.2 | Discrimination factors

Overall, there was no significant effect of lipid extraction on the $\delta^{13}C$ or $\delta^{15}N$ values of sea turtle skin samples (paired t-tests, $\delta^{13}C$ p = 0.17, t = 1.6539, df = 4; $\delta^{15}N$ p = 0.71, t = 0.3993, df = 4); therefore, only NLE skin samples were used for determination of the $\Delta^{13}C$ and $\Delta^{15}N$ values (Table 2; Table S2, supporting information).

However, one of the five captive turtle NLE skin samples appeared to have been incorrectly sampled, seeming to include a subcutaneous layer beneath the epidermis, as indicated by its higher C:N ratio of 4.2 (Table S2, supporting information). For the four skin samples with C:N ratios <3.5, lipid extraction increased the $\delta^{13}C$ values by a range of 0.1 to 0.8 ‰, while lipid extraction on the one skin sample with C:N ratio = 4.2 increased the $\delta^{13}C$ value by 2.9 ‰ (Table 2; Table S2, supporting information). Therefore, as the higher C:N ratio indicated that the sample was contaminated with other tissue, and for consistency, we omitted this sample from further analysis.

Both skin and bone tissues had higher $\delta^{13}C$ and $\delta^{15}N$ values than those from the NLE and LE diet (Table 2; and Table S2 and Figure S1, supporting information). The calculated $\Delta^{13}C$ values, relative to the NLE diet, ranged from 1.3 to 2.7 ‰ for bone (mean 2.1 ± 0.6 ‰) and 1.9 to 2.6 ‰ for skin (mean 2.3 ± 0.3 ‰; Table 2). The $\Delta^{15}N$ values ranged from 4.1 to 6.8 ‰ for bone (mean 5.1 ± 1.1 ‰) and 3.5 to 4.4 ‰ for skin (mean 4.1 ± 0.4 ‰; Table 2). Relative to the LE diet, the $\Delta^{13}C$ values were all slightly lower than in the NLE diet, and ranged from 0.9 to 2.3 ‰ for bone (mean 1.7 ± 0.6 ‰) and 1.5 to 2.2 ‰ for skin (mean 1.9 ± 0.3 ‰; Table 2).

The mathematically predicted, or expected, bone $\Delta^{13}C$ and $\Delta^{15}N$ values, estimated using skin discrimination values from two previously published studies[31,34] and the current study, in relation to the difference between skin and bone tissues from the wild turtles (see section 2 and below), ranged from −0.4 to 1.7 ‰ (mean 1.0 ± 1.0 ‰) and 3.6 to 4.9 ‰ (mean 4.5 ± 0.6 ‰), respectively (Table 4). The mathematically predicted value was very similar to the calculated mean bone $\Delta^{13}C$ values for the NLE and LE diets (2.1 ± 0.6 ‰ and 1.7 ± 0.6 ‰, respectively) and the calculated $\Delta^{15}N$ values (5.1 ± 1.1 ‰; Table 3).

3.3 | Tissue-to-tissue SI conversions

We assessed the SI ratios from skin and bone of both the captive and the wild turtles for the tissue-to-tissue conversion analysis. Two skin samples and three bone samples from five different wild turtles had high C:N ratios (>3.5) and therefore the $\delta^{13}C$ values for these samples were omitted from further analysis, as was done for one captive turtle skin sample as noted above. The bone samples from both captive (n = 5) and wild turtles (n = 25) had higher mean $\delta^{15}N$ values than skin and the values for the two tissues ($\delta_{bone} - \delta_{skin}$) differed by 1.0 ± 0.9 ‰ (p = 0.06) and 0.8 ± 1.0 ‰ (p = 0.0003), respectively, whereas only the mean $\delta^{13}C$ values from bone and skin collected from wild turtles (n = 20; 5 samples omitted) were different, with lower (more negative) mean $\delta^{13}C$ values from skin (−0.6 ± 0.9 ‰; p = 0.011; Figure 2, Table 3). The mean $\delta^{13}C$ values from bone and skin from the captive turtles (n = 4; 1 sample omitted) were not different (bone mean: −18.2 ± 0.4 ‰ vs skin mean: −18.2 ± 0.3 ‰; p = 0.94) (Figure 2, Table 3). Finally, linear regression equations using all the data from wild and captive turtles together described significant relationships between the SI ratios of bone vs skin:

$$\delta^{13}C_{bone} = 0.54\left(\delta^{13}C_{skin}\right)\text{-}8.31, \text{Adj.}R^2 = 0.21, p = 0.0137$$

$$\delta 15N_{bone} = 0.89\left(\delta^{15}N_{skin}\right) + 2.55, \text{Adj.}R2 = 0.61, p<0.0001$$

and the slope was significantly different from 1 (p <0.0001) for $\delta^{13}C$ values, but not for $\delta^{15}N$ values (p = 0.44) (Figure 3). The equations for calculating the tissue-to-tissue conversions by group, captive and wild are shown in Figure 3.

TABLE 2 The $\delta^{13}C$ and $\delta^{15}N$ values (‰) from turtle tissues and diet (corrected for concentration dependence; see Table 1 and section 2), carbon to nitrogen (C:N) ratios, and the stable carbon ($\Delta^{13}C$) and nitrogen ($\Delta^{15}N$) isotope discrimination factors (‰) for each individual and mean values (± SD)

	Skin		Bone		Diet (corrected)		C:N		
Turtle	NLE $\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	NLE $\delta^{13}C$	LE $\delta^{13}C$	$\delta^{15}N$	NLE skin	bone
1	−18.6	14.1	−18.6	14.7	−20.5	−20.1	10.1	2.9	3.1
2	−20.9*	14.1	−19.2	14.2				4.2*	3.1
3	−17.9	14.5	−17.8	15.8				2.8	3.1
4	−18.1	14.5	−17.8	16.9				2.9	3.2
5	−18.1	13.6	−18.5	14.3				2.8	3.2
Mean±SD	−18.7±1.2	14.2±0.4	−18.4±0.6	15.2±1.1				3.1±0.6	3.1±0.1

Discrimination factors						
	Skin			Bone		
Turtle	NLE $\Delta^{13}C$	LE $\Delta^{13}C$	$\Delta^{15}N$	NLE $\Delta^{13}C$	LE $\Delta^{13}C$	$\Delta^{15}N$
1	1.9	1.5	4.0	1.9	1.5	4.6
2	NA	NA	4.0	1.3	0.9	4.1
3	2.6	2.2	4.4	2.7	2.3	5.7
4	2.4	2.0	4.4	2.7	2.3	6.8
5	2.4	2.0	3.5	2.0	1.6	4.2
Mean±SD	2.3±0.3	1.9±0.3	4.1±0.4	2.1±0.6	1.7±0.6	5.1±1.1

For samples with C:N >3.5, marked with a *, the $\delta^{13}C$ values were excluded from the mean and further analysis. The $\Delta^{13}C$ values were determined by comparing isotope ratios from bone (corrected, see section 2) and non-lipid-extracted (NLE) skin from captive, juvenile turtles with C:N <3.5 (n = 4) with those from their LE and NLE diet, whereas the $\Delta^{15}N$ values were calculated using the $\delta^{15}N$ values from NLE turtle tissues and NLE diet (see diet values in Table 1). All turtles were 3-year-old males and reared in captivity on a known diet for their entire lives. All data, including LE and NLE skin values, are given in Table S2 (supporting information).

TABLE 3 Stable carbon ($\delta^{13}C$ values) and nitrogen ($\delta^{15}N$ values) isotope ratios (‰), all non-lipid extracted from skin and bone (corrected, see Methods), used for tissue-to-tissue conversion and relationship values

	$\delta^{13}C$		$\delta^{15}N$		Tissue-to-tissue ($\delta_{bone} - \delta_{skin}$)		C:N	
Captive turtle	bone	skin	bone	skin	$\delta^{13}C$	$\delta^{15}N$	bone	skin
1	−18.6	−18.6	14.7	14.1	0.0	0.6	3.1	2.9
2	−19.2	−20.9*	14.2	14.1	NA	0.1	3.1	4.2*
3	−17.8	−17.9	15.8	14.5	0.1	1.3	3.1	2.8
4	−17.8	−18.1	16.9	14.5	0.3	2.4	3.2	2.9
5	−18.5	−18.1	14.3	13.6	−0.4	0.7	3.2	2.8
Mean ± SD	−18.2 ± 0.4	−18.2 ± 0.3	15.2 ± 1.1	14.2 ± 0.4	0.0 ± 0.3	1.0 ± 0.9	3.1 ± 0.1	3.1 ± 0.6
Wild turtle								
1	−17.5	−16.8	15.2	15.6	−0.6	−0.4	3.4	3.2
2	−16.6	−16.0	17.0	16.4	−0.7	0.5	3.2	3.1
3	−16.5	−16.2	14.9	15.2	−0.3	−0.2	3.3	3.1
4	−17.5	−16.4	17.8	17.6	−1.1	0.2	3.1	3.1
5	−18.0	−19.7*	16.6	15.4	NA	1.3	3.2	5.6*
6	−17.6	−16.6	17.1	16.6	−1.0	0.5	3.2	2.8
7	−16.3	−17.6	17.2	16.7	1.3	0.5	3.1	3.3
8	−17.9	−16.7	19.4	17.7	−1.2	1.7	3.0	3.0
9	−16.2	−16.9	15.2	14.1	0.6	1.1	3.3	3.1
10	−17.8	−16.7	16.2	14.5	−1.1	1.7	3.1	2.9
11	−16.6	−16.0	14.7	13.9	−0.6	0.7	3.5	2.8
12	−18.3	−18.5	18.9	16.3	0.2	2.6	3.1	3.4
13	−15.6	−16.3	14.8	12.6	0.6	2.2	3.2	2.7
14	−17.6	−17.9	17.8	17.3	0.3	0.4	3.1	3.3
15	−19.6*	−16.2	17.8	16.9	NA	0.9	4.1*	3.0
16	−16.0	−16.3	14.6	15.8	0.3	−1.2	3.3	3.2
17	−18.7	−16.6	15.6	15.4	−2.1	0.3	3.4	3.0
18	−18.0	−16.7	18.0	17.2	−1.3	0.8	3.2	2.9
19	−16.1	−15.9	15.5	15.9	−0.2	−0.4	3.0	2.8
20	−18.0	−22.4*	18.3	16.4	NA	1.9	3.2	8.7*
21	−19.1*	−16.9	17.5	17.0	NA	0.4	3.7*	3.1
22	−17.7	−16.1	18.5	16.3	−1.6	2.2	3.1	2.8
23	−18.1	−16.2	17.2	15.3	−1.9	1.9	3.3	2.8
24	−18.5	−16.9	18.6	17.0	−1.6	1.6	3.3	3.0
25	−19.9*	−18.4	16.6	17.0	NA	−0.4	3.9*	3.2
Mean±SD	−17.3±0.9	−16.7±0.7	16.8±1.5	16.0±1.3	−0.6±0.9	0.8±1.0	3.2±0.3	3.0±0.2

All $\delta^{13}C$ values for tissue samples with a carbon/nitrogen ratio (C:N) >3.5 are marked with * and were omitted from the final analysis.

4 | DISCUSSION

4.1 | Discrimination factors

The SI discrimination factors for bone presented here are the first calculated for any sea turtle species. The cortical bone $\Delta^{13}C$ and $\Delta^{15}N$ values, reflecting isotopic measurements of collagen, are near those previously reported for bone collagen from primarily terrestrial mammals and birds, ~2 to 6 ‰ for both $\Delta^{13}C$ and $\Delta^{15}N$.[23,35,36,41,61] The discrimination factors that we measured between turtle skin and their diet were within ~0.5 ‰ of those reported in a previous study detailing discrimination factors from juvenile captive turtles ($\Delta^{13}C_{skin}$: 1.9 ± 0.6 ‰ and $\Delta^{15}N_{skin}$: 3.8 ± 0.4 ‰ [34]), whereas the differences were slightly greater between our results and those of Seminoff et al[31] ($\Delta^{13}C_{skin}$: 0.17 ± 0.03 ‰ and $\Delta^{15}N_{skin}$: 2.80 ± 0.11 ‰). Seminoff et al[31] used juvenile turtles in the same size class as our study, but their turtles were reared on a pellet diet, and the isotope ratios were from LE skin and diet items.

The agreement observed between our mathematically predicted and experimentally derived $\Delta^{13}C$ and $\Delta^{15}N$ values shows that this mathematical approach could be used in other studies where sampled tissues may lack experimentally derived discrimination factors. Our experimentally derived $\Delta^{13}C$ and $\Delta^{15}N$ values that we directly determined from turtles reared on the known diet were near the predicted Δ_{bone} values that we calculated based on the sum of the Δ_{skin} values from previous studies and the bone-to-skin (tissue-to-tissue) differences that we measured from the wild turtle samples (Table 4). This provides independent support that the mathematically calculated

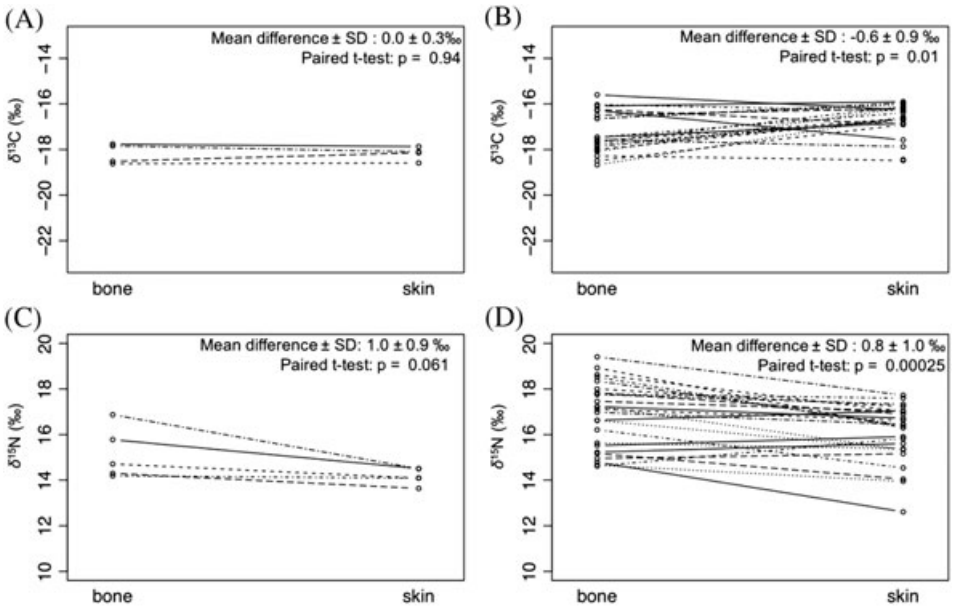

FIGURE 2 Stable isotope ratios from paired bone and skin samples: $\delta^{13}C$ values from (A) captive (n = 4) and (B) wild turtles (n=20) and $\delta^{15}N$ values from (C) captive (n = 5) and (D) wild (n=25) turtles

predicted Δ_{bone} values from the current study fall within a reasonable range. Importantly, future studies could apply this approach to (1) estimate discrimination factor values for new tissues, and (2) assess the applicability of using experimentally derived discrimination factors for wild populations. For example, inert tissues such as some bone tissue, teeth, hair, nails/claws, and shell are better preserved than soft tissues such as skin, blood, or muscle, and can be collected after an animal dies. Collection of such samples is possible from animals killed as a result of several causes, including bycatch, changes in environmental conditions (i.e. storms, floods, cold stunning events, etc.), road kill, or other fatal interactions with humans or anthropogenic structures (i.e. wind turbines, glass windows, hunting, etc.). Yet the stable isotope interpretation of these preserved tissues for trophic or other ecological studies is greatly limited without previously determined discrimination factors.

The differences between the mathematically derived and experimental discrimination factor values (Table 4) are probably caused by variation among individual turtles and may include diet preferences (i.e. consumption of more or less of a certain prey type), growth rates, age, as well as other health and physiological differences. Our results for cortical bone-diet $\Delta^{13}C$ values, which reflect the collagen SI ratios,[47] were in the range of previous studies (~2 to 6 ‰[23,35-37,39-41]). The carbonate content in sea turtle cortical bone is low,[47] so we only estimated Δ values for the organic collagen and did not measure the SI ratios from the bioapatite. Bioapatite is the mineral, inorganic crystalized structure of bone, whereas the collagen is the proteinaceous, organic matrix interwoven with the apatite,[35] and the $\delta^{13}C$ values of these two bone components differ as a result of varying metabolic and routing pathways.[23,35,46] Previously determined bone-diet $\Delta^{13}C$ values for bioapatite from rodents and ungulates[36] ranged from ~9 to 14 ‰.[36]

We added the lipid extraction step for the skin samples because current protocol for SI analysis of sea turtle skin is evolving with regard to the necessity of lipid extraction. Most recently, lipid extraction has been shown to be unnecessary for sea turtle skin (i.e. [34]), particularly when care is taken to separate the surface epidermis layer from the dermis tissue. Epidermis samples are expected to have C:N ratios <3.5,[34] which is the recommended threshold for lipid extraction or for mathematical correction for the presence of lipids for aquatic animals before SI analysis.[49]

Despite the small sample size of captive turtles used in this study, we present these discrimination factor results for turtle bones as a useful starting place given the difficulty of accessing dead sea turtles on a known diet, and the lack of other published data that could be used to inform the interpretation of sea turtle bone isotopic data. In our direct comparison of the tissue-to-tissue values from captive and wild turtles, the relationships between bone and skin SI ratios were similar and the differences in mean SI ratios were 1 ‰ or less for both $\delta^{13}C$ and $\delta^{15}N$ values (Table 3). This suggests that the discrimination factors calculated from captive turtles in this study are appropriate for application to turtles in wild populations.

The captive turtles in our study were fed an omnivorous diet, composed of over 50% (by weight) animal matter (fish, shrimp, squid). Different species and even populations of sea turtles are known to have widely varying diets (e.g. [5,34,48,59,62]) and the discrimination factors and tissue-to-tissue conversion values may be more applicable to omnivorous marine turtles. For instance, east Pacific green turtles are known to ingest benthic (e.g. sea pens, anemones, nudibranchs) and pelagic invertebrate species (e.g. red crabs, fish, squid[5,45,62-65]), along with kelp and macroalgae. This may be especially true as the type and amount of protein in a consumer's diet (all plant vs mixed vs all animal) is known to affect the $\Delta^{13}C$ and $\Delta^{15}N$ values for other vertebrates (e.g. [22]).

Consumers with diets higher in animal-derived protein typically exhibit higher $\Delta^{15}N$ values. This may be due to an increase in bioavailable dietary protein as animal matter is more digestible than plant material.

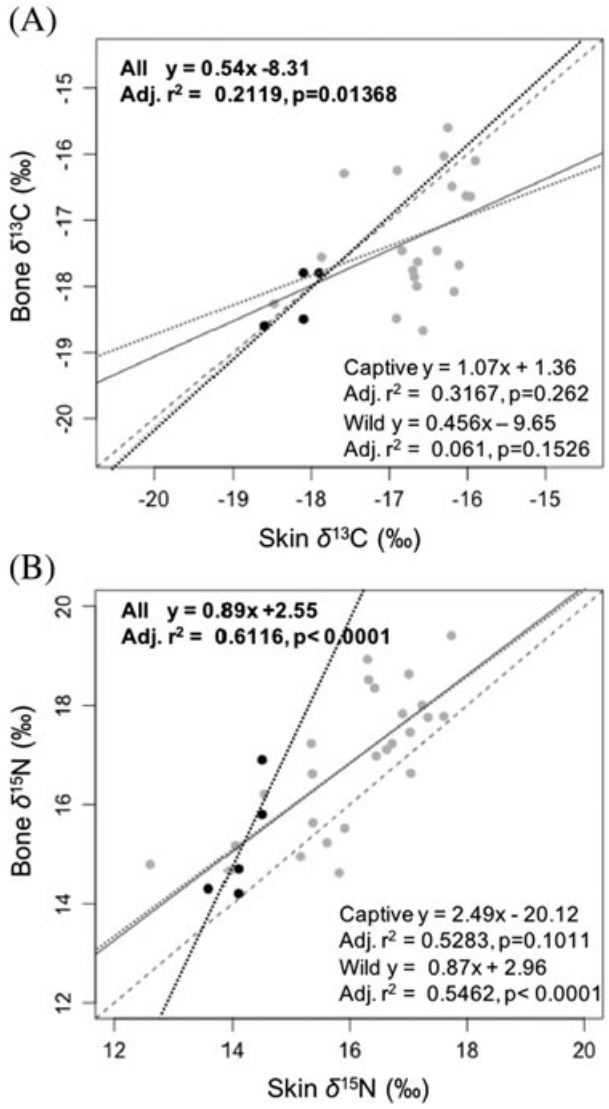

FIGURE 3 Relationships between stable isotope ratios from paired bone and skin samples: (A) $\delta^{13}C$ values and (B) $\delta^{15}N$ values from captive (black dots and dotted line) and wild (grey dots and dotted lines) turtles. The 1:1 relationship is shown by the dashed black line. The linear relationships for all turtles (captive and wild) are shown by the solid black line and in bold at the top, while individual linear relationships are shown separately for captive and wild turtles at the bottom right.

This, in turn, leads to an increase in the waste production to shed extra dietary protein. The nitrogen in waste contains more of the preferentially excreted ^{14}N, so the remaining nitrogen in the body pool becomes increasingly enriched in the heavier ^{15}N isotope,[22,34,36] causing higher $\Delta^{15}N$ values. The $\Delta^{13}C$ values may also be affected by dietary protein content, amino acid contributions, and even animal physiology (for reviews, see [22,36]). Some data suggest that diets containing multiple sources with varying $\delta^{13}C$ values can cause noticeable variations in diet-tissue $\Delta^{13}C$ values,[22,41] but the mechanisms for this are not known. More study on the effects of isotopic routing and diet type, among other factors (i.e. age, growth, reproductive status), on animal $\Delta^{13}C$ and $\Delta^{15}N$ values is needed and should be considered when applying our results to populations of primarily herbivorous green turtles.

4.2 | Tissue-to-tissue

Different tissues commonly have different isotopic ratios due to several factors including differential fractionation, amino acid/biochemical composition, macronutrient routing, protein turnover times, and even diet, age, growth rate, and life stage of the individual animal.[10,22,33,34] One might expect these isotopic differences to be uniform between tissues, but the differences in the $\delta^{13}C$ and $\delta^{15}N$ values from bone and skin that we observed were not uniform across turtles, even for those raised in captivity on a constant diet, and this is similar to previous studies comparing SI ratios among different tissues.[20] The $\delta^{15}N$ values were higher for bone than skin for all the captive turtles, and for 20 of the 25 wild turtles, and the $\delta^{15}N$ difference between all bone and skin pairs ranged from −1.2 to +2.6 ‰ (mean 0.9 ± 0.9 ‰, $p < 0.0001$). There was similar non-uniformity for the $\delta^{13}C$ values, and in the opposite direction, where two of the four analyzed captive turtles and only six of the 20 analyzed wild turtles had higher $\delta^{13}C$ values from bone than from skin, and the $\delta^{13}C$ differences between all bone and skin pairs ($\delta_{bone} - \delta_{skin}$) ranged from −2.1 to +1.3 ‰ (mean −0.5 ± 0.9 ‰, $p = 0.012$). This may be due to differences in the amino acid composition of the diet ingested by the captive and wild turtles.[22] The captive turtles were offered all diet items, but some may have preferentially ingested variable amounts of plant and/or animal matter, thereby causing differences in the isotope ratios of their tissues, and a similar disparity may also be present for the wild turtles. Determining the exact mechanisms of the observed variation between bone and skin was beyond the scope of the current

TABLE 4 Green turtle discrimination factor values (‰) for skin from this and previously published studies, used to calculate expected bone discrimination factor values (expected Δ_{bone} = published Δ_{skin} + ($\delta_{bone} - \delta_{skin}$); see text for details), compared with calculated bone discrimination factor values from this study (calculated $\Delta_{bone} = \delta_{bone} - \delta_{diet}$). For the current study, all skin $\delta^{13}C$ values used to calculate discrimination factor values were from intact, non-lipid-extracted skin samples (see text)

Published Δ_{skin}			Expected Δ_{bone}		Calculated Δ_{bone}	
Study	C	N	C	N	C	N
34[a]	1.87±0.56	3.77±0.40	1.3	4.6		
31[b]	0.17±0.03	2.80±0.11	−0.4	3.6		
This study[a]	2.3±0.3	4.1±0.4	1.7	4.9	2.1±0.6	5.1±1.1
This study[b]	1.9±0.3	4.0±0.4	1.3	4.8	1.7±0.6	5.0±1.1
		Mean±SD:	1.0±1.0	4.5±0.6		

[a]Values in relation to a non-lipid-extracted diet.

[b]Values in relation to a lipid-extracted diet.

study, but research investigating this would further improve interpretation of isotopic results. Regardless of mechanism, it does not appear that simply subtracting the skin $\delta^{13}C$ or $\delta^{15}N$ value from those from bone, or using the mean difference ($\delta_{bone} - \delta_{skin}$), is sufficient to relate the isotope ratios between these tissues, and this emphasizes the importance of an approach that quantitatively relates the values as recommended by Vander Zanden et al[20] and performed in our study. We found significant linear relationships between the $\delta^{13}C$ and $\delta^{15}N$ values from bone and skin tissues from both wild and captive turtles combined (Figure 3). Therefore, the presented linear equations may be more suitable for relating the isotope ratios of these two tissues in future studies. The adjusted r^2 values of the linear regressions ($\delta^{13}C$: 0.21; $\delta^{15}N$: 0.61) also indicate there were other factors affecting the variation in the $\delta^{13}C$ and $\delta^{15}N$ values between the two tissues and this warrants further investigation.

5 | CONCLUSIONS

We present the stable carbon and nitrogen isotope discrimination factors for cortical bone from sea turtles, and also provide equations for describing the relationships between the $\delta^{13}C$ and $\delta^{15}N$ values from skin and bone, and present a new approach to mathematically predict stable isotope discrimination factor values. These findings will improve ecological interpretations of bone SI ratios for reconstructing foraging behavior and locations, and allow for the application of stable isotope mixing models to investigate sea turtle trophic ecology over time from studies using sequentially sampled bone. Furthermore, as skin is much more commonly sampled from marine turtles than bone, our results allow for comparisons between this and other studies that sample either bone or skin and facilitate a better understanding of the relationships between isotope ratios from different tissues in the same animals. Finally, our results invite future stable isotope studies to mathematically predict the stable isotope discrimination factors for species' tissues that have not yet been experimentally derived.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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