九州大学学術情報リポジトリ
Kyushu University Institutional Repository

δ^15N and δ^13C measurements from the hawksbill turtle, Eretmochelys imbricata, used for scute sourcing

Ishibashi, Hiroshi
Lavoratory of Material Information, Graduate School of Social and Cultural Studies, Kyushu University

Diaz-Fernandez, R
Faculty of Biology, Havana University

Carrillo, E
Fisheries Research Center, Ministry of Fishery, Cuba

Koike Hiroko
Lavoratory of Material Information, Graduate School of Social and Cultural Studies, Kyushu University

https://doi.org/10.15017/8628

出版情報：比較社会文化. 6, pp.37-45, 2000-03-01. 九州大学大学院比較社会文化研究科
バージョン：
権利関係：

KYUSHU UNIVERSITY

『比較社会文化』第6巻（2000）37～45頁
Bulletin of the Graduate School of Social and Cultural Studies, Kyushu University
vol.6 (2000), pp.37～45

$\delta^{15}N$ and $\delta^{13}C$ measurements from the hawksbill turtle, *Eretmochelys imbricata*, used for scute sourcing

H. ISHIBASHI[1], R. DÍAZ-FERNÁNDEZ[2], E. CARRILLO[3] and H. KOIKE[1]

Keywords: Carbon isotope, nitrogen isotope, hawksbill turtle, Cuba, scute sourcing

Abstract

The hawksbill turtle, *Eretmochelys imbricata* is listed the IUCN Red List as critically endangered. At the COP 10 of the CITES meeting held in 1997, Cuba proposed to downlist the population of hawksbills from Appendix I to Appendix II to allow limited trade. In this report $\delta^{15}N$ and $\delta^{13}C$ measurements were used for scute-sourcing, where $\delta^{13}C$ values allow differentiation between oceanic plankton ecosystems and coral reef ecosystems, and $\delta^{15}N$ values indicate the consumer's trophic level in a regional food web. A total of 118 scute samples were analysed from the Caribbean Sea region including Cuba, Haiti and Mexico, from the Pacific Ocean region and from the Indian Ocean region. Breeding individuals from Cuba had higher $\delta^{15}N$ values than wild individuals from Cuba, indicating the isotope analysis will be useful for distinguishing the scutes of breeding populations, which will be targeted for export approval under CITES, from the wild populations specimens obtained by traditional fishing. The samples from the Caribbean Sea region are characterized by less negative $\delta^{13}C$ values than samples from the Pacific Ocean region, suggesting a higher dependance on coral ecosystems. The most positive $\delta^{15}N$ values were from the Seychelles in the Indian Ocean, suggesting a higher dependance on sponges or other animal species.

1. Introduction

The hawksbill turtle, *Eretmochelys imbricata* (Linnaeus, 1766) is taxonomically classified as Order Testudinata, Family Cheloniidae. The hawksbill turtle has a global distribution in the tropical zones of the Atlantic, Pacific and Indian Oceans (Fig. 1). The hawksbill turtle is generally considered the least migratory of sea turtles and appears to favor shallow waters in coral reefs, bays, estuaries and keys (Witzell, 1983).

Since scutes of the carapace for the hawksbill turtle are thicker than any for other turtle, they are used for ornaments and musical instruments. The Convention on International Trade in Endangered Species of Flora and Fauna (CITES) lists all marine turtles in its Appendix I (Prohibited from international trade from or to signatory countries). The World Conservation Union (IUCN) has listed *Caretta caretta* as vulnerable, and all other sea turtle species (except *Natatordepressus*) as endangered. In 1996, the hawksbill turtle was listed in the IUCN/Red Lists as "critically endangered" (Mrosovsky, 1997). At the COP 10 of the CITES meeting held in June 1997 in Harare, Zimbabwe, Cuba proposed to downlist the population of hawksbills in its waters from Appendix I to Appendix II, to allow limited trade (Republic of Cuba, 1997).

To identify the source of scutes from hawksbill turtles, we have utilized stable isotope measurements, trace element analysis and DNA sequencing. Mitochondrial DNA, using extracted DNA from the scute, provided genetic information to identify the local populations of the hawksbill turtle (Okayama *et al.*, 1999; Diaz *et al.*, 1999). Trace element content, obtained by ICP-MS analysis of the scutes, provided environmental information on sea water in their habitat (Tanabe and Sakai, 1997). Carbon and nitrogen isotope analyses from the scutes provided import and ecological information on the diet of the animals (Koike, 1996).

Standard approaches to food web analysis include gut content analysis and direct observation, both in the field and laboratory. The hawksbill turtle is a carnivorous species and feeds upon a wide variety of organisms in tropical reef habitats. Stomach content analyses (Pritchard, 1979;

[1] Laboratory of Material Information, Graduate School of Social and Cultural Studies, Kyushu University, Fukuoka, Japan.
[2] Department of Biochemistry, Faculty of Biology, Havana University, Havana, Cuba.
[3] Turtle Project Group, Fisheries Research Center, Ministry of Fishery, Havana, Cuba.

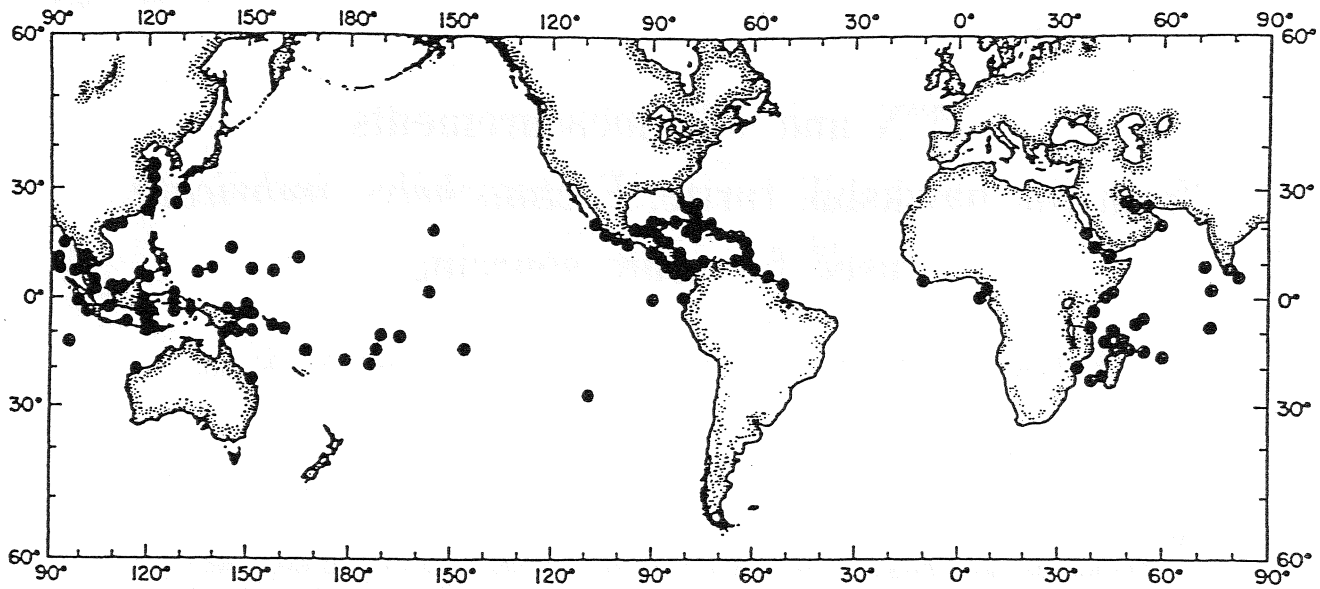

Fig. 1. Distributions of nesting rookeries for the hawksbill turtle. (from witzell, 1983)

Ernst and Barbour, 1989; Anderes and Uchida, 1994) have revealed that sponges figure pro-minently in the adults diet, but coelenterates, ectoprocts, echinoderms, gastropods and bivalve molluscs, barnacles, crustaceans, ascidians and fish have also been reported. Adult hawksbill turtles have specialized on a diet of sponges.

Analysis of gut contents requires few tools and equipment. However, some organisms digest their prey quickly, making identification difficult. Also, some prey tend to lose their morphological characteristics more quickly than others. Stable isotope analysis has more recently been used as an alternative, and in some cases, better tool for food web analysis. With the development of automated systems, a broad survey of organisms in a food web system can be performed with a reasonable amount of data.

Carbon isotopic ratios ($\delta^{13}C$*1) of animals reflect those of the diet within about 1 ‰ (Haines, 1976; DeNiro and Epstein, 1978; Fry *et al.*, 1978a; Haines and Montague, 1979; Teeri and Schoeller, 1979; Rau, 1981; Rau and Anderson, 1981. This conservative transfer of carbon isotopic compositions to the animal from the diet can be useful in tracing foodwebs in systems where there are food sources with large differences in $\delta^{13}C$ values, such as C_3 versus C_4 plants, or marine versus terrestrial systems (Haines, 1976; Fry *et al.*, 1977, 1978b; DeNiro and Epstein, 1978; Rau, 1981; Schoeninger and DeNiro, 1984).

As with carbon, nitrogen isotope ratios ($\delta^{15}N$*2) in the organism reflect those of the diet, but in most cases the whole animal is enriched in $\delta^{15}N$ relative to the diet (DeNiro and Epstein, 1981). Field studies show an average+3.2‰ enrichment in animal $\delta^{15}N$ versus diet, which is reflected as a trophic level effect in food web studies (Michener and Schell, 1994). Minagawa & Wada (1984) found a $\delta^{15}N$ enrichment of+3.4±1.1‰ per trophic level, independent of habitat. A survey of bone collagen by Schoeninger & DeNiro (1984) for 66 species of vertebrates resulted in an average+3‰ enrichment per trophic level.

In this report, $\delta^{15}N$ and $\delta^{13}C$ values were obtained for scute samples from the Caribbean Sea region of Cuba, Haiti and Mexico, from the Pacific Ocean region of the Solomon Islands, Fiji, Indonesia and the Philippines, and from the Indian Ocean region of Seychelles, Morjib and some unknown countries in Africa. $\delta^{13}C$ values allow differentiation between oceanic plankton ecosystems and coral reef ecosystems, both of which might provide primary foods for the turtle. $\delta^{15}N$ values indicate the consumer's trophic level in a regional food web, wherein $\delta^{15}N$ values for the secondary producers are usually around +5 and those for higher producers are around+10‰.

2. Materials

A total of 118 scute samples were analysed as follows:

*1 Ratios of ^{13}C to ^{12}C for samples are compared with that ratio for a standard, which is the internationally used PDB standard ($^{13}C/^{12}C$standard =0.0112372), a fossil belemnite from the PeeDee layer, South Carolina, and the results expressed as:

$$\delta^{13}C(‰) = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard}}{(^{13}C/^{12}C)_{standard}} \times 1000$$

from the Caribbean Sea region (Table 1) of Cuba (58 samples), Haiti (10 samples), and Mexico (1 sample); from the Pacific Ocean region (Table 2) of the Solomon Islands (11 samples), Fiji (2 samples), Indonesia (5 samples) and the Philippines (3 samples); and from the Indian Ocean region (Table 3) of Seychelles (19 samples), Morjib (8 samples), and from unknown African countries (4 samples). The 55 scute samples from Cuba were collected by the Cuban Fishery Department, and grouped into two categories: "breeding" individuals from Pinos Island (11 individuals) and "wild" individuals (44 individuals). All other scute samples were provided by the Japan Bekko Association, which could only identify the exporting country for samples obtained before the CITES agreement came into effect.

3. Methods

In each case about 20 mg of sample was taken from the innersurface of the scute using a clean knife, avoiding the white-wax-like residues on the surface. To remove lipids, the samples were treated with chloroform-methanol (2:1), and then freeze-dried.

δ^{15}N and δ^{13}C measurements were done using an ANCA-MS (Automatic Nitrogen and Carbon Analyzer mass-spectrometry, Europa Scientific Ltd.) following the method developed by Dr. C.M. Scrimgeour (Handley *et al.*, 1997). Two analyses on 700 mg of each sample were done to obtain % N, %C and δ^{13}C data. For δ^{15}N analysis, the composition obtained from the first run was used to weigh out samples containing 100 μg/N.

4. %N and %C Values

C/N, that is, ratio of % C to % N, was obtained from total ^{12}C and total ^{14}N data by the ANCA-MS. C/N for the scute samples (Fig. 2) fell along the expected regression line for this protein, suggesting that these scute samples were well purified protein.

5. δ^{15}N and δ^{13}C results for the scute samples

Caribbean Sea region (Fig. 3)

Breeding individuals from Cuba: The δ^{13}C and δ^{15}N distributions for the 11 samples from the breeding center at Pinos Island were concentrated between -11.4 and -12.8‰ for the δ^{13}C values, and between $+6.7$ and $+8.3$ ‰ for the δ^{15}N values. Clear separation of the breeding individuals from wild population was observed. The breeding individuals gave an average and a standard deviation of -12.3 ± 0.5‰ for δ^{13}C values, and $+7.5\pm0.6$ ‰ for δ^{15}N values. The breeding individuals had higher δ^{15}N values than wild individuals. Standard deviations for the breeding individuals were ±0.5‰ for δ^{13}C and ±0.6 ‰ for δ^{15}N which coincides with experimental data for monkeys (Koike *et al.* 1988).

Wild population individuals from Cuba: The δ^{13}C values for 44 individuals from the wild Cuban population had a wide distribution; between -9 to -16‰ with one outlier at -19.5‰. The average and standard deviation for the Cuban wild population was -13.9 ± 2.7‰ suggesting a strong dependancy on coral reef ecosystems.

The δ^{15}N values for the Cuban wild population were between $+3$ and $+7$‰ with one exception at $+12.3$‰. Their average and standard deviation was $+5.7\pm1.8$‰ which was characterized by relatively low nitrogen values, suggesting that their diet must be from lower trophic levels.

Samples from Haiti: δ^{13}C values for the 10 scute samples from Haiti clustered around -11 to -14‰ with an average of -13.5‰ and a standard deviation of ±0.9‰. The δ^{15}N values for these samples were between $+6$ and $+10$ ‰ with an average of $+8.6\pm1.2$‰. The δ^{15}N for the Haitian wild population was higher than those of the Caribbean samples, suggesting a relatively stronger dependance on sponges or higher-trophic level animals.

The Pacific Ocean region (Fig. 4)

Samples from the Solomon Islands: δ^{13}C values for the 11 scute samples from the Solomons were distributed between -12 and -17‰ with an average of -14.9‰ and a standard deviation of ±1.6‰. Their more negative δ^{13}C values showed a relatively higher dependance on oceanic ecosystems. The δ^{15}N values for these samples were between $+6$ and $+11$‰ with an average of $+9.4$ ±2.2‰.

Samples from Fiji: The δ^{13}C values for the 2 samples from Fiji were -14.9 and -16.1‰ and the δ^{15}N values were relatively low, at $+7.9$ and $+8.1$‰.

Samples from Indonesia: δ^{13}C values for the 5 scute samples from Indonesia clustered around -16 to -18‰

*2 Ratios of ^{15}N to ^{14}N for samples are similarly compared with the ratio for a standard (^{15}N/^{14}Nstandard $=0.0036765$), which is atmospheric nitrogen, and the results expressed as:

$$\delta^{15}\mathrm{N}(‰) = \frac{(^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{sample}} - (^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{standard}}}{(^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{standard}}} \times 1000$$

Table 1. List of samples for the Caribbean Sea region.

Lab. No.	country	category	original No.	measurement		Average±STD			
				delta 13C	delta 15N	delta 13C	delta 15N	delta 13C	delta 15N
95TS-187	Cuba	Breeding	2054(sakai-R)	−12.3	7.7	−12.3	+7.5	−12.3	+7.5
95TS-188	Cuba	Breeding	2054(sakai-R)	−11.4	7.6	±0.5	±0.6	±0.5	±0.6
95TS-189	Cuba	Breeding	2054(sakai-R)	−11.6	7.8				
95TS-190	Cuba	Breeding	2054(sakai-R)	−11.9	7.7				
95TS-191	Cuba	Breeding	2054(sakai-R)	−12.3	6.7				
95TS-192	Cuba	Breeding	2054(sakai-R)	−12.2	7.2				
95TS-193	Cuba	Breeding	2054(sakai-R)	−12.3	6.7				
95TS-194	Cuba	Breeding	2054(sakai-R)	−12.8	6.7				
95TS-195	Cuba	Breeding	1013/5R(kobayashi)	−12.5	7.4				
95TS-196	Cuba	Breeding	1013/6R(kobayashi)	−12.8	8.3				
95TS-197	Cuba	Breeding	1013/7R(kobayashi)	−12.9	8.3				
93TS09	Cuba	Wild	Cuba-1	−19.5	7.2	−13.9	+5.7	−13.7	+6.2
93TS10	Cuba	Wild	Cuba-2	−14.6	5.4	±2.7	±1.8	±2.5	±2.1
93TS11	Cuba	Wild	Cuba-3	−14.9	7.7				
93TS12	Cuba	Wild	Cuba-4	−14.9	5.8				
93TS13	Cuba	Wild	Cuba-5	−13.4	6.3				
93TS20	Cuba	Wild	Cuba-6	−16.0	5.9				
93TS21	Cuba	Wild	Cuba-7	−16.1	8.3				
94TS09	Cuba	Wild	Cuba-8	−9.8	2.7				
94TS10	Cuba	Wild	Cuba-9	−13.0	5.0				
94TS11	Cuba	Wild	Cuba-10	−14.0	4.1				
94TS12	Cuba	Wild	Cuba-11	−15.4	6.4				
94TS18	Cuba	Wild	Cuba-12	−13.4	4.2				
94TS19	Cuba	Wild	Cuba-13	−15.1	6.6				
94TS20	Cuba	Wild	Cuba-14	−14.8	5.5				
94TS21	Cuba	Wild	Cuba-15	−14.8	6.8				
94TS22	Cuba	Wild	Cuba-16	−14.5	5.8				
94TS23	Cuba	Wild	Cuba-17	−14.9	5.1				
94TS24	Cuba	Wild	Cuba-18	−14.9	7.2				
94TS25	Cuba	Wild	Cuba-19	−10.8	3.2				
94TS26	Cuba	Wild	Cuba-20	−15.5	5.7				
94TS27	Cuba	Wild	Cuba-21	−13.2	4.6				
94TS28	Cuba	Wild	Cuba-22	−14.0	6.0				
94TS29	Cuba	Wild	Cuba-23	−15.5	9.2				
94TS30	Cuba	Wild	Cuba-24	−11.0	6.3				
94TS31	Cuba	Wild	Cuba-25	−12.5	5.4				
94TS32	Cuba	Wild	Cuba-26	−15.8	5.3				
94TS33	Cuba	Wild	Cuba-27	−14.2	5.6				
94TS34	Cuba	Wild	Cuba-28	−15.0	6.8				
94TS35	Cuba	Wild	Cuba-29	−16.6	12.3				
95TS-198	Cuba	Wild	951013/4(sakai-R)	−12.6	4.1				
95TS-199	Cuba	Wild	951016/1(sakai-R)	−12.8	5.0				
95TS-200	Cuba	Wild	951016/2(sakai-R)	−11.2	4.6				
95TS-203	Cuba	Wild	951017/1(sakai-R)	−14.5	4.7				
95TS-204	Cuba	Wild	951017/2(sakai-R)	−15.3	7.2				
95TS-205	Cuba	Wild	951024A/1(sakai-R)	−8.8	5.1				
95TS-206	Cuba	Wild	951024A/2(sakai-R)	−12.3	4.6				
95TS-207	Cuba	Wild	951024A/3(sakai-R)	−14.3	4.7				
95TS-208	Cuba	Wild	951024A/4(sakai-R)	−14.9	5.9				
95TS-209	Cuba	Wild	951024A/5(sakai-R)	−14.8	5.0				
95TS-210	Cuba	Wild	951024A/6(sakai-R)	−13.9	4.8				
95TS-211	Cuba	Wild	951024A/7(sakai-R)	−10.9	5.6				
95TS-212	Cuba	Wild	951024A/8(sakai-R)	−13.7	4.4				
95TS-213	Cuba	Wild	951024A/9(sakai-R)	−13.5	4.0				
95TS-214	Cuba	Wild	951024A/10(sakai-R)	−12.4	3.3				
97TS20	Haiti	wild	Haiti-1	−14.0	8.0	−13.5	+8.6		
97TS21	Haiti	wild	Haiti-2	−14.3	9.1	±0.9	±1.2		
97TS22	Haiti	wild	Haiti-3	−14.2	9.6				
97TS23	Haiti	wild	Haiti-4	−11.6	8.0				
97TS24	Haiti	wild	Haiti-5	−12.9	9.3				
97TS25	Haiti	wild	Haiti-6	−13.7	9.6				
97TS26	Haiti	wild	Haiti-7	−14.5	10.1				
97TS27	Haiti	wild	Haiti-8	−13.2	6.0				
97TS28	Haiti	wild	Haiti-9	−12.4	7.5				
97TS29	Haiti	wild	Haiti-10	−14.0	8.8				
94TS13	Mexico	wild	Mexico-1	−15.7	8.4	−15.7	+8.4		

Table 2. List of samples for the Pacific Ocean region.

Lab. No.	country	category	original No.	measurement		Average±STD			
				delta 13C	delta 15N	delta 13C	delta 15N	delta 13C	delta 15N
93TS05	Solomon	wild	Solomon-1	−16.9	10.5	−14.9	+9.4	−15.6	+8.9
93TS06	Solomon	wild	Solomon-2	−16.2	5.7	±1.6	±2.2	±1.5	±1.9
93TS07	Solomon	wild	Solomon-3	−14.3	8.7				
93TS08	Solomon	wild	Solomon-4	−14.3	5.0				
93TS18	Solomon	wild	Solomon-5	−13.4	10.7				
93TS19	Solomon	wild	Solomon-6	−11.6	10.0				
94TS03	Solomon	wild	Solomon-7	−14.3	9.9				
94TS04	Solomon	wild	Solomon-8	−16.1	11.1				
94TS05	Solomon	wild	Solomon-9	−16.0	11.3				
94TS06	Solomon	wild	Solomon-10	−14.2	10.2				
95TS-164	Solomon	wild	Solomon-11	−16.1	10.8				
94TS07	Fiji	wild	Fiji-1	−14.9	7.9	−15.5	+8.0		
94TS08	Fiji	wild	Fiji-2	−16.1	8.1	±0.8	±0.1		
93TS01	Indonesia	wild	Indonesia-1	−15.7	9.5	−16.9	+8.1		
93TS02	Indonesia	wild	Indonesia-2	−16.8	9.1	±0.9	±0.3		
93TS03	Indonesia	wild	Indonesia-3	−17.6	9.0				
93TS04	Indonesia	wild	Indonesia-4	−18.0	4.6				
95TS-162	Indonesia	wild	Indonesia-5	−16.6	8.2				
94TS01	Philippines	wild	Philippines-1	−16.2	8.5	−16.3	+9.0		
94TS02	Philippines	wild	Philippines-2	−16.3	9.8	±0.1	±0.7		
95TS-163	Philippines	wild	Philippines-3	−16.2	8.6				

Table 3. List of samples for the Indian Ocean region.

Lab. No.	country	category	original No.	measurement		Average±STD			
				delta 13C	delta 15N	delta 13C	delta 15N	delta 13C	delta 15N
97TS01	Seychelles	wild	Seychelles-1	−14.0	11.5	−13.0	+12.9	−13.6	+11.8
97TS02	Seychelles	wild	Seychelles-2	−8.1	10.7	±2.1	±2.4	±2.1	±2.7
97TS03	Seychelles	wild	Seychelles-3	−14.4	15.2				
97TS04	Seychelles	wild	Seychelles-4	−14.8	13.6				
97TS05	Seychelles	wild	Seychelles-5	−11.1	13.5				
97TS06	Seychelles	wild	Seychelles-6	−11.0	13.4				
97TS07	Seychelles	wild	Seychelles-7	−10.8	14.5				
97TS08	Seychelles	wild	Seychelles-8	−12.0	13.0				
97TS09	Seychelles	wild	Seychelles-9	−8.8	13.5				
97TS10	Seychelles	wild	Seychelles-10	−14.5	13.3				
97TS11	Seychelles	wild	Seychelles-11	−13.5	8.6				
97TS12	Seychelles	wild	Seychelles-12	−15.0	9.2				
97TS13	Seychelles	wild	Seychelles-13	−14.1	15.6				
97TS14	Seychelles	wild	Seychelles-14	−13.9	15.3				
97TS15	Seychelles	wild	Seychelles-15	−14.2	15.1				
97TS16	Seychelles	wild	Seychelles-16	−13.7	9.0				
97TS17	Seychelles	wild	Seychelles-17	−14.2	14.4				
97TS18	Seychelles	wild	Seychelles-18	−14.4	9.9				
97TS19	Seychelles	wild	Seychelles-19	−15.0	15.7				
93TS22	Morjib	wild	Morjib-1	−16.2	10.8	−14.9	+9.2		
93TS23	Morjib	wild	Morjib-2	−15.9	10.0	±1.5	±1.5		
93TS24	Morjib	wild	Morjib-3	−16.7	10.1				
94TS14	Morjib	wild	Morjib-4	−16.2	7.8				
94TS15	Morjib	wild	Morjib-5	−14.0	9.8				
94TS16	Morjib	wild	Morjib-6	−13.7	10.3				
94TS17	Morjib	wild	Morjib-7	−13.3	6.5				
95TS-165	Morjib	wild	Morjib-8	−12.9	7.8				
93TS14	African	wild	African-1	−16.6	8.7	−16.4	+12.9		
93TS15	African	wild	African-2	−16.3	11.1	±0.4	±2.4		
93TS16	African	wild	African-3	−16.8	10.9				
93TS17	African	wild	African-4	−15.9	5.5				

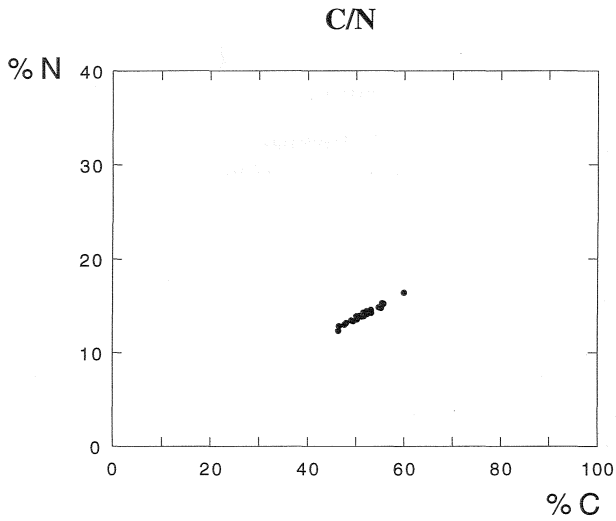

Fig. 2. C/N distributions for scute samples analysed

with an average of -16.9 ± 0.9‰. They had the most negative $\delta^{13}C$ values of samples from the Pacific Ocean region, indicating a relatively strong dependance on oceanic ecosystems. The $\delta^{15}N$ values for these samples were around +9‰ except for one sample at +4.6‰ giving an average of $+8.1\pm0.3$‰.

Samples from the Philippines: The $\delta^{13}C$ values for the 3 samples from Philippines were around −16‰ and the $\delta^{15}N$ values were between +8 and +10‰ which placed them between the values of the samples from the Solomons, with higher $\delta^{15}N$ values, and those from Fiji.

In general, samples from the Pacific Ocean region gave $\delta^{13}C$ values less than −14‰, suggesting a relatively high dependance on oceanic ecosystems, and $\delta^{15}N$ values higher than +10‰.

The Indian Ocean region (Fig. 5)

Samples from the Seychelles: $\delta^{13}C$ values for the 19 scute samples from the Seychelles had a wide distribution which formed three clusters: (1) a cluster characterized by less negative $\delta^{13}C$ values distributed between −8 and −12‰ and $\delta^{15}N$ values between +10 and +14‰, (2) a cluster characterized by higher $\delta^{15}N$ values distributed around −14‰ and $\delta^{15}N$ values between +13 and +15‰, and (3) a cluster characterized by almost the same distribution of $\delta^{13}C$ values around −14‰ but with lower $\delta^{15}N$ values from +8 to +11‰. The first cluster, with the less negative $\delta^{13}C$ values did not overlap with any other samples analyzed, and the third cluster, with the lower $\delta^{15}N$ values and around −14‰ in $\delta^{13}C$ values was overlapped with those from Haiti shown in Fig. 3. Averages and standard deviations for $\delta^{13}C$ values of the Seychelles samples were -13.0 ± 2.1‰, and for $\delta^{15}N$ values were $+12.9\pm2.4$‰.

Samples from Morjib: $\delta^{13}C$ values for the 8 scute samples from Morjib had a relatively wide distribution of $\delta^{13}C$ values from −12 to −17‰ and of $\delta^{15}N$ values from +6 to +11‰. These distributions seem to separate into two clusters based on $\delta^{13}C$ values: those with less negative $\delta^{13}C$ values of around −13‰ and those with more negative $\delta^{13}C$ values around −16‰. The former cluster had a similar distribution as the third cluster from the Seychelles, and the latter cluster overlapped with the samples from Africa. Averages and standard deviations for the Morjib were -14.9 ± 1.5‰ for $\delta^{13}C$ values, and

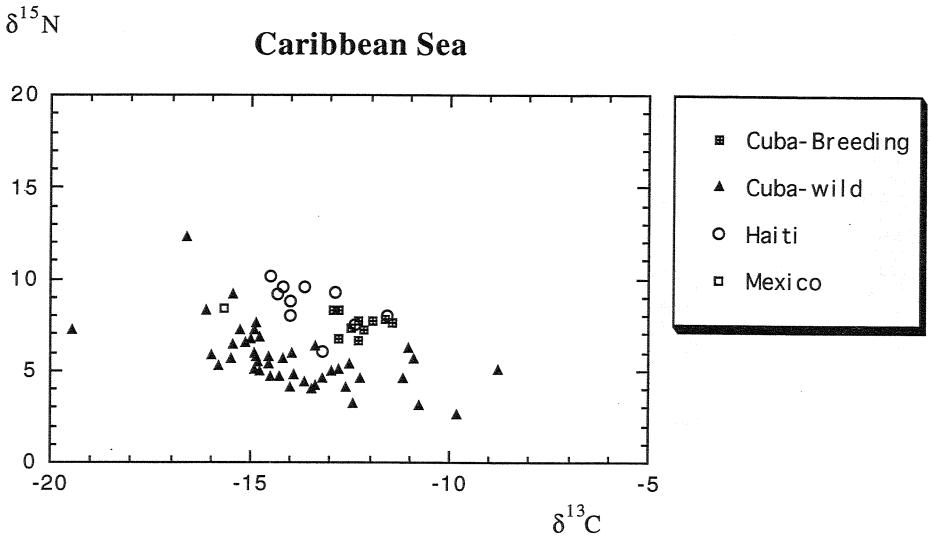

Fig. 3. $\delta^{15}N$ and $\delta^{13}C$ distributions for scute samples from the Caribbean Sea region.

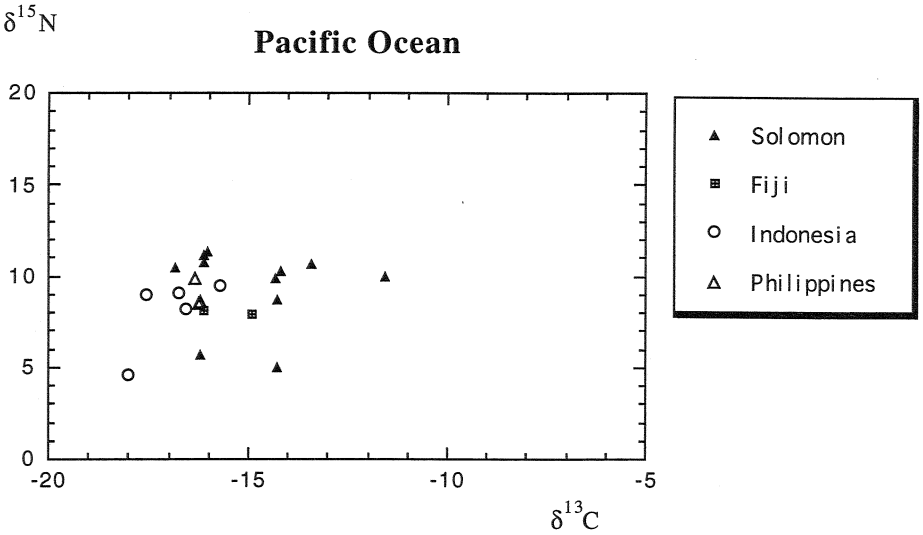

Fig. 4. $\delta^{15}N$ and $\delta^{13}C$ distributions for scute samples from the Pacific Ocean region.

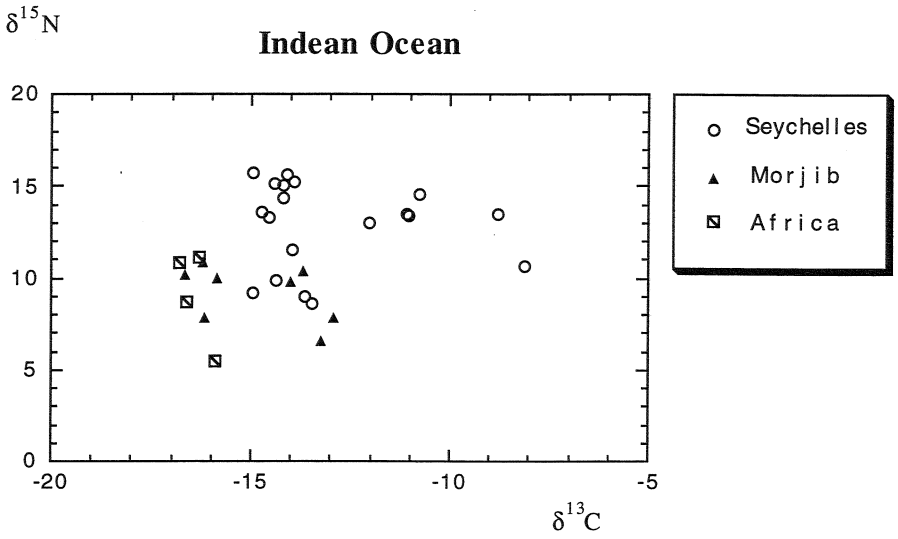

Fig. 5. $\delta^{15}N$ and $\delta^{13}C$ distributions for scute samples from the Indian Ocean region.

$+9.2\pm1.5$‰ for $\delta^{15}N$ values.

Samples from unknown African countries: $\delta^{13}C$ values for the 4 scute samples from Africa were distributed around -16‰ (-16.4 ± 0.4‰) and from $+6$ to $+11$‰ (-12.2 ± 2.8‰) for $\delta^{15}N$ values. These distributions had the most negative $\delta^{13}C$ values of samples from the Indian Ocean region, indicating a strong dependance on the oceanic ecosystems.

6. Discussion

$\delta^{13}C$ and $\delta^{15}N$ distribution for the 118 scute samples from the Caribbean Sea region, Pacific Ocean region, and the Indian Ocean region. are shown in Fig. 6.

Breeding individuals from Cuba had higher $\delta^{15}N$ values than wild individuals from Cuba, since sardines or other small fish were fed to the breeding individuals. A clear separation in the results indicates that isotope analysis will be useful for distinguishing the scutes of breeding populations, which will be targeted for export approval under CITES, from the wild populations specimens obtained by traditional fishing (Moncada et al., 1997).

The samples from the Caribbean Sea region are characterized by less negative $\delta^{13}C$ values (less negative than -16‰) than samples from the Pacific Ocean region. This suggests that the hawksbill turtles in the Caribbean Sea region must have a higher dependance on coral ecosystems than do those from the Pacific Ocean region.

Values more positive than -8‰ generally indicate an dietary intake of sponges or higher-trophic-level animals. The most positive values obtained from our samples were from the Seychelles in the Indian Ocean, suggesting a higher dependance on sponges or other animal species.

Risk *et al.* (1994) examined cross-continental shelf trends in $\delta^{13}C$ for coral from the Great Barrier Reef, Australia. The correlation between values for tissue and zooxanthellae in both species was highly significant and strongly linear. Although their previous data derived from POC (particulate organic carbon) in sediments have implied that the limit of the terrigenous influence was 10 to 20 km, both tissue and zooxanthellae of *Pontes lobata* and *Acropora formosa* gave $\delta^{13}C$ values which increase linearly with distance from the shore, increasing from -16 to -11‰, indicating that inshore corals derive much of their nutrients from terrigenous sources, and that a terrigenous influence on diet is measurable out to the edge of the continental shelf, ca 110 km offshore.

According to Yamamuro *et al.* (1992), tissues of coral reef specimens had $\delta^{13}C$ values between -15 and -11 ‰ which are clearly different from oceanic plankton values of -20 to -16‰.

Biological nitrogen fixation, whereby nitrogen is

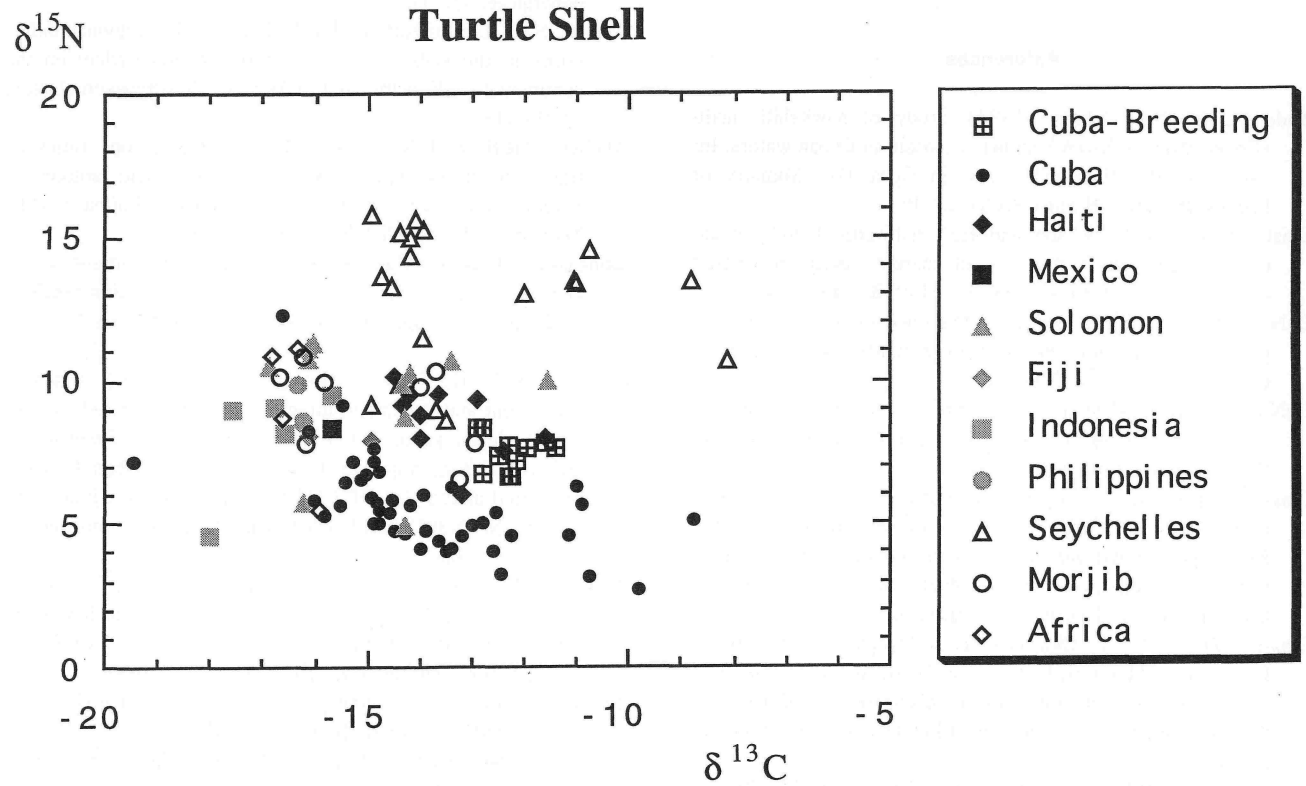

Fig. 6. General distribution of δ^{15} N and δ^{13} C values for scute samples analyzed.

enzymatically converted to NH_3 by microorganisms, results in plant tissues that are ^{15}N-depleted relative to tissues from non-nitrogen-fixing plants. In ecosystems where nitrogen fixers are important primary producers, consumers may be ^{15}N-depleted relative to animals that eat materials derived from plants that do not fix nitrogen. An example of this isotopic pattern was documented by Keegan and DeNiro (1988) in modern organisms inhabiting a coral-reef system in the Bahamas. They detected ^{15}N-depletion in the tissues of certain reef invertebrates and fish, relative to the typical values for marine fish. This ^{15}N-depletion presumably resulted from the fixation of molecular nitrogen by marine micro organisms found in association with corals and mangroves (Macko *et al.*, 1984).

Samples from the coral reef in Indonesia (Halim, personal communication) showed that tissues of the corals Goniopora sp. and Labophylia sp. had $\delta^{13}C$ values between -12.1 and -15.6‰ and $\delta^{15}N$ values between $+3.7$ and 4.5‰ (Halim, personal communication). Their secondary producers (the bivalve Malleus malluesand pearl oysters) had $\delta^{13}C$ values between -8.5 and -15.7 ‰ and $\delta^{15}N$ values between $+4.8$ and $+6.4$‰. Carnivore snails (*Strombus* sp.) had $\delta^{13}C$ values between -7.2 and -9.0‰ and $\delta^{15}N$ values between $+9.1$ and 10.0‰. These values seem to be normal $\delta^{5}N$ values rather than indicating the ^{15}N-depletion by coral fixation suggested by Keegan and DeNiro (1988).

References

Anderes, B. & I. Uchida (1994) Study of hawksbill turtle (*Eretmochelys imbricata*) stomach content in Cuban waters. In: "Study of the Hawksbill turtle in Cuba (I)" Ministry of Fishing Industry, Havana, Cuba: 27-40.

Chisholm, B.S., D.E. Nelson and H.P. Schwarez (1982) Stable isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science, 216:1131-1132.

DeNiro M.J. & S. Epstein (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta, 42:495-506.

DeNiro M.J. & S. Epstein (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta, 42:495-506.

Díaz-Fernández, R., T. Okayama, Y. Baba, *et al.* (1998) Genetic marker for the Cuban population of the Hawksbill turtle, *Eretmochelys imbricata*, and its contribution to the foraging population. Bulletin of the Graduate School of Social and Cultural Studies, Kyushu University, 4:119-135.

Díaz-Fernández, R., T. Okayama, T. Uchiyama, E. Carrillo, G. Espinosa, R. Marquez, C. Diez and H. Koike (1999) Ge-netic sourcing for the hawksbill turtle, *Eretmochelys imbricata*, in the Northern Caribbean Region. Chelonian Conservation and Biology, 3:296-300.

Ernst, C.H. & R.W. Barbour (1989) "Turtles of the World". Smithsonian Institution Press. Washington. 313 pp.

Fry, B., R.S. Scalan & P.L. Parker (1977) Stable carbon isotope evidence for two sources of organic matter in coastal sediments: seagrasses and plankton. Geochimica et Cosmochimica Acta, 41:1875-1877.

Fry, B., W. Jeng, R.S. Scalan, P.L. Parker & J. Baccus (1978a) $\delta^{13}C$ food web analysis of a Texas sand dune community. Geochimica et Cosmochimica Acta, 42:1299-1302.

Fry, B., A. Joern & P.L. Parker (1978b) Grasshopper food web analysis: use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. Ecology, 59:498-506.

Haines, E.B. (1976) Relation between the stable carbon isotope composition of fiddler crabs, plants, and soils in a salt marsh. Limnology and Oceanography, 21:880-883.

Haines, E.B. & C.L. Montague (1979) Food sources of estuarine invertebrates analysed using 13C/12C ratios. Ecology, 60:4856.

Handley, Y. L. L. & C. M. Scrimgeour (1997) Terrestrial plant ecology and 15N natural abundance: The present limits to interpretation for uncultivated systems with original data from a Scottish old held. In: Advances in Ecological Research. VOL. 27. (eds M. Begon & A. H. Fitter) Academic Press, New York: 133-212.

Ishibashi, H., T. Takeuchi, I. Whyte, & H. Koike (1999) $\delta^{13}C$/$\delta^{15}N$ measurements from the African elephant, *Loxodonta africana*, for ivory sourcing. Bulletin of the Graduate School of Social and Cultural Studies, Kyushu University, 5:1-8.

Keegan, W.F. & M.J. DeNiro (1988) Stable carbon and nitrogen-isotope ratios of bone collagen used to study coral-reef and terrestrial components of prehistoric Bahamian diet. American Antiquity, 53:320-336.

Koike, H. and B. Chisholm (1988) An Application of stable-carbon isotopic analysis to diet analysis in modern mammals. Journal of the College of Liberal Arts, Saitama University 24. Natural Science.

Koike, H. (1996) Origin-sourcing using turtle-shell (Bekkou) by isotope analysis. Annual Report for 1995s project. 1-36.

Linnaeus, C (1766) "Systema Naturae", 12th ed. Halae Magdeborgicae. 1:1-532.

Macko, S.A., L.Enterzoth & P.L.Parker (1984) Regional differences in the stable isotopes of nitrogen and carbon on the continental shelf of the Gulf of Mexico. Naturwissen-shaften, 72:243-249.

Michener, R.H. & D.M. Schell (1994) Stable isotope ratios as tracers in marine aquatic food webs. in "Stable Isotopes in Ecology and Environmental Science" (eds.K. Lajtha & R.H. Michener), Blackwell Publisher Ltd.: 138-157.

Minagawa, M. & E. Wada (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta, 48: 1135-1140.

Moncada, F.G., H. Koike, G. Espinosa, *et al.* (1997) Annex 8. Movement and population integrity. In: An annotated transfer of the Cuban population of Hawksbill Turtles (*Eretmochelys imbricata*) from Appendix I to Appendix II. Cuban Proposal presented at the 10 COP CITES meeting. Harare, June 1997.

Mrosovsky, N. (1997) IUCN's credibility critically endangered. Nature, 389:436.

Okayama, T., R. Díaz-Fernandez, Y. Baba, M. Halim, O. Abe, N. Azeno & H. Koike (1999) Genetic diversity of the haw-ksbill turtle in the Indo-Pacific and Caribbean Regions. Chelonian Concervation and Biology, 3:362-367.

Pritchard, P.C.H. (1979) Encyclopedia of turtles. T.F.H. Publications, INC., Neptune, New Jersey, 895pp.

Rau, G.H. (1981) Hydrothermal vent clam and tube worm: further evidence of nonphotosynthetic food sources. Science, 213:338-340.

Rau, G.H. & N.H.Anderson (1981) Use of 13C/12C to trace

dissolved and particulate organic matter utilization by populations of an aquatic invertebrate. Oecologia, 48:19-21.

Risk, M.J., P.W.Sammarco & H.P. Schwarcz (1994) Cross-continental shelf trends in $\delta^{13}C$ in coral on the Great Barrier reef.Mar. Ecol. Prog. Ser., 106:121-130.

Republic of Cuba (1997) An annotated transfer of the Cuban population of Hawksbill Turtles (*Eretmochelys imbricata*) from Appendix I to Appendix II. Cuban Proposal presented at The 10 COP CITES meeting. Harare, June 1997.

Schoeninger, M.J. & M.J. DeNiro (1984) Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica Acta, 48:625-639.

Tanabe, S. and H. Sakai (1997) Trace element analysis of turtoise-shell (Bekkou) using ICP-MS and AAS, Annual Report for 1996 Project: 1-9.

Teeri, J.A. & D.A. Schoeller (1979) $\delta^{13}C$ values of an herbivore and the ratio of C_3 to C_4 plant carbon in its diet. Oecologia, 39:197-200.

Yamamuro, M. , M. Minagawa & H. Kayane (1992) Preliminary Observation on food webs in Shiraho coral reef as determined from carbon and nitrogen stable isotopes. Proceedings of the 7th International Coral Reef Symposium, Vol. 1: 358-362.

Witzell, W. (1983) Synopsis of biological data on the Hawksbill turtle Eretmochelys imbricata (Linneus, 1766). FAO Fisheries Synopsis, 137:1-78.

(Received October 1, 1999; Accepted December 22, 1999)