Long-term Marking of Green Turtles with Ir

Takeshi Umezu,[*1] Mamoru Murai,[*2] Makoto Miki,[*3]
and Yôji Kurata[*2,4]

(Received December 28, 1990)

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By neutron activation analysis (NAA), the Ir content of scutes and bone was determined in hatchlings over a 1,550 day period, following feeding with 100–200 mg Ir per capita with a 2000 ppm diet for 14 days. In the specimen of No. 198 Ir ranged from 0.48 to 167 ppb in 46 bone fragments on day 1,110, in which the highest Ir occurred in the portion with the slowest growth (target), located in the middle of peripheral bone, under the scute junction, near the margin on the ventral side. After 30 years Ir remains at about a 0.7 ppb level, this determined by the regression line between Ir content and time, calculated from 90 results. To discriminate the Ir mark depends on the Ir content in bone of natural turtles (=0.01 ppb), and the detection limit is estimated to be 0.1 ppb. The Ir mark is therefore very useful up to 30 years or more, however, Eu is not useful in turtles.

The green turtle, *Chelonia mydas* making extenive migrations, occurs in the tropical and subtropical waters of the world. Like other marine turtles, this species is in danger of becoming extinct, due to the intensive hunting and polluting activities of man. Around the Bonin Islands, 1,850 turles were caught in 1880, following which the number had greatly fallen to 32 turtles in 1923.[1] Annual catches are now leveling off around 100–200 turtles. Since 1897, many turtles have been released there, marked with tags and/or notches on their shell so that their movements could be tracked. However, many tags and notches have been lost, possibly because of the growth from 10^2 g hatchlings to 10^5 g mature turtles. Little is known about the life history of green turtles, because they spend most of their life in the vast expanse of the open ocean. To know more about the life history, migration and the population of green turtles, we are in need of an advanced method—a long term, mass-producible and simple method of marking. Here, we tried to apply Ir and Eu as activable tracers for marking green turtles.

Materials and Methods

To select 15 turtles for mark-test and 15 controls, we collected natural hatchlings hatched on Aug 7, 1985 at the Kita-hatsune-ura beach of Chichijima of the Bonin Islands. For two weeks we adapted them to food and aquarium (100×120×100 cm depth) with 10 cm deep running seawater in the Tokyo Metropolitan Ogasawara Fisheries Center. During Aug 21–Sept 3, we fed Eu containing diet (2,000 ppm in wet basis) and during Sept 4–17, Ir diet (2,000 ppm). Crushed fish pellets and minced fish meat were mixed well with $EuCl_3 \cdot 6H_2O$ or $IrCl_4 \cdot H_2O$ separately. Mean initial carapace length in a straight line (SCL) was 7.6 cm (7.1–8.0) with body weight 79 g (67–90). Turtles ate almost all diet fed daily about 5% of their body weight, so that uptake of Eu or Ir per capita was estimated to be $1–2 \times 10^2$ mg during the 14 days feeding. Thereafter, they were transferred to larger aquariums with growth until the end of the experiment.

After the end of Ir feeding (sept 17, 1985), we killed three mark-test turtles and two controls on day 60 (Nov 15, 1985), day 400 (Oct 18, 1986), day 740 (Sept 29, 1987) and day 1,110 (Sept 26, 1988) to sample scutes and bone: central (C_2, C_3), lateral

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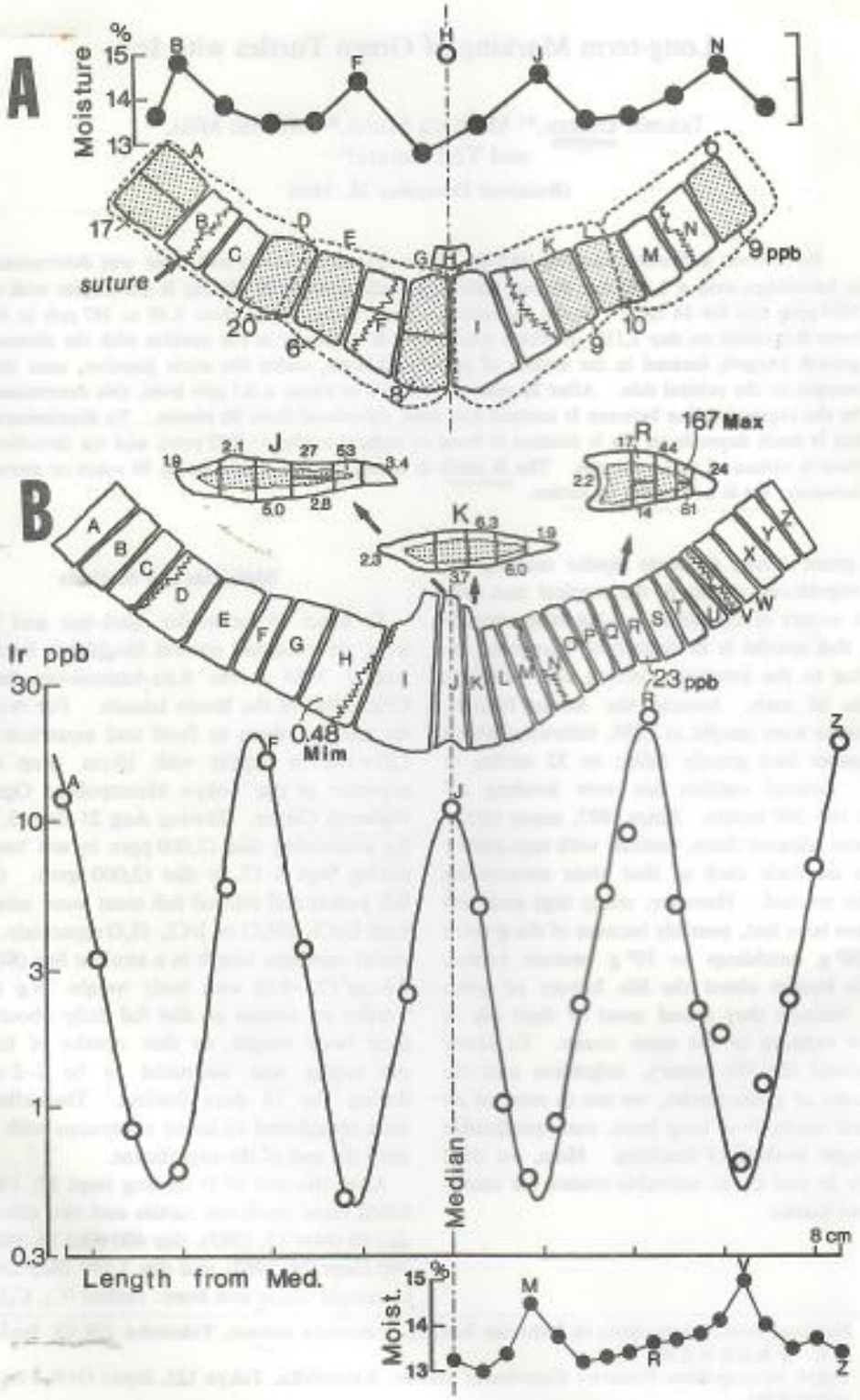

Fig. 1. Ir distribution and moisture in peripheral and pigal bone on day 1110. A) Dorsal view of No. 199 specimen: SCL 41.7 cm—9.3 kg. B) Ventral view of No. 198 44.4 cm—11.9 kg. Numbers show Ir content in dry bone (ppb).

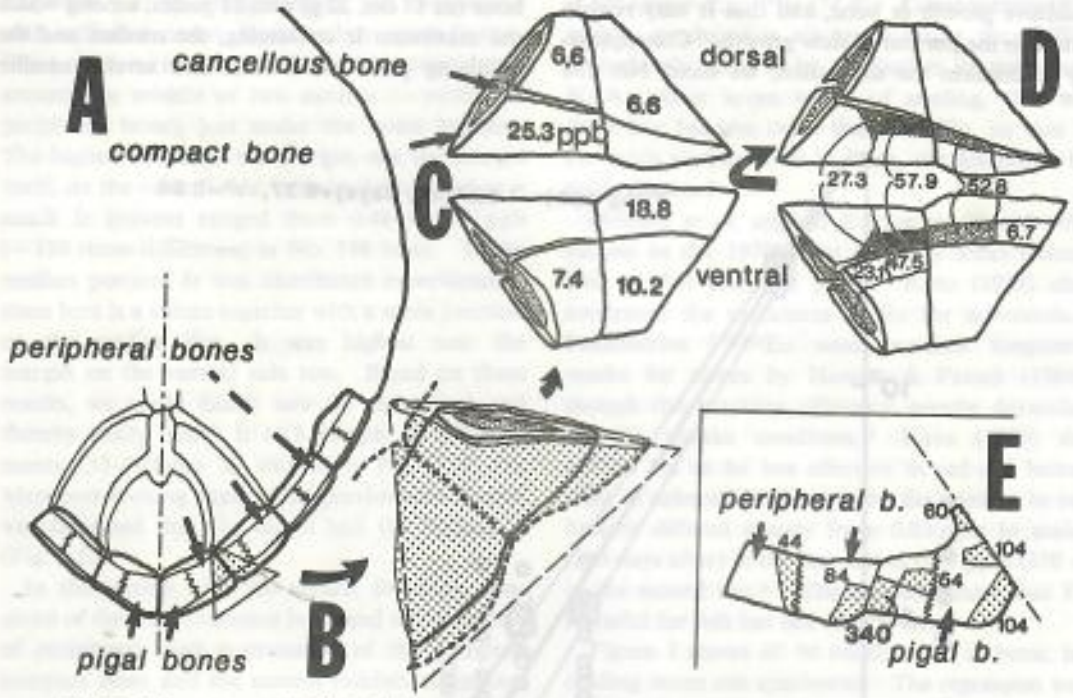

Fig. 2. Target sawed off (A, B) and Ir distribution in divided (C) and redivided (D) bone on day 1110 No. 216: 45.1 cm—11.6 kg, except E) Specimen on day 400 No. 212: 30.4 cm—4.12 kg.

(right L_2, left L_4) and the rearmost marginal+postcentral sucte (right, left) with peripheral+pigal bone (right, left). Finally on day 1,550 (Dec 13, 1989) we sawed off a triangular piece of bone (=6 g raw), which is referred to as the target hereafter, from two living turtles from the mark-test and one control. We dried scutes and bone at 100°C, ashed some of them at 450°C and sealed divided bone (60–1,500 mg) and ashed specimens (10–60 mg), which were irradiated at 10^{13} n, cm^{-2}, sec^{-1} for 24–48 hr by TRIGA MARK-II of St. Paul University. After several weeks of cooling, we determined the radioactivities of the specimens by plural Ge(Li) detectors with 4,000 channel PHAs for 6×10^{5}–10^{5} sec. Ir and Eu content were calculated from the 316 and 468 keV peaks of ^{192}Ir (half life 74 d) and the 122, 344 and 1,408 keV of ^{152}Eu (13.3 y) with filter paper on which the atomic absorption standard of Ir or Eu (Sigma) was pipetted.

In addition, we determined activities of minute fragments of bone (−20 mg), cancellous bone, compact bone and bone fractions treated with HCl (2.4%) and dilute KOH or NaOH (1–7%). In 1983–5 we preliminary tried Eu mark-test with 200 ppm diet. After one and two years, specimens of organs and tissues (100–200 mg) were irradiated at JJR-4 (3×10^{14} n) for 30 min and specimens of the scute's surface (60–90 mg) cut off from two years after were irradiated at TRIGA-MARK II for 15 hr.

Results

We detected about 3 ppm Ir (2.6, 3.5, 3.6) in bone and 0.25 ppm Ir (0.1–0.6, n=18) in scutes on day 60 specimens, followed by detection of 38–340 ppb Ir in all specimens of bone, except scutes on day 400 and 740 turtles. These results hinted to us that Ir may distribute heterogeneously in bone, so that day 1110's bone No. 199 (ca 15 cm, 13 g) was divided into 18 pieces and moisture measured (dry/air dry) and the Ir content counted with count times of 10^{5} sec. Four pieces have a suture which showed higher moisture (=15%) than the others (13–14%), and moreover seven of the 18 pieces had significant Ir ($N>3\sigma$: net counts larger than three times the standard deviation). Ir was lower around the suture and higher around the middle of the two suturae, just under the junction of scutes, which suggested that the portion of higer moisture may be the site of

years or more.

Here, we look at the results of red sea breams marked with Ir by Kato (1990).[1] Comparative were the variables: Ir content of diet, the amount of diet fed and feeding period, growth rates, specimen weight and integrated neutron flux in his test and ours. Count times differed greatly from 10^5 sec for sea breams to 6×10^5–10^6 sec for turtles, as a result the detection limit was higher in sea breams than in turtles by about 10^2 times. He also determined Ir content of liver and scales, and the long residence of Ir in liver is of interest. Comparing roughly above 0.1 ppm in two cases, we could regard the results as the same, though Ir content was a little higher in sea breams than in turtles around 200–400 days. Regardless of the difference between Class Reptilia and Class Osteichthyes, Ir turns over similarly, a fact which is interesting biochemically. On the other hand, Eu behaves dissimilarly between fish and turtles.

Looking at Eu and Ir in the marine environment, Eu (0.1 ng/kg)[8] exists about 10^5 times greater than Ir (1.3 pg/kg)[9] in seawater and in marine organisms Eu (≒10 ppb dry basis)[10] is 10^{2-3} times Ir (≒20 ppt)[7]. Nevertheless, Eu was not detected in bone, which may be derived from the difference in chemical form of Eu and Ir in diet and in natural food, as well as seawater, and from the different chemical behavior of Eu and Ir in turtle body.

Ir is an effective mark for turtles over 30 years or more, however, with Ir we can only mark one year or one site of release, and we need more useful elements as activable tracers for turtles.

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