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Title: Long-term trends in the foraging ecology and habitat use of an endangered species: an isotopic perspective

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I. Sample Collection

Blood was drawn from the dorsal cervical sinus or a rear flipper using standard serum collection tubes that contain anticoagulants (heparin) to prevent clotting (Dutton 1996). Samples were frozen at -20°C at the field station and stored long-term at -80°C until further analysis. The bulk δ^{15}N from whole blood from turtles reflect data integrated over ~six months before the time of tissue collection (Seminoff et al. 2007), and thus adequately reflect a time frame indicative of leatherback re-migration from their foraging grounds to St. Croix. We limited our analyses to samples collected from recently arrived leatherbacks at the start of the nesting season, as their δ^{15}N values would best reflect those from their pre-nesting locations.

II. Isotope Analyses

We used 500-600 μg of dried blood for bulk isotope analyses, where we determined bulk δ^{15}N ($\delta^{15}N_{bulk}$) values using a CE1108 Elemental analyzer interfaced via a CONFLO III device to a

Thermo-Electron Delta Plus XP mass spectrometer at the University of California, Santa Cruz. We report stable nitrogen isotope values in δ notation relative to atmospheric N_2.

For CSIA-AA we prepared ~ 10 mg of dried blood by acid hydrolysis followed by derivatization of amino acids (see Popp et al. [2007] and Hannides et al. [2013] for details). Samples were hydrolyzed (6N HCl, 150 °C for 70 minutes), esterified (4:1 isopropanol:acetyl chloride), derivatized (3:1 methylene chloride:trifluoroacetyl anhydride), and analyzed using a Trace GC gas chromatograph and a Thermo Delta XP mass spectrometer through a GC-C III combustion furnace (980°C), reduction furnace (680°C), and a liquid nitrogen cold trap. Samples were injected (split/splitless, 5:1 split ratio) with a 180°C injector temperature and a constant helium flow rate of 1.4 mL min^{-1}.

We analyzed the samples in triplicate, and corrected the $\delta^{15}N$ values to internal reference compounds norleucine and aminoadipic acid as these have known nitrogen isotope compositions and were co-injected with each sample. We confirmed quality control by analyzing an amino acid suite, with known $\delta^{15}N$ values of 12 amino acids, before and after each triplicate sample run. We analyzed the $\delta^{15}N$ values of 18 amino acids, although, some were not abundant enough for detection on all chromatographs. We grouped amino acids into standard 'trophic' and 'source' categories, based on previous studies (McClelland and Montoya 2002, Popp et al. 2007, Chikaraishi et al. 2009).

III. Estimating Trophic Position (TP) using variations of Eqn 1

We estimated TP using three variations of Eqn 1 in the main text of the manuscript, which rely on somewhat different approaches. First, we used one source ($\delta^{15}N_{phe}$) and one trophic ($\delta^{15}N_{glu}$) amino acid, an approach from Chikaraishi et al. 2009:

$$TP_{Glu-Phe} = \frac{(\delta^{15}N_{Glu} - \delta^{15}N_{Phe}) - 3.4}{7.6} + 1 \quad \text{(SI 1)}$$

where $TP_{Glu\text{-}Phe}$ is the TP based on mean δ^{15}N values from the trophic and source amino acids glutamic acid ($\delta^{15}N_{Glu}$) and phenylalanine ($\delta^{15}N_{Phe}$), respectively, the trophic discrimination factor (TDF) is 7.6 ‰ and represents the ^{15}N enrichment of $\delta^{15}N_{Glu}$ with respect to $\delta^{15}N_{Phe}$ per trophic step, and β represents $\delta^{15}N_{Glu}$- $\delta^{15}N_{Phe}$ in primary producers (3.4 ± 0.9 ‰).

More recent studies have indicated that using multiple source and trophic amino acids, along with a lower TDF to estimate TP, may provide more realistic TP estimates for certain taxa. Thus, we also estimated TP using approaches from Bradley et al. (2015), which was derived from a meta-analysis of teleosts:

$$TP_{Trp-Src} = \frac{\left(\delta^{15}N_{Trp} - \delta^{15}N_{Src}\right) - 3.6}{5.7} + 1 \quad \text{(SI 2)}$$

where $TP_{Trp\text{-}Src}$ is the TP based on the weighted mean δ^{15}N values of the trophic (Trp) amino acids alanine, glutamic acid, and leucine, and the source (Src) amino acids glycine, lysine, and phenylalanine, 3.6 ‰ is the ß value, and the TDF is 5.7 ‰.

Similar to Bradley et al. (2015), we also estimated TP using an equation derived from a recent meta-analysis of fish from Nielsen et al. 2015:

$$TP_{Trp-Src} = \frac{\left(\delta^{15}N_{Trp} - \delta^{15}N_{Src}\right) - 2.9}{5.7} + 1 \quad \text{(SI 3)}$$

where $TP_{Trp\text{-}Src}$ is the same as SI Eqn 2 above, but the ß value is 2.9 ‰, and the TDF is 6.6 ‰.

IV. Estimating Trophic Position using a Bayesian Approach

Lastly, we used a novel Bayesian approach, where we can estimate the TPs of consumers relative to baseline isotope data. Here, we used the R package ‘tRophicposition’ (Quezada-Romegialli et al. 2018), which combines an interface to JAGS (Just Another Gibbs Sampler) in R, using the

library “rjags”. JAGS uses Markov Chain Monte Carlo (MCMC) simulations to create Bayesian models, and ‘rjags’ couples JAGS with R, which allows us to sample posterior density distributions from the MCMC simulations. The MCMC simulations are coupled with stable isotope data to create Bayesian models to estimate TP. See Quezada-Romegialli et al. (2018) for more details on this approach.

There are several advantages to this approach. We can include variability in two key parameters that are used to estimate TP: the baseline isotope value of primary producers and the trophic discrimination factors. Thus, our TP estimates are likely more robust than relying on the aforementioned approaches to estimating TP. The equation used to in ‘tRophicposition’ is:

$$\delta^{15}N_{consumer} = \delta^{15}N_{baseline} + \mathrm{TDF}(\mathrm{TP} - \lambda) \quad \text{(SI 4)}$$

where λ represents the TP of the baseline isotope data. The ‘tRophicposition’ package was built for bulk isotope data. However, we used a novel approach to apply this package to amino acid isotope data, where we used $\delta^{15}N_{phe}$ as our baseline, $\delta^{15}N_{glu}$- ß as our consumer value, a TDF of 5.7 ± 0.9 ‰ from Bradley et al. (2015) and set λ = 1, since $\delta^{15}N_{phe}$ represents primary consumer values.

We defined a Bayesian model using the function jagsOneBaseline, where we defined our priors as a normal distribution of an *a priori* TP and standard deviation (3 ± 0.5). Then, we fed the Bayesian model with isotope data using the function TPmodel, and defined the number of parallel chains (2) and adaptive iterations (10000). We ran the model to generate large numbers of posterior density distributions of TP and then sampled these posterior trophic position estimates to obtain summary statistics for the posterior estimates.

V. Calculating Remigration Intervals

Since the remigration interval is the number of years between nesting events, calculating this value required nesting information for turtles over multiple nesting seasons. We calculated the remigration intervals for individuals in which blood samples and nesting data were available for the same years. Therefore, the remigration interval reflected the number of years, prior to the year of our sample collection, since the turtle was documented at Sandy Point National Wildlife Refuge (SPNWR). Blood samples were often taken on the first sighting of turtles at SPNWR, so nesting information prior to our sample collection was not always available, and thus, we could not calculate remigration intervals for all individuals. Based on previous knowledge about leatherback remigration intervals (Dutton et al. 2005, Price et al. 2005), we excluded remigration intervals > 6 years because those individuals likely nested elsewhere in the years between documentation at SPNWR.

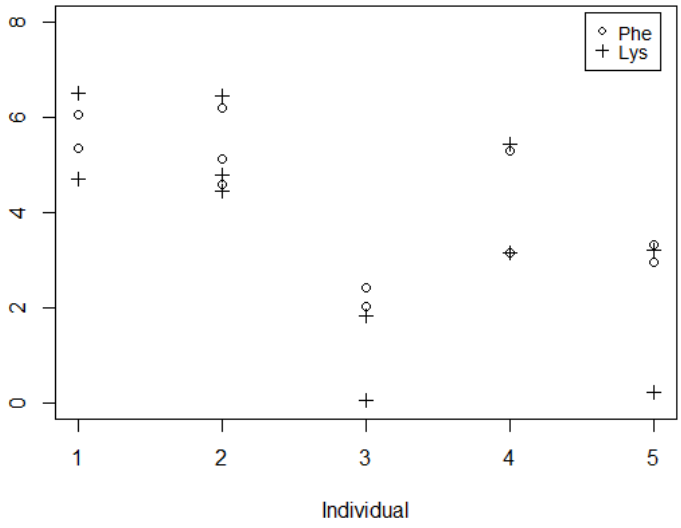

Fig. 1. The δ^{15}N values for the source amino acids phenylalanine (Phe) and lysine (Lys) for five individual turtles sampled during multiple nesting years.

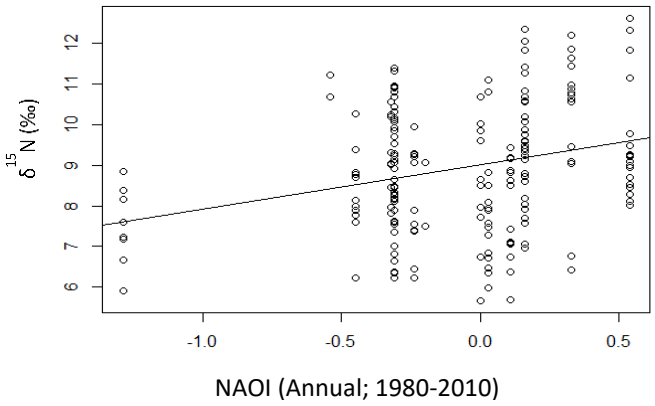

Fig. 2. Relationship between $\delta^{15}N_{bulk}$ values and the North Atlantic Oscillation Index., Adj. R^2 = 0.06, $F_{(1,197)} = 17.1$, $p < 0.00001$.

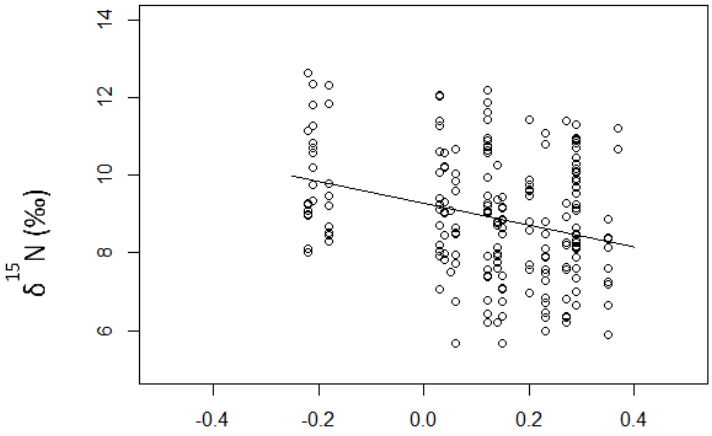

Fig. 3. Relationship between the $\delta^{15}N_{bulk}$ values and the Atlantic Multidecadal Oscillation Index, Adj. $R^2 = 0.08$, $F_{(1,197)} = 18.7$, $p < 0.00001$.

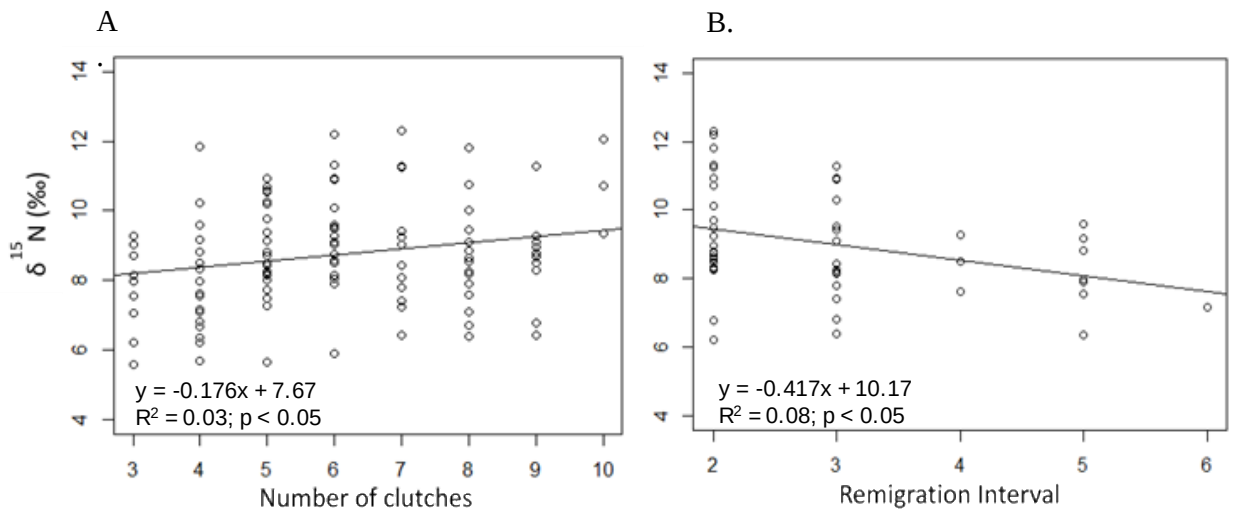

Fig. 4. Linear relationships between the $\delta^{15}N_{bulk}$ values from leatherback turtle blood and A) the number of clutches laid by individual leatherback turtles (i.e. clutch productivity) during the nesting season in which we collected blood samples for stable isotope analysis, and B) leatherback remigration intervals (years) prior to blood sample collection.

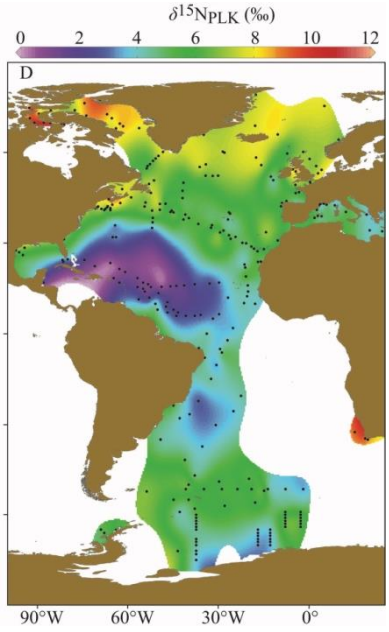

Fig 5. Nitrogen isoscape of plankton δ^{15}N values from McMahon et al. (2013). Here, the low-latitude region in the northwestern Atlantic with characteristically low δ^{15}N values is apparent. This region has lower δ^{15}N values at the base of the food web compared to the rest of the North Atlantic Ocean due to excess nitrogen fixation (Gruber and Sarmiento 1997, Montoya et al. 2002, Somes et al. 2010, McMahon et al. 2013), which is then reflected in the δ^{15}N values from the base of the food web.

Table 1. The mean (±SD) $\delta^{15}N_{bulk}$ values from leatherback turtles for each year of sample collection at Sandy Point National Wildlife Refuge, St. Croix, USVI.

Year	**n**	**δ^{15}N (‰) ± SD**
1992	9	9.5 ± 1.5
1993	11	10.7 ± 0.9
1994	9	9.6 ± 1.5
1997	2	8.3 ± 1.1
1998	3	9.2 ± 3.1
1999	15	9.7 ± 2.3
2000	14	9.7 ± 1.6
2001	12	8.2 ± 1.2
2002	13	8.5 ± 1.4
2003	15	7.9 ± 1.5
2004	12	9.0 ± 1.2
2005	31	9.2 ± 1.3
2006	11	7.9 ± 1.6
2007	14	7.9 ± 1.2
2008	11	8.3 ± 1.1
2009	9	9.2 ± 1.0
2010	10	7.4 ± 1.1
Total	201	8.9 ± 1.6

Table 2. Stable isotope data from five individual leatherbacks that were sampled during multiple nesting seasons, with years sampled, bulk isotope values ($\delta^{15}N_{bulk}$), mean source and trophic amino acid ($\delta^{15}N_{phe}$ and $\delta^{15}N_{glu}$) values, and the differences between sampling events in: phenylalanine values ($\Delta\ \delta^{15}N_{phe}$), and a proxy for trophic position ($\Delta\ \delta^{15}N_{glu}$ - $\delta^{15}N_{phe}$). Individual sample numbers correspond to the superscripts in the Sample ID column in Table 1.

Individual	Year	$\delta^{15}N_{bulk}$ (‰)	$\delta^{15}N_{phe}$ (‰)	$\delta^{15}N_{glu}$ (‰)	$\delta^{15}N_{glu}$ - $\delta^{15}N_{phe}$ (‰)
1	2000	12.1	6.0	21.0	15.0
1	2005	10.3	5.3	20.6	15.3
Δ	**5**	**1.8**	**0.7**	**0.4**	**-0.3**
2	1993	12.3	6.2	22.2	16.0
2	1999	6.8	5.1	20.1	14.9
2	2005	9.9	4.6	19.9	15.3
Δ 93-99	**6**	**5.6**	**1.1**	**2.1**	**1.1**
Δ 93-05	**12**	**2.4**	**1.6**	**2.3**	**0.7**
Δ 99-05	**6**	**-6.0**	**0.2**	**-0.4**	**-3.1**
3	1999	6.4	2.0	12.5	10.5
3	2003	7.9	2.4	15.9	13.5
Δ	**4**	**-1.5**	**-0.4**	**-3.4**	**-3.0**
4	1999	11.4	5.3	19.4	14.1
4	2002	9.8	3.2	17.9	14.7
Δ	**3**	**1.6**	**2.1**	**1.5**	**-0.7**
5	2002	5.7	3.3	13.4	10.1
5	2007	6.4	3.0	15.6	12.7
Δ	**5**	**-0.7**	**0.3**	**-2.3**	**-2.6**

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