

Age determination of long-lived reptiles : some techniques for seaturtles (*)

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Détermination de l'âge des reptiles de grande longévité : techniques appliquées aux tortues de mer.

INTRODUCTION

The time between a seaturtle's entry into the sea and its return to a nesting beach as an adult, the « Lost Year », has been variously estimated, often speculatively so, with very little data. Age estimates have been based frequently on the rapid growth rate of hatchling turtles raised in captivity, commonly yielding time estimates from hatching to sexual maturity of less than ten years (e.g., MARQUEZ, 1972 ; WITZELL, 1980). Recently, recapture data for juvenile seaturtles have shown that growth rates are geographically variable and very slow in some populations (e.g., *Chelonia mydas* in the Hawaiian chain ; BALAZS, 1982).

Because there are only a few seaturtle research programs that are long term and that are sampling juveniles regularly, estimates of growth from recapture data are sparse. Skeletochronology offers a methodology for estimating ages and calculating growth rates without the investment of time necessary for a mark-recapture study. My research in skeletochronology has emphasized seaturtles, the principal goal being the determination of the average age of sexual maturity for different populations and species. This research has encountered problems and solutions that are common to all skeletochronological research and ones that are specific to seaturtles. In the following discussion, I will examine a few of these problems and solutions.

PROBLEMS AND SOLUTIONS

Seaturtles grow from 35-50 mm sCL (straight-line carapace length) at hatching to 650-950 mm at sexual maturity. This 18-20X size differential from hatchling to adult suggests major structural reorganization within the skeleton, and indeed, resorption-remodeling is evident in all bones. In many bony elements, only the current periosteal layer is unchanged. The humerus and femur retain numerous periosteal layers in the middle of the diaphysis. I have used the humerus exclusively, taking cross-sections from the diaphysis immediately distal to the deltopectoral crest ; this area is the narrowest region of the humerus and contains the greatest number of unmodified periosteal layers (see *fig. 3* in ZUG *et al.*, 1986). Although the humerus is least remodeled in this area, its core has undergone successive deposition-resorptions, and the earlier periosteal layers have been destroyed. In my initial studies of seaturtle skeletochronology (ZUG and BALAZS, 1985 ; ZUG *et al.*, 1986), I measured the thickness (width) of each periosteal layer (= MSG, mark of skeletal growth), calculated the average MSG width, and divided the radius of the bone by the average MSG width. The resulting value was an estimate of the total number of MSGs, and if one MSG equaled one year, the total number equaled the age of the turtle. Comparison of growth curves from the MSG age estimates and recapture age data

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showed that they had nearly identical shapes and similar positions. In spite of the similarities, the average width data is likely to overestimate the actual age, particularly so in older turtles. The earliest MSGs are wider owing to faster growth in young turtles, but the average width derives from the later slower growth phase and its narrower layers. Hence dividing the bone's radius by the average width yields more MSGs than actually existed.

I have been searching for a technique that would yield more accurate and objective estimates of age. In an ongoing study of *Lepidochelys kempii*, we have modified HEMELAAR's (1985) analytical technique for the calculation of resorbed periosteal growth layers and have developed a protocol that provides an explicit and repeatable method for obtaining skeletochronological age estimates, also presumably more accurate than the average MSG-width technique.

Whereas Hemelaar's technique uses a graphical and statistical approach for predicting the number of resorbed MSGs and the animal's age, the sea-turtle protocol relies on a set of ranking statements and a tabular organization of the data. The data set is identical for Hemelaar's analytical method and the ranking protocol: the diameters of each periosteal growth ring, diameter of the resorption core (= marrow), and diameter of the entire bone's cross-section. The steps of the protocol evaluate these diameters and make assignments to growth-cycle classes in the following manner:

- 1) Rank the bone sections in order of increasing resorption core diameters.
- 2) Starting with the section with the smallest core, assign the innermost (smallest) periosteal diameter to the lowest growth class possessing an appropriate range of MSGs' diameters.
- 3) The succeeding (outer) MSGs' diameters are placed in successive classes. No classes are skipped. (Table 1)
- 4) The class containing the outermost diameter of the bone is the estimate of the number of growth cycles.
- 5) Each growth cycle presumably equals one year.

Ideally, a full set of growth stages are represented in the study sample. Such a set allows the investigator to establish the range of diameters for the earliest growth classes. The initial *Lepidochelys kempii* sample (ZUG and KALB, 1989) lacked individuals from the 5-22 cm sCL and individuals > 45 cm sCL. The latter range does not affect assignments by the ranking protocol, but does affect the calculation of growth curves (discussed in a subsequent section). The unavailability of smaller individuals did not allow an unequivocal identification of first year MSGs in the specimens, so a hypothetical range of first year MSG diameters (3.0-7.6 mm) was propo-

sed on the basis of hatchling bone diameters and the rapid growth rate of captive Ridelys. All individuals with innermost MSG diameters within this range are assigned to class 1 and, if the innermost diameter is slightly larger, to class 2. These assignments of the innermost diameter fix the assignments of the more external diameters to successive classes, thereby producing ranges of diameters for the successively older classes. Subsequent assignment of bone samples depends upon these diameter ranges derived from smaller and less remodeled bone samples. This stepwise assignment of bones with increasingly larger resorption cores and innermost MSG diameters maintains a rigorous objectivity by preventing the investigator from making arbitrary class assignments. This ranking protocol is in principle Hemelaar's analytical technique without its statistical rigor.

Independent data sets and statistical analysis

My experience in skeletochronology has involved iguanas and sea turtles, all of which have shown extensive resorption and other structural features causing difficulties in recording the skeletochronological data. Because of this, I have repeatedly emphasized that my data are estimates of actual ages and that the entire sample of age estimates is the unit for drawing cautious interpretations on the demographics of the species or population studied. I wish to encourage those working with problem skeletochronological samples to practice independent data recording and statistical testing. For example, in our study of *Iguana iguana* (ZUG and RAND, 1987), the two sets of age data recorded by two observers independently from the same histological sections were significantly different (Wilcoxon matched-pairs signed-rank test); two data sets recorded by the same observer at an interval of 6-9 months were not significantly different. A similar strategy was used in the initial phases of the *Lepidochelys* study (ZUG and KALB, 1989); however, in this case, the two observers based their age estimates on different sections and different measurement axes from the same bone. In spite of the difference in data capture, the two data sets were not significantly different.

Timing the periosteal growth cycle

The basic tenet of skeletochronology is the cyclic nature of bone growth with each cycle representing an equal time interval. The cycle is usually assumed to be annual. Although an annual cycle has been demonstrated for a number of amphibians and reptiles, it has remained an unconfirmed assumption in sea turtles. A recent Virginia Institute of Marine Science study (KLINGER, 1988) of *Caretta caretta* in the Chesapeake Bay and the adjacent western Atlantic Ocean has demonstrated an annual growth

TABLE I. - A sample of the long axis diameter data from juvenile *Lepidochelys kempii*, arranged by the ranking protocol. Specimens C, E, I and O are assigned to class 1 because the arbitrary range of the first year's MSG diameter is 3.0-7.6 mm; S, T and U are assigned to class 3 because resorption may have eliminated the second year's MSG.

TABLEAU I. - Exemple de données à partir d'un juvénile de *Lepidochelys kempii*, présentées selon le « ranking protocol » (voir texte). Les spécimens C, E, I and O sont rangés dans la classe 1 compte tenu de la taille de leur marque de croissance de première année qui va de 3 à 7, 6 mm. S, T et U sont rangés dans la classe 3 car la résorption peut avoir éliminé la marque de deuxième année.

Specimen	Bone Resorption Core	Diameters (mm)							Age (yr)
		1	2	Periosteal Layers or Rings					
				3	4	5	6	7	
A	00.8	hatchling							0
B	03.5	.	10.9	2
C	03.6	7.1	08.1	10.6	3
D	03.9	.	12.3	12.7	3
E	03.9	6.6	11.7	2
F	04.1	.	09.6	2
G	04.4	.	08.7	09.5	09.9	10.6	.	.	5
H	04.5	.	08.2	09.7	13.7	.	.	.	4
I	04.5	5.2	08.3	2
J	05.0	.	11.4	13.8	3
K	05.2	.	08.5	10.1	12.6	.	.	.	4
L	05.4	.	11.6	2
M	06.1	.	08.2	08.3	08.7	09.0	.	.	5
N	06.2	.	08.4	10.7	3
O	06.2	7.6	08.8	09.1	10.1	11.0	.	.	5
P	06.7	.	12.8	15.1	3
Q	07.7	.	11.3	12.6	3
R	08.0	.	08.4	09.7	09.9	12.3	.	.	5
S	08.5	.	.	11.2	17.0	.	.	.	4
T	09.3	.	.	10.6	14.9	18.3	.	.	5
U	10.9	.	.	12.7	14.1	16.9	19.7	.	6
V	12.5	.	12.9	14.2	15.4	15.9	16.1	16.3	7

cycle in juveniles (> 40 cm sCL). Turtles accidentally captured in fishermen's nets were injected with tetracycline and released. Nine of these turtles were recaptured in subsequent years, and bone biopsies were taken from the right humerus of each prior to re-release. Three of these turtles lacked tetracycline layers in the biopsies; one turtle recaptured one year after the injection had no separation between the tetracycline layer and the periosteum; and in the remaining five turtles, the number of periosteal growth layers above the tetracycline layer equaled the number of years since the injection. These latter data confirm an annual periosteal growth cycle in seaturtles, specifically in juvenile *Caretta caretta*. Nonetheless, questions remain unanswered. For example, do younger *Caretta* (< 40 cm sCL) have an annual growth cycle, particularly if they are residents of the Gulf Stream gyre? Do species or individuals

living in or migrating to tropical seas have cyclic growth patterns?

Estimating a population's average age of maturity

Once age estimates are available, they are used with the associated size data to calculate growth curve equations for the sample. Most statistical software packages have programs for examining the curvilinear relationships of a bivariate data set and for estimating a best-fit equation. Two growth equations (logistic and von Bertalanffy) have been used regularly (e.g., FRAZER and SCHWARTZ, 1984) in the analysis of seaturtle growth. The advantage of using a growth equation rather than the actual age estimates is that the equation derives from the entire data sample, whereas an investigator is likely to overemphasize a portion of the sample, the portion best

matching his biases. Outliers in the data set may have a disproportionate influence on the derived equation, so equations should be calculated with and without the outliers for comparison.

Two other points are noteworthy. Average hatching size at day one (do not use $t_0=0$) should be included as part of the age data set for the calculation of the growth equations. It is a known value and serves to establish a realistic origin for the growth curve. Emphasizing a comment in an earlier section, skeletochronological samples should include representatives from the full growth series if the equation/curve is to reflect accurately the growth pattern and the sizes at hatching and maturity.

Sclerotic ossicles : an unusual bone for skeletochronology

In limbed reptiles, skeletochronology has relied on the presence of periosteal layering in elements of the appendicular skeleton. The limbs of *Dermochelys coriacea* show a mammalian-like growth pattern, hence there is no periosteal layering. A preliminary study of *Dermochelys* sclerotic ossicles shows distinct layering and lines of arrested growth. These growth layers may provide data for ageing these unusual searturtles. Currently, our analysis is impeded by the absence of ossicles from juvenile Leatherbacks, hence we have no reliable means to estimate the periosteal diameters of earlier growth stages.

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