Downloaded from rsbl.royalsocietypublishing.org

biology letters

The 'lost years' of green turtles: using stable isotopes to study cryptic lifestages

Kimberly J Reich, Karen A Bjorndal and Alan B Bolten

Biol. Lett. 2007 **3**, 712-714
doi: 10.1098/rsbl.2007.0394

References This article cites 14 articles, 1 of which can be accessed free
http://rsbl.royalsocietypublishing.org/content/3/6/712.full.html#ref-list-1

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Biol. Lett.* go to: **http://rsbl.royalsocietypublishing.org/subscriptions**

This journal is © 2007 The Royal Society

Downloaded from rsbl.royalsocietypublishing.org

biology letters

Biol. Lett. (2007) **3**, 712–714
doi:10.1098/rsbl.2007.0394
Published online 18 September 2007

Population ecology

The 'lost years' of green turtles: using stable isotopes to study cryptic lifestages

Kimberly J. Reich*, Karen A. Bjorndal and Alan B. Bolten

Archie Carr Center for Sea Turtle Research, Department of Zoology, PO Box 118525, University of Florida, Gainesville, FL 32611, USA
**Author for correspondence (kreich@zoology.ufl.edu).*

Ignorance of the location or inaccessible locations of lifestages can impede the study and management of species. We used stable isotopes of carbon and nitrogen to identify the habitats and diets and to estimate the duration of a 'missing' lifestage: the early juvenile stage of the green turtle, *Chelonia mydas*. Stable isotopes in scute from young herbivorous green turtles in shallow-water habitats revealed that they spend 3–5 years as carnivores in oceanic habitats before making a rapid ontogenetic shift in diet and habitat. Stable isotopes in persistent and continuously growing tissues, such as some fish scales, bird bills and claws and mammal hair and claws, can be used to evaluate the ecology of inaccessible lifestages.

Keywords: ontogenetic shift; stable isotopes; marine turtles

1. INTRODUCTION

Species with cryptic lifestages—in unknown or inaccessible locations—pose a special challenge to scientists and conservationists. Our study demonstrates how stable isotopes can be used to evaluate the ecology of an unknown or inaccessible lifestage of an organism. We used stable isotopes of carbon and nitrogen to study the early juvenile stage of green turtles, *Chelonia mydas*, a lifestage of unknown location. We solved a 50-year mystery in the biology of marine turtles posed by Archie Carr in 1952: how do green turtles spend their first years of life?

After leaving the nesting beach as 5 cm hatchlings, green turtles 'disappear' until they recruit to neritic habitats—waters over continental shelves—as greater than 20 cm juveniles and feed primarily on seagrasses and algae. Carr (1952) identified finding where 'hatchling and post-hatchling turtles go and what they do' during their 'lost' years as critical for the restoration of green turtle populations.

In 1986, Carr postulated that the early juvenile stage of all sea turtle species was spent in the surface waters of oceanic habitats (Carr 1986, 1987). Since that time, we have learned that North Atlantic loggerheads, *Caretta caretta*, conform to Carr's hypothesis, and spend their first 10 years in oceanic habitats feeding primarily on sea jellies and salps (Bolten *et al.* 1998; Bjorndal *et al.* 2003; Bolten 2003*a*). Carr's hypothesis has been generally accepted as the working hypothesis for other sea turtle species (Musick & Limpus 1997). However, extensive searching in the North Atlantic has yielded thousands of sightings of loggerheads, but green turtles are rarely seen (Witherington 2002; Bolten 2003*a*,*b*). Therefore, whether green turtles undergo an ontogenetic shift from oceanic to neritic habitats remains a question.

Stable isotopes of nitrogen and carbon have been used to study migration, feeding ecology and trophic structure in marine and terrestrial ecosystems (Hobson & Welch 1992; Post 2002; Cerling *et al.* 2006). Levels of ^{15}N are used to determine trophic position, and, in marine environments, carbon isotopes can distinguish between oceanic and neritic habitat use. Stable isotope values in keratinized tissues have been used to track changes in diet and habitat of baleen whales (Hobson & Schell 1998).

We tested Carr's hypothesis with the stable isotope record stored in green turtle scute tissue—the hard, keratinized tissue covering the bony shell of most chelonians. Scute is continually produced over the entire surface; hence, as a turtle grows and the bony shell increases in area, scute accumulates and becomes thicker over the older areas, while areas of recent growth expansion are covered only by thin, young scute tissue. Once produced, scute is inert and, although it is susceptible to wear, retains a history of diet and habitat. We used stable isotope values from young loggerheads in oceanic habitats around the Azores to evaluate the diets and habitats of 'lost year' green turtles. If Carr was correct, the oldest scute removed from green turtles newly recruited to neritic foraging grounds should contain a stable isotope signature similar to that of the oceanic-stage loggerheads and the signature of the youngest tissue should approach that of resident green turtles in neritic habitats (figure 1*a*).

2. MATERIAL AND METHODS

Scute samples were collected at a long-term study site off Great Inagua, Bahamas (Bjorndal *et al.* 2005) from 16 previously untagged green turtles (recruits) less than 36 cm in straight carapace length, assumed to have recruited to the study area in the previous year. Samples were also collected from 28 green turtles tagged in previous years and thus known to have been resident for at least 1 year (residents). We used sterile 6 mm biopsy punches to remove scute samples at a posterior (oldest) and anterior (youngest) site on the second lateral scute (figure 2). The oldest scute tissue was from the surface of the posterior sample; youngest tissue was from the anterior sample. We micro-sampled posterior scute from eight green turtle recruits in successive layers (50 μm) to provide a chronological sequence (figure 2). Standard procedures were employed for stable isotope analyses. See the electronic supplementary material for detailed methods.

3. RESULTS

Signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were significantly different between the oldest and youngest scute tissues from green turtles that had recruited within the previous year to neritic seagrass habitats (Wilcoxon signed-rank tests for paired data, $p<0.0001$, $n=16$, 16; figure 1*b*). Isotope signatures were not significantly different between small oceanic loggerheads (A. B. Bolten 2005, unpublished data, $n=12$)

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2007.0394 or via http://www.journals.royalsoc.ac.uk.

Received 23 July 2007
Accepted 28 August 2007

This journal is © 2007 The Royal Society

Downloaded from rsbl.royalsocietypublishing.org

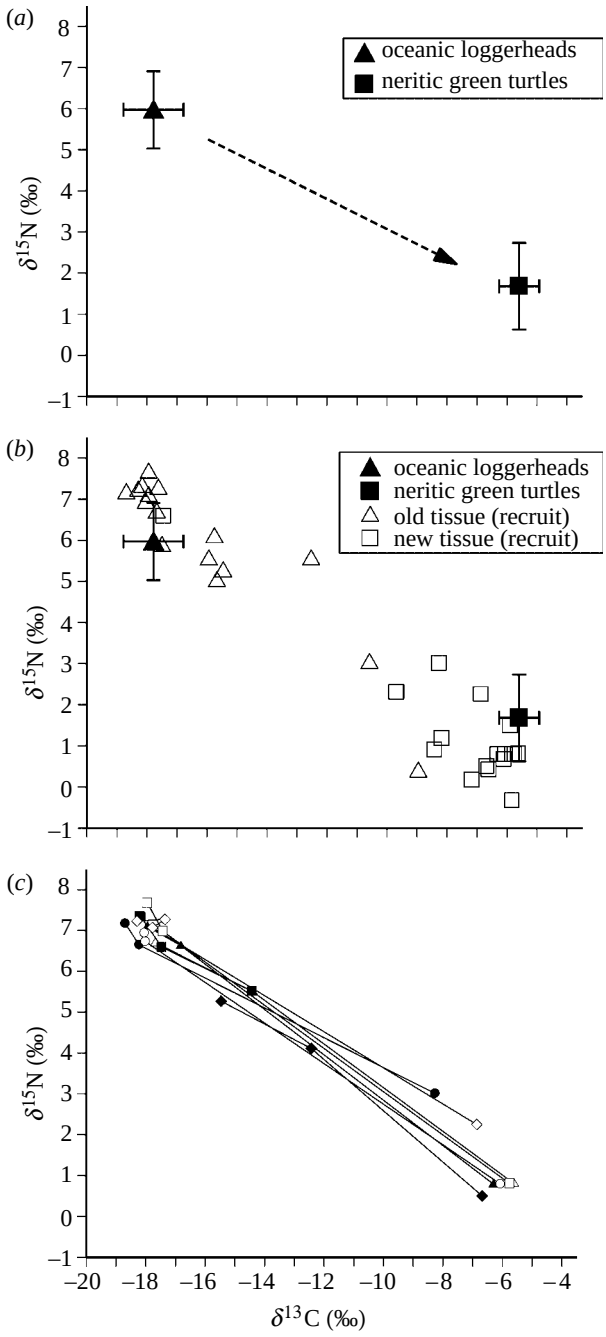

Figure 1. (*a*) Mean values (±1 s.d.) of δ^{13}C and δ^{15}N (‰) from oceanic-stage loggerheads ($n=12$) and neritic green turtles resident in seagrass habitat ($n=28$). If Carr's hypothesis is correct, these values should be equivalent to the shift in stable isotope values (indicated by arrow) from the oldest to the youngest scute tissues from green turtles recently recruited to neritic habitats. (*b*) Values of δ^{13}C and δ^{15}N (‰) from 16 green turtle recruits, added to (*a*). (*c*) Values of δ^{13}C and δ^{15}N for successive scute layers from eight green turtles. Each line is an individual; each point is a layer: from left to right, oldest to youngest.

and the oldest tissues from green turtle recruits (Wilcoxon rank–sum tests, $n=12$, 16; $p=0.423$ for δ^{13}C and $p=0.593$ for δ^{15}N). The youngest scute tissues from green turtle recruits and those of green turtles resident in the same neritic seagrass habitat for at least 1 year (residents) did not differ significantly in δ^{15}N values (Wilcoxon rank–sum tests, $n=16$, 28; $p=0.150$), but differed in δ^{13}C ($p=0.0003$).

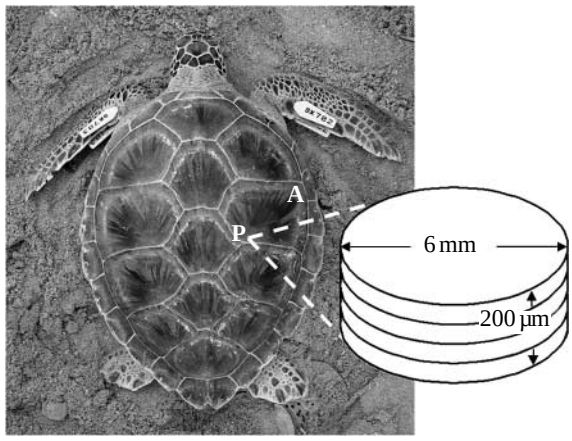

Figure 2. Image of a green turtle showing the two sampling sites anterior (A) and posterior (P). Diagram illustrates the sequential sample layers from posterior scute samples. Paler tissue around the anterior and lateral sides of each scute is new tissue.

4. DISCUSSION

Stable isotopes in scute tissue revealed that, before recruiting to neritic habitats, juvenile green turtles occupy similar habitats and feed at the same trophic level as do oceanic-stage loggerheads. As predicted, the isotope values of the youngest scute tissue from recent recruits approached those of residents on neritic foraging grounds. The δ^{15}N values were not significantly different between these two groups, but the δ^{13}C values were significantly lower in recruits, indicating that incorporation of the new nitrogen signature into scute tissue was more rapid than that of carbon. This pattern matches the relative rates of N and C incorporation into scute in captive juvenile loggerheads (K. J. Reich *et al.* 2006, unpublished data) and provides further support that N and C incorporations can be uncoupled and cannot be assumed to be equal (Hobson & Stirling 1997; Hobson & Bairlein 2003; Carleton & Martínez del Rio 2005).

Because successive layers of scute store a chronological record of diets and habitats, we can draw conclusions about the rates of change if the rate of scute deposition is used as a proxy for time. These conclusions must be considered with caution because our 50 µm sampling layers were based on the minimum amount of sample needed for analysis; we do not know the biological significance of this depth. A relatively rapid and direct ontogenetic shift from oceanic to neritic habitats is indicated by the paucity of values between the primary oceanic and neritic signatures (figure 1*c*) and the oceanic signature still present in the youngest scute tissue of one turtle caught on neritic foraging grounds (figure 1*b*). The oldest two to three layers in most turtles had the same oceanic foraging signature (figure 1*c*), suggesting that these isotopic values represent either the entire or a major portion of the lifestage between hatching and recruitment to neritic habitats.

A few data points in figure 1*b* do not conform to the general pattern. The youngest scute point (square) in the midst of the oldest scute points (triangles) represents an individual that had just recruited and had not yet incorporated into a neritic

Downloaded from rsbl.royalsocietypublishing.org

biology letters

signature. The oldest scute point that falls within the youngest scute points probably represents an individual that had recruited earlier but had escaped tagging in the previous year. The two points for the oldest tissue which fall between the two clusters may represent (i) sampling layers that combined tissues with the oceanic and neritic signatures, (ii) a transition habitat and diet between the oceanic and neritic signatures, or (iii) a different habitat and diet in the early lifestage of these two individuals.

The similarity of diets, or trophic levels, between oceanic-stage green turtles and loggerheads suggests that growth rates of young green turtles may be similar to those of loggerheads. Therefore, we estimated the duration of the oceanic stage of green turtles as the time required for loggerheads to grow to 25–35 cm (sizes at which green turtles recruit to neritic habitats). Because oceanic-stage loggerheads in the eastern Atlantic reach 25 and 35 cm in approximately 2.8 and 4.6 yr, respectively (Bjorndal *et al.* 2003), we estimated that the duration of the green turtle oceanic-stage is approximately 2.8–4.6 yr, as well. This range is similar to an estimate for green turtles based on skeletochronology of 3–6 yr (Zug & Glor 1999). Of course, variation in temperature, diet quality and food availability would affect the growth rates of green turtles.

Other populations of green turtles and, apparently, other species of sea turtles share similar oceanic habitats and diets in early juvenile stages and provide additional support for Carr's hypothesis. Samples of the oldest scute tissue from small green turtles stranded dead in Florida ($n=11$), hawksbills (*Eretmochelys imbricata*, $n=4$) and a Kemp's ridley (*Lepidochelys kempii*, $n=1$) indicate that all have a similar oceanic signature (figure A-1 in the electronic supplementary material).

Stable isotopes of scute provided insights into the early juvenile stage of green turtles, a lifestage whose geographical location remains unknown, but whose oceanic habitat has now been confirmed. Tissues that are continually produced and retain a stable isotope record, such as turtle scutes, baleen, mammal hair and claws, bird bills and claws and fish scales, provide a powerful tool for studying inaccessible lifestages.

This study was conducted under the University of Florida Institutional Animal Care and Use Committee protocol (permit Z097).

We thank C. Martínez del Rio, L. Chapman, D. Hodell, and B. MacFadden for advice; S. Luciano, N. Osman, J. Pfaller, K. Hyndman and P. Eliazar for assistance; the Light Stable Isotope Lab, University of Florida and J. Curtis for assistance with stable isotope analyses; and B. Wallace for comments on the manuscript. This project was in cooperation with the Bahamas National Trust and was funded by Disney Wildlife Conservation Fund, National Marine Fisheries Service, US Fish and Wildlife Service, and Florida Fish and Wildlife Conservation Commission Marine Turtle Grants Program. All necessary research and CITES permits were obtained.

Bjorndal, K. A., Bolten, A. B., Dellinger, T., Delgado, C. & Martins, H. R. 2003 Compensatory growth in oceanic loggerhead sea turtles: response to a stochastic environment. *Ecology* **84**, 1237–1249. (doi:10.1890/0012-9658(2003)084[1237:CGIOLS]2.0.CO;2)

Bjorndal, K. A., Bolten, A. B. & Chaloupka, M. Y. 2005 Evaluating trends in abundance of immature green turtles, *Chelonia mydas*, in the Greater Caribbean. *Ecol. Appl.* **15**, 304–314. (doi:10.1890/04-0059)

Bolten, A. B. 2003*a* Active swimmers–passive drifters: the oceanic juvenile stage of loggerheads in the Atlantic system. In *Loggerhead sea turtles* (eds A. B. Bolten & B. E. Witherington), pp. 63–78. Washington, DC: Smithsonian Institution Press.

Bolten, A. B. 2003*b* Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages. In *The biology of sea turtles*, vol. II (eds P. L. Lutz, J. A. Musick & J. Wyneken), pp. 243–257. Boca Raton, FL: CRC Press.

Bolten, A. B., Bjorndal, K. A., Martins, H. R., Dellinger, T., Biscoito, M. J., Encalada, S. E. & Bowen, B. W. 1998 Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. *Ecol. Appl.* **8**, 1–7. (doi:10.1890/1051-0761(1998)008[0001:TDMOLS]2.0.CO;2)

Carleton, S. A. & Martínez del Rio, C. 2005 The effect of cold-induced increased metabolic rate on the rate of ^{13}C and ^{15}N incorporation in house sparrows (*Passer domesticus*). *Oecologia* **114**, 226–232. (doi:10.1007/s00442-005-0066-8)

Carr, A. 1952 *Handbook of turtles: the turtles of the United States, Canada, and Baja California*. Ithaca, NY: Cornell University Press.

Carr, A. 1986 Rips, FADS, and little loggerheads. *Bioscience* **36**, 92–101. (doi:10.2307/1310109)

Carr, A. 1987 New perspectives on the pelagic stage of sea turtle development. *Conserv. Biol.* **1**, 103–121. (doi:10.1111/j.1523-1739.1987.tb00020.x)

Cerling, T. E., Wittemyer, G., Rasmussen, H. B., Vollrath, F., Cerling, C. E., Robinson, T. J. & Douglas-Hamilton, I. 2006 Stable isotopes in elephant hair document migration patterns and diet changes. *Proc. Natl Acad. Sci. USA* **103**, 371–373. (doi:10.1073/pnas.0509606102)

Hobson, K. A. & Bairlein, F. 2003 Isotopic fractionation and turnover in captive garden warblers (*Sylvia borin*): implications for delineating dietary and migratory associations in wild passerines. *Can. J. Zool.* **81**, 1630–1635. (doi:10.1139/Z03-140)

Hobson, K. A. & Schell, D. M. 1998 Stable carbon and nitrogen isotope patterns in baleen from eastern Arctic bowhead whales (*Balaena mysticetus*). *Can. J. Fish. Aquat. Sci.* **55**, 2601–2607. (doi:10.1139/cjfas-55-12-2601)

Hobson, K. A. & Stirling, I. 1997 Low variation in blood $\delta^{13}C$ among Hudson Bay polar bears: implications for metabolism and tracing terrestrial foraging. *Mar. Mamm. Sci.* **13**, 359–367. (doi:10.1111/j.1748-7692.1997.tb00645.x)

Hobson, K. A. & Welch, H. E. 1992 Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. *Mar. Ecol. Prog. Ser.* **84**, 9–18.

Musick, J. A. & Limpus, C. J. 1997 Habitat utilization and migration in juvenile sea turtles. In *The biology of sea turtles* (eds P. L. Lutz & J. A. Musick), pp. 137–163. Boca Raton, FL: CRC Press.

Post, D. M. 2002 Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**, 703–718.

Witherington, B. E. 2002 Ecology of neonate loggerhead turtles inhabiting lines of downwelling near a Gulf Stream front. *Mar. Biol.* **140**, 843–853. (doi:10.1007/s00227-001-0737-x)

Zug, G. R. & Glor, R. E. 1999 Estimates of age and growth in a population of green sea turtles (*Chelonia mydas*) from the Indian River lagoon system, Florida: a skeletochronological analysis. *Can. J. Zool.* **76**, 1497–1506. (doi:10.1139/cjz-76-8-1497)