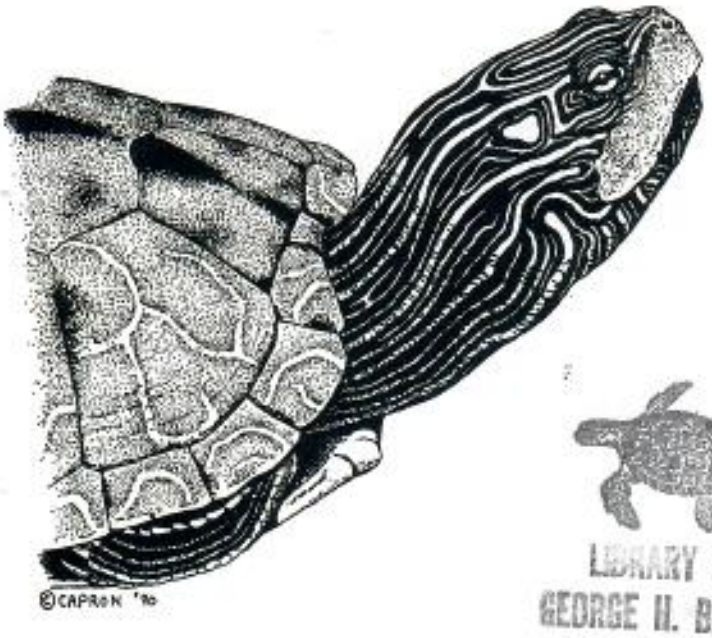
AGE DETERMINATION IN TURTLES

GEORGE R. ZUG



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Cover drawing of a female Common Map Turtle *(Graptemys geographica)* by Martin Capron, Oxford, Kansas.

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INTRODUCTION

Much of the biology of turtles, for that matter of all animals, is age dependent or time correlated. All inquiries into turtle growth and development, reproductive biology, and population biology require some knowledge or estimation of turtle age. Yet, age determination of research specimens is difficult and often highly subjective.

Mark-release-recapture is the only method for accurately determining the actual (absolute or true) age of individuals, but requires the marking of hatchlings and a long term commitment to the study of a marked population. The time honored aging method for turtles is counting scute annuli. This method is a handy field technique for age estimation, but it has uncertainties. Obviously, no age determination technique is free of difficulties in its application and interpretation. However, the importance of knowing turtle ages for understanding their biology outweighs the difficulties and dictates that we place more emphasis on age determination. The goal of the present review is to examine the age determination techniques currently used in turtle research and those used for other vertebrates that might be used with turtles, and to present each technique so that the reader can perform the technique and interpret the results without reference to additional publications. Each technique is presented in the following standardized format: 1) principle upon which the technique is based; 2) the methodology, supplies and equipment necessary for collecting the data and the method for interpreting or analyzing the data; 3) an evaluation of assumptions, an assessment of advantages and shortcomings of the technique, and a general discussion of the technique relative to earlier studies.

This literature review is not intended to be all inclusive. The emphasis has been to identify the publications that will allow the reader to confirm the information presented here and to delve further into the subject. In addition, the bibliography lists a few articles not cited in the text in order to provide better access to the literature on age determination.

KNOWN-AGED SAMPLES

The ultimate method for age determination is to follow individual turtles from hatching to death. The data derived from this method are actual ages and potentially accurate to a week or even a day, if such fine resolution is required. It is these data upon which the calibration and verification of the age estimates derived from other techniques depend.

Hatching is the logical point in time to set as a turtle's "birth" date (i.e., age 0); however, the actual date of hatching is usually unknown for wild-caught turtles, because the eggs are buried and hatching is not observed. As a

substitute, emergence from the nest may serve best as age 0. Upon emergence, hatchlings are both visible and free-living; they have departed their site of embryonic development and are beginning their first period of postembyronic growth. The use of emergence as age 0 establishes an uniform standard for all turtles whether they have prompt or delayed emergence and avoids the semantic argument of what is hatching (i.e., Pipping the egg shell, exiting the egg shell, or exiting the nest?).

The preceding comments on age 0 highlights another aspect of aging: how old a turtle is means different things to different researchers. For example, age can be presented as the number of activity seasons, the number of growth cycles, or the number of winters (temperate zone) or dry seasons (tropics) survived. These ages may or may not relate directly to a period of time (mo, yr) from emergence/hatching. Thus, users of age data must determine each researcher's counting technique, and authors must be explicit in describing their method of counting and establishing age 0.

Mark-Release-Recapture

Principle. — Actual age can be determined only by following an animal from its hatching/birth to the time of age determination for living turtles or death for dead ones.

Practice. — Age data are gathered as one aspect of a long-term population study. Generally such a study begins with an emphasis on the capture and marking of juveniles and adults which are then released. These individuals can be aged by one or more of the indirect methods presented later. With good planning and luck, nests will be located in the first field season. The nest sites should be marked and, several weeks prior to hatching and emergence of the young turtles, enclosed within a cage so that the hatchlings can be marked before they disperse. Marking and coding methods are described by Ferner (1979) and Swingland (1978). Each hatchling may be uniquely marked, or all members of a clutch or a year class may share the same mark. Upon recapture, these marked turtles can be aged accurately since their emergence dates are known.

Evaluation. — Ages and associated life history and meristic data are dependent upon repeated recaptures of the marked individuals. Only the individuals marked as hatchlings will provide actual ages or precise minimum ages for those species that delay emergence after hatching. Individuals marked as juveniles or older animals will have estimated ages, the reliability of which will depend upon the accuracy of the indirect method used to determine the ages at first capture.

Since the likelihood of each hatchling surviving to adulthood is slight, the investigator must mark and release large numbers of hatchlings to obtain a

small sample of known-aged animals in subsequent years. Nonetheless, the importance of these known-aged animals for calibrating the age estimates of the remainder of the marked population repays the marking of emerging hatchlings whenever possible. Marking may, however, modify survivorship and growth rate.

Examples. — *Chrysemys picta* (Tinkle et al., 1981), *Clemmys guttata* (Ernst, 1976), *Emydoidea blandingii* (Congdon et al., 1983), *Geochelone gigantea* (Swingland and Lessells, 1979), *Kinosternon subrubrum* (Gibbons, 1983), *Terrapene carolina* (Schwartz and Schwartz, 1974).

Captive Rearing

Principle. — Actual age can only be determined by following an animal from its hatching/birth to the time of its age determination or death.

Practice. — Eggs are gathered at the time of egg laying and incubated artificially or hatchlings are collected as they emerge from the nests. These captive animals may be maintained in completely artificial conditions or in semi-natural enclosures. For biologically useful results, samples of ten or more animals are raised under these conditions, and each animal must be uniquely marked. These marks eliminate the possibility of confusing the animals of different year classes as the size differences of the cohorts disappear in adulthood.

If facilities permit, the captive environment should match the natural environment to produce growth and aging phenomena as close as possible to those of free-living (wild) animals. The most obvious features to regulate are light and temperature regimes; food intake in natural quantities and quality is usually unknown and often the amount of food provided captive animals is excessive for the reduced energy requirements of captive animals. Semi-natural enclosures will match the climatic regime if geographically close to the site of capture, but a concerted effort is still required to reproduce a natural feeding regime.

Evaluation. — Captive rearing easily yields known-aged animals. The major problem is whether or not the size and life history data are valid in the sense that captive animals reach sexual maturity in the same length of time as wild animals. Captive turtles commonly show evidence of either stunted or accelerated growth. Stunted growth is common in pet turtles that have experienced one or more of the following: irregular feeding or inadequate amounts of food, poor quality food (e.g., dried insects), suboptimal temperature regime, crowded or small captive containers, injury, bacterial or fungal infections. At the other extreme, captives may show accelerated growth due to overfeeding, higher quality food, higher than natural temperatures, no seasonal change in food resources or in climatic regime. For example,

Hildebrand (1932) observed in captive raised *Malaclemys terrapin* that those young fed through their first winter gained a full year's growth and matured one year earlier than young terrapins allowed to hibernate in response to the natural climatic regime. The quantity of food given captive *Caretta caretta* determines growth rate; juveniles receiving the maximal daily amount of food showed the maximum growth rate (Nuitja and Uchida, 1982). Similarly diet quantity and quality are evident in the growth rates and ages of sexual maturity in wild turtle populations with access to different resource levels, e.g., *Chrysemys picta* (Gibbons, 1967), *Geochelone gigantea* (Bourn and Coe, 1978), *Kinosternon sonoriense* (Hulse, 1976), *Trachemys scripta* (Parmenter, 1980). Because of these variables, the actual ages of captive animals should be used cautiously in interpreting age-related characteristics of free-living turtles.

Examples. — *Batagur baska* (Moll, 1980); *Emydura krefftii* (Banks, 1987), *Gopherus agassizii* (Patterson and Brattstrom, 1972); *Lepidochelys kempii* (Márquez, 1972), *Malaclemys terrapin* (Hildebrand, 1929).

MEASURES OF SIZE AS SUBSTITUTES FOR AGE

Hatchling and new-born reptiles grow and become larger with increasing age, at least until or shortly after sexual maturity. This direct association of increasing size with age may allow the substitution of a body measurement for an animal's age in those situations where there is no other means or opportunity for obtaining an estimate of actual age. These measurements are estimates of relative age and typically are partitioned into classes from small to large paralleling age cohorts of young to old. As age estimates, size classes are unsuitable for demographic studies of a population, because each cohort possesses animals of different sizes owing to differences in size at hatching/birth and different growth rates, thus each size class likely contains representatives from two or more age cohorts. Nonetheless, size classes can be used to examine age-related phenomena of individuals and populations, although the results must be considered tentative and interpreted cautiously. If size and age data are closely linked, a growth table or formula can be used to estimate the ages of all the actively growing individuals.

Body Length or Mass

Principle. — As a turtle grows, its length and mass increase in a manner directly proportional to its age.

Practice. — When captured, turtles are weighed and measured for carapace or plastron length, often other dimensions as well. These data can be divided into size classes by several different methods, although the emphasis

should be in the recognition of biologically meaningful groups, e.g., hatchlings, sexually mature males, etc. Recognition of such groups establishes minimum and maximum values for delimiting some of the size classes. By examining the size frequency distribution of a monthly sample (or a sample from some other discrete and narrow time interval), a multimodal clustering often appears; each cluster may represent a single age cohort (no examples for turtles but for snakes, see Fitch [1960:table 21], Voris and Jayne [1979:figs. 1,2]). The size ranges of the cohort provide a means for delineating the dimensions of the size classes. If no clusters are apparent in the size frequency graphs, the investigator must arbitrarily define the dimensions of the size classes. The classes should comprise equal size ranges (except for the lower- and uppermost classes, i.e., < and >) and be mutually exclusive (e.g., an individual is assigned to one class and only one class; see Fig. 1). Rarely there may be evidence to suggest that classes have unequal ranges owing to strikingly faster growth rates of the youngest juveniles and the subadults. The

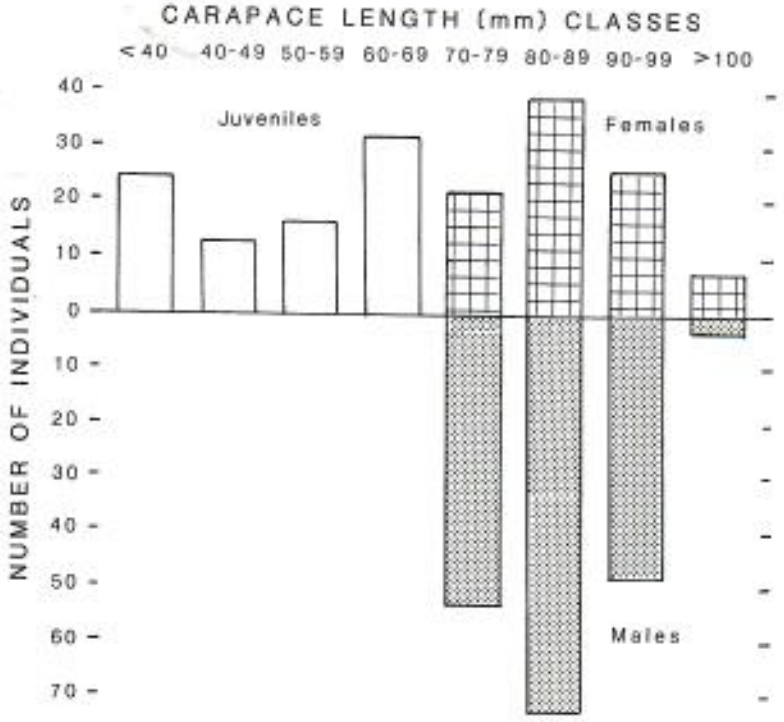

Figure 1. Size frequency distribution of *Sternotherus depressus* in Sipsey Fork, Black Warrior River basin, Alabama. Data from Dodd et al. (1986).

broadest size-range class would presumably be assigned to the youngest animals, i.e., those growing the fastest, since younger turtles would traverse a broader size range faster than older, slower growing turtles.

Evaluation. — As relative age estimates, length or mass classes are most appropriately used to examine "age" aspects of populations that can be sampled only over a brief time period and only once. Further, the preference would be to use carapace or plastron length rather than body mass owing to the variability of the latter caused by recency of food intake, bladder water, and similar factors. Skeletal length measurements, such as carapace length, are also variable but are not subject to daily variations found with mass measurements.

The relationship between age and size are directly associated, i.e., growing animals become larger through time, but the linkage between the two is highly variable. The variability derives from numerous sources. First as noted in the preceding section, growth rate is greatly influenced by quality and quantity of food, so that individuals in different populations and even in the same population will possess different growth rates. Turtles can slow or stop growth depending upon the availability of food or as an adjustment to adverse weather conditions (Pough [1980] addresses the adaptability of adjustable growth rates). Hatchling size is affected by the hydric environment of the incubating eggs and the degree of dehydration (Packard and Packard, 1986); also, larger hatchlings may grow faster and reach larger adult sizes than smaller hatchlings. Health and genetic factors affect growth rates, for individuals from the same clutch have different growth rates when grown under identical conditions (Bourke et al., 1977). In those species where multiple clutches are produced annually, a year class may have two or more size classes of animals (Gibbons, 1976) and the late hatchlings of one year may be more similar in size to the early hatchlings of the subsequent year than to their early hatching yearmates.

All the preceding factors and others confound size class data (also see Halliday and Verrell, 1988, for discussion on the relation of age and size). Nonetheless, circumstances may require the use of such data. Turtles of several families (e.g., Cheloniidae, Trionychidae) do not produce visible external markers for estimating age, yet it is valuable to obtain estimates of population age structure (e.g., Frazier, 1984, *Lepidochelys*, and Plummer, 1977a, *Trionyx*). Such size class data must be used cautiously in interpreting a population's demographic features, although a cautious interpretation based on data is better than no answer or a guess without supporting data. Furthermore, males and females should be analyzed separately owing to likelihood of differential growth rates between the sexes, as well as differential growth producing different shapes (proportions) in adult females and males.

Carapace or plastron lengths from marked-recaptured turtles can be used

to calculate a growth curve, and in turn, the growth curve can be used to estimate the ages for turtles of different sizes. This manner of age estimation has been used predominantly in the study of seaturtles (e.g., *Caretta caretta*, Frazer and Ehrhart, 1985; *Chelonia mydas*, Balazs, 1982, Frazer and Ladner, 1986) for estimating the age of sexual maturity for different populations; growth curves have also been used to estimate age for other turtles (e.g., *Geochelone gigantea*, Bourn and Coe, 1978; *Trionyx muticus*, Plummer, 1977b). The utility of this technique rests upon the recapture of various sized turtles over several years in moderate to large numbers.

Examples. — *Geochelone elephantopus* (MacFarland et al., 1974), *Gopherus agassizii* (Turner et al., 1987), *Graptemys pulchra* (Shealy, 1976), *Malaclemys terrapin* (Hurd et al., 1979), *Trachemys scripta* (Gibbons et al., 1980), *Trionyx muticus* (Plummer, 1977a).

Lens Mass

Principle. — The eye lens grows continually, but not continuously, through an animal's life. Owing to its unique location, the lens experiences little wear and steadily gains mass and increases in diameter.

Procedure. — Lens mass as an estimator of age is little tested in reptiles (Kheruvimov et al, 1977; Frazier et al., 1982; see discussion in the following evaluation section). The following procedures derive largely from Friend (1968) as modified by Morris (1972) for mammals. Lens mass can be obtained as fresh wet mass, preserved wet mass, or preserved dry mass; in all cases, a balance capable of weighing to 0.001 g, preferably to 0.01 mg, is needed. Preserved dry mass would seem to introduce the least measuring error in the data.

The eye should be removed immediately upon the death of the turtle and fixed in neutral (buffered) 4% formalin. Each eye must be removed carefully to avoid damage to the lens. The eye should be slit open in the rear to permit the direct entry of formalin and rapid preservation; the volume of formalin should be no less that 5 times the volume of the eye. Experience with other reptilian tissues indicates that the lens will be well fixed in 2–3 days. The lenses are then removed, air-dried, and stored temporarily in open vials (but kept dust free) until ready to be weighed.

The preserved lens is carefully removed from the eye, and any adherent tissue stripped gently from the lens. The lens may be air or oven dried. Measurement error is reduced by oven drying (60–80°C) to a constant mass, preferably in large lots for standardization of treatment. The lens is removed from the oven, cooled in a desiccator (anhydrous calcium chloride is recommended) and weighed immediately in order to prevent the hygroscopic lens from absorbing water and gaining mass. Since lenses are very light (e.g.,

means of 1.80–5.15 mg per lens for strains of laboratory mice), small differences in mass will confound the accuracy of age estimates, hence this procedure calls for gentle, careful, and speedy handling.

Lens masses are relative age estimates and may be analyzed in the same manner as body size measurements. However, the goal in mammalian studies has been to develop a lens mass-age curve. From this curve, an animal's age can be estimated once its lens mass is determined.

Evaluation. — Eye lens mass provides a relative age estimate unless lens masses can be linked directly with the known-aged animals; however, compared to other morphological measurements, lens mass appears to correlate more directly with age. Unlike body mass, lens mass is less affected by seasonal or daily changes in diet, reproductive and physiological state. As an ectodermal structure, the lens continues to grow throughout life and presumably in a more uniform manner than the spurt-like patterns of body mass and length measurements.

The lens shows a number of molecular changes associated with aging. These changes include the ratio of alpha and delta crystallins, racemization of aspartic acid, and several other molecular changes (summarized in Frazier, 1982). Although these molecular changes may prove useful, it would seem premature to rush into complex biochemical analyses prior to testing the reliability of increasing lens mass as an age determination technique.

Morris (1972) recommended the removal of both lens to ensure a high reliability of results. He and others working with mammalian lenses found that left and right lenses freshly removed from an individual will differ in mass by only about 1%. Differences significantly higher than 1% result commonly from damage through careless handling or tissue deterioration by decay. Large differences between left and right lens obviously require the removal of that specimen from the aging sample; however, the upper limit of acceptance must be selected by the investigator for each species studied. If only one lens per specimen is used, each lens must be carefully examined to ensure the lens is undamaged (e.g., no nicks or gouges) and/or decayed (e.g., fresh lens are clear, smooth, and nearly spherical when dried in contrast to discoloring, pockmarking, and shape distortion of decayed lens).

Because lenses are light, small differences in mass result in disproportionate differences in the estimation of age. Procedures should be standardized wherever possible. For example, the entire sample should be oven-dried, cooled, and weighed at the same time under identical conditions to minimize variation due to handling. Friend (1968) observed that unequal fixation time resulted in mass difference and also that alcohol was a poor fixative because it extracted lipids, thereby reducing lens mass. Freezing lenses prior to preservation and/or weighing is not recommended; freezing produces highly variable results.

The value of lens mass for age estimation remains to be demonstrated in turtles. Only a single study (unpublished) has examined lens masses in turtles (*Chelonia mydas*; Frazier et al., 1982). These investigators examined the correlation of lens masses (fresh wet) with age in known-aged, captive turtles (3, 15, 39 and 128 mo old turtles). Left and right lens masses (n = 36) commonly differed by less than 4%, and the heavier lens was equally likely to be the left as the right. The average mass of each pair was plotted against age and showed a high correlation ($r>0.98$). Further, turtles of the same age but of greatly different body masses had highly similar lens masses. These results encourage a further testing in smaller species and wild turtles.

Examples. — There are no published studies for turtles, but see Schroeder and Baskett (1968) for the frog *Rana catesbeiana*.

INCREMENTAL GROWTH MARKERS FOR AGE ESTIMATION

Scute Growth Zones

Principle. — Epidermal scutes provide an externally visible record of the growth sequences of individuals. Scute growth responds to seasonal changes of the environment, and a new scute is produced during each major growth season. This seasonal periodicity can be used as a timing mechanism to estimate age.

Practice. — Turtles display two patterns of epidermal scute growth with the exception of the leathery, uncornified epidermal shell covering of trionychids, carettochelyids and dermochelyids. The actual production of a new scute does not appear to differ between the two growth patterns, but the final phase, loss or retention of the preceding year's scute, does differ and results in different counting techniques and level of reliability.

A brief description of scute growth is necessary to permit a researcher to distinguish between cyclic (annual in areas of predictable seasonal climatic changes: hot-cold, wet-dry) and acyclic (unpredictable and brief pauses due to drought, injury, etc.) growth. The following description is derived largely from Legler (1960) and Moll and Legler (1971) and is greatly generalized; in fact, there have been no detailed histological or developmental investigations of the various epidermal coverings of turtle shells. Each scute develops from the epidermal sheath covering the entire shell (Fig. 2). The grooves or sutures between each scute are merely indentations of the epidermis into the dermis lying beneath it. The indentations seldom match the suture pattern of the underlying bones. Each period of growth is best seen at the margin of the scutes. When growth begins after a major period of dormancy, the germinal layer of the entire epidermal sheath grows, not just at the edge of the scutes. A new scute is formed beneath the scute of the preceding growth cycle and

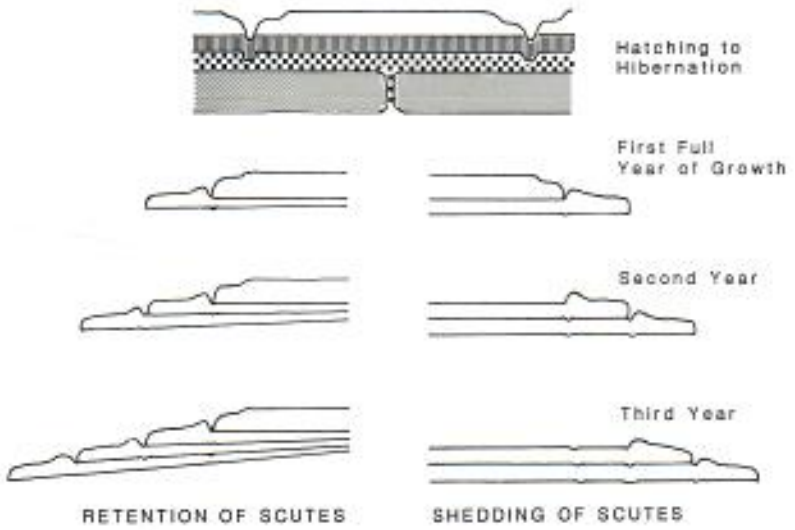

Figure 2. Diagrammatic representation of cyclic epidermal scute growth in turtles. The sequence on the left depicts a non-shedding pattern, that on the right a shedding pattern. Information for this sketch derives principally from Legler (1960) and Moll and Legler (1971). Clear area represents dead, cornified tissue (scute); vertical lines, living epidermis; squares, dermis; stippling, bones of the shell.

lifts the older scute off the living epidermis.

In some species (e.g., *Chrysemys picta*, *Emydura krefftii*), the older scute is shed, but the indentation or the suture line between scutes of the preceding growth periods remains on the new scute; this indentation becomes shallower and less evident in scutes of succeeding years. In other species (e.g., *Terrapene ornata*, *Rhinoclemmys annulata*), the older scutes remain attached to the newly formed scutes and are lost only after years of abrasion. In the latter growth pattern, the portion of the new scute lying beneath the older scute is much thinner than the portion lying beyond the edge of the older scute; in contrast, the new scutes in shedding species are of equal thickness throughout. In both types, minor cessations of growth may occur owing to temporary lack of food, illness or injury; as growth slows, stops and starts again a slight indentation is formed in the scute. Importantly, these pauses produce only indentations; only a major cessation and the beginning of a new growth cycle initiate the formation of a new scute.

To estimate age in non-shedding species, the investigator counts the layers of scutes, of course taking care to differentiate minor growth marks from the major growth marks as well as being attentive to the loss of the earliest scutes (Fig. 3). Typically, the counts are made from the fourth vertebral (e.g.,

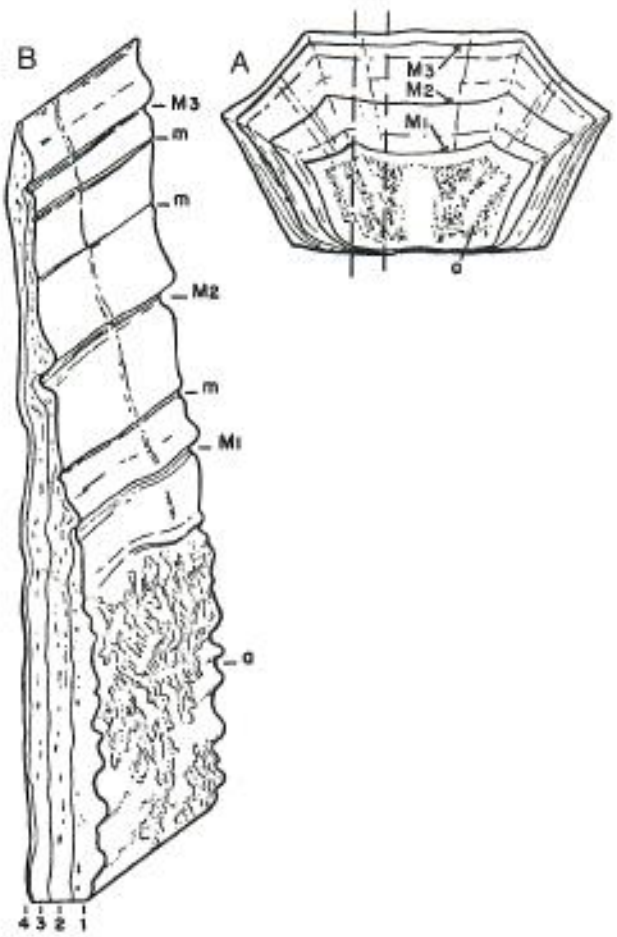

Figure 3. Second vertebral scute of a juvenile *Terrapene ornata* in its third full season of growth showing layers produced in successive seasons of growth. A. Entire scute from above; dashed line shows portion removed in parasagittal section. B. Diagonal view of parasagittal section removed from scute in "A," thickness of layers exaggerated. Each layer ends at major growth mark (M 1–3) that formed by the cessation of growth in late summer and the initial spurt of growth the subsequent spring; minor growth marks (m), form during the growing season by a slowing of growth. Apparently a new scute or epidermal layer forms only if growth stops totally. Note the granular texture of the areola (a); the smooth zone between the areola and M1 shows amount of growth in the season of hatching. Reproduced through the courtesy of the Museum of Natural History, University of Kansas and Dr. John M. Legler; from Legler (1960).

Chelydra serpentina, Galbraith and Brooks, 1987; *Testudo graeca*, Lambert, 1982) or abdominal (e.g., *Chrysemys picta* and *Sternotherus odoratus*, Mitchell, 1988) scutes. The counts should always be taken from the same scute on each turtle to avoid increasing sampling error, although an investigator may wish to record the number of layers from several different scutes to test for variability of intra-individual growth and sampling technique. The majority of studies have examined temperate-zone turtles and have assumed that only one scute is formed each year, hence number of scute layers is equal to number of years of life.

The same assumption has applied in the estimation of age for scute shedding species, but in these, the investigator counts the number of rings (annuli) formed by the suture indentations of previous years' scutes (Fig. 4). The counts are taken typically from the abdominal scute.

The scutes used for counting should also be measured, thus the investigator obtains both growth and age data from one technique. The importance of these measurements for age estimation is that they permit the investigator to age older turtles that have lost the indentations of the earliest scutes. The technique is based on obtaining means, ranges, and standard deviations of the lengths of the same scute in a large sample (Sexton, 1959). In each turtle, the midline scute length of each major growth period is recorded. These data are divided into size classes (see Table 1) that permit the investigator to determine the approximate age of the earliest visible scute indentation and, thus, an age estimate of the turtle in hand. For example, if the internal-most scute length in a turtle is 18.5 mm and three indentations lie externally, the estimated age is 6 yrs because the 18.5 mm is near the mean length for the scute at three years and the three indentations indicate three additional growth periods, hence 3 + 3 = 6 yr. This technique requires a segregation of the length classes by sex and the development of an aging table (Table 1) for each population studied.

Another advantage of measuring scute lengths is that these data may be used to estimate growth parameters. The Sergeev equation $L_1/L_2 = C_1/C_2$ provides estimates of annual growth for an individual through the relationship of plastron length (L_1) and abdominal scute length (C_1) at time 1 to plastron (L_2) and abdominal scute (C_2) lengths at the next time period. Since the relationship between the abdominal scute and plastron lengths are nearly unchanged through time (isometric), measurements of the scute growth zone lengths and the plastron length of a turtle allow the estimation of the turtle's plastron lengths at the end of preceding growth seasons (e.g., *Clemmys guttata*, Ernst, 1975).

Evaluation. — Estimation of age by counting the number of stacked scutes in non-shedding turtles or the number of major growth zones (scute marks) in shedding turtles is based on several assumptions. First, scute growth possesses a regular, cyclic pattern and that each major cycle produces a

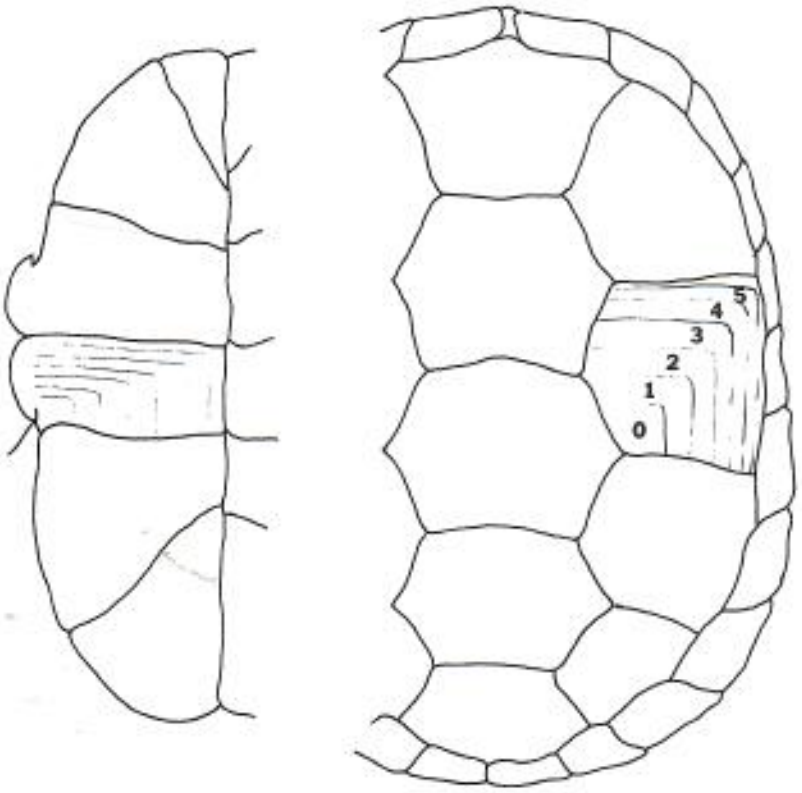

Figure 4. Diagrammatic representation of the carapace and plastron of an emydine turtle. The number 0 indicates the scute formed prior to hatching; 1–5 denote the scutes or scute growth zones formed during the five subsequent growing seasons.

distinct and visible mark. Second, the cyclic growth is directly associated with regular climatic events so that each scute mark or layer represents a specific interval of time. Third, either the scute marks remain visible throught the life of the turtle or, if lost, the number of marks lost can be accurately estimated.

These assumptions are variably fulfilled in different species and populations of turtles. All temperate-zone turtles possess a regular, cyclic growth pattern that is turned on by rising daily temperatures and off by falling daily temperatures (photoperiod likely involved as well; Licht, 1972), hence each major growth period represents one year. This one to one association has been directly confirmed in a number of turtles by long-term, mark-release-recapture studies, e.g., *Trachemys scripta* in central (Cagle, 1946) and southeastern

United States (Gibbons, 1970), *Chrysemys picta* in north central United States (Sexton, 1959), *Clemmys guttata* in northeastern United States (Ernst, 1975), *Terrapene ornata* in west central United States (Legler, 1960), *Graptemys pulchra* in southeastern United States (Shealy, 1976), *Chelydra serpentina* in eastern Canada (Galbraith and Brooks, 1987), *Gopherus agassizii* in southwestern United States (Germano, 1988) and *Testudo graeca* in central Europe (Benedetti, 1926). The one to one association is obtained by carefully reading the growth layers with the ability to differentiate the marks left by minor pauses in growth from the major seasonal pauses (Fig. 3). Even with great care, the cyclic record of growth cannot be read accurately in all temperate-zone turtles; Woodbury and Hardy (1948) and Dobie (1971) could find no strong correlation between age in years and the number of major growth marks in *Gopherus agassizii* and *Macroclemys temminckii*, respectively. While not denying their observations, I wish to note that these investigators were attempting to find a correlation in samples composed predominantly of adult turtles. Other investigators (e.g., Sexton, 1959; Gibbons, 1983) have observed that a direct correlation does exist between known age in years and number of scutes or major scute growth zones in subadult and immature turtles; once maturity is obtained, growth slows or ceases, and it become increasingly difficult to recognize the annual growth marks.

Table 1. Aging table based on the medial length of the right abdominal scute in *Chrysemys picta*. Data from Sexton (1959), slightly modified and abbreviated. F = female; M = male.

Growing Season		Length at End of Growing Season (mm)		
Year		Mean	SD	Range
1		11.3	1.2	9–14
2		15.4	1.3	13–18
3	F	18.6	1.4	15–23
3	M	17.9	1.7	15–21
4	F	21.1	1.8	18–25
4	M	20.2	1.5	17–23
—				
—				
7	F	27.8	1.9	24–31
7	M	25.0	1.9	23–29
—				
—				
13	F	34.1	—	—
13	M	29.8	—	—

There have been few long-term, mark-release-recapture studies of subtropical and tropical turtles, and the conclusions on the reliability of the scute-growth-zone technique are variable. *Geochelone gigantea* does not shed its scutes, and Gaymer (1968) found a strong correlation of the size and growth rate to number of scute layers in young tortoises and proposed an annual cycle of growth. Panamanian *Trachemys scripta* and Malayasian *Batagur baska* shed their scutes regularly. The growth marks are poorly defined in *Batagur* and likely of little of value in the estimation of age even in young turtles (Moll, 1980). In contrast, Panamanian *T. scripta* possess distinct growth marks, but mark-release-recapture data demonstrates that several growth marks (i.e., growth of completely new scutes) can be and often are produced during a single year (Moll and Legler, 1971). Nonetheless, Moll and Legler were able to recognize a cyclic growth pattern from the scute marks (growth marks within a single annual growth season were commonly tightly grouped) and linked each major scute mark with slow or no growth in the wet season. Thus, they were able to age sexually immature turtles and used these ages to predict growth rates. The marking and recapture of a single *Chelodina longicollis* (Stott, 1988) indicated that individuals within this particular population may have two growth-no growth periods matching a diphasic annual population cycle of its major prey species.

Scute layers or marks are unquestionably the most useful age determination criteria, because they allow an investigator to estimate a turtle's age upon first capture without killing it. Nonetheless, this technique must be used cautiously and, whenever possible, tested with known-aged animals.

Examples. — Cited above.

Claws and Rhamphothecae

Principle. — Claws and rhamphothecae (jaw sheaths) are epidermal structures and grow continuously throughout the life of turtles. Growth is governed by environmental factors and will undergo periods of rapid, slow and no growth. Alternation of growth and no growth may produce a layering in the keratin. If these layers are linked to regular seasonal events, they may serve as age estimators.

Practice. — As yet no investigations have revealed a regular layering in the claws or rhamphothecae of turtles. Since the scutes show a layering, the possibility exists that a regular-interval layering exists in the claws and jaw sheaths of some turtles. The potential advantage of layering in claws would be the ability to remove a digit tip from a marked animal, release it, and later determine that animal's age.

The search for layering should center on the examination of claws from juvenile and subadult animals. Growth patterns are most evident in these actively growing age classes. The claws may be examined as whole mounts

or as histological preparations, with transmitted light in both cases. Histology of thick keratinous structures is frequently difficult so Bouin's solution is recommended for fixation (including postfixation), since this solution softens keratin and allows easier sectioning.

Evaluation. — This technique is untried. If layers are present, they will share the same assumptions and will require the same verification and calibration as the other techniques using incremental growth layers.

Examples. — No known published studies.

Skeletochronology

Principle. — A skeletal element grows larger through the deposit of new bone on its outer surface. This periosteal growth responds to seasonal changes of the environment and produces layers in bones that show periods of growth and no growth. Seasonal periodicity equals a specific unit of time and can be used to estimate age.

Practice. — The use of skeletochronology requires dead turtles. In other reptiles, regular periosteal layers have been found in phalanges, but only humeri, femora and sclerotic ossicles of turtles show distinct periosteal layers and permit unambiguous counts. Either the humerus or femur is recommended, and it is advisable to use the same element on the same side for all specimens to reduce sampling error.

Similarily, it is advisable to use samples that are processed identically. The bone may be removed from formalin preserved specimens or from dead animals and either macerated or air dried. A section is removed from the narrowest portion (middle) of the humeral or femoral shaft. This section is stored in buffered formalin for a minimum of 24 hr if it is to be sectioned histologically; if it is to be polished, it is prepared as a dry section.

Polished sections are prepared by grinding and polishing one surface of the bone section. The general procedure is to cut a thin (1–2 mm) section with a thin-bladed bone saw, preferably not by hand since both faces should be parallel and smoothly planar. One face is further polished by rubbing across a piece of glass coated with carborundum powder. The section may be ground to a transparent thin section permitting examination by transmitted light. The visibility of the layers may be enhanced by light hematoxylin staining or treatment with a silver nitrate solution. Polishing can be time-demanding, and a researcher may wish to examine thicker sections by reflected light. Further details of this technique are reviewed by Erickson and Seliger (1969) and Morris (1972).

Histological preparation requires decalcification of bone samples. Numerous decalcifications solutions are available; however our best results (Zug et al., 1986) were obtained with a weak hydrochloric-formic acid

solution. More concentrated solutions proceed too rapidly and damage the histological structure of the bones. Even weak (5%) nitric acid has this damage potential because of its speed and corrosive power. Decalcification time depends upon the thickness and porosity of the bone sample, e.g., 5–7 mm thick sections from the humerus of adult *Caretta* require 4–8 days at 20–24° C. Decalcification must be watched closely; even in the weakest solutions, over-decalcification will destroy bone structure, making the recognition of growth layers impossible. Also, the bone sample must be thoroughly washed (several hours to a day in running water) to remove any trace of acid, which will affect later staining of the thin sections.

Thin sections may be obtained by cutting the bone with a razor blade (not recommended), freezing microtome or standard microtome. Sectioning with a microtome provides sections of uniform thickness and as thin as 5–6 μm. The preparation, sectioning and staining are described in numerous textbooks (e.g., Preece, 1965; Sheehan and Hrapchak, 1980) and laboratory manuals (e.g., Coolidge and Howard, 1979) and will not be repeated here. It is advisable, however, to seek the assistance of an experienced histologist or practice and experiment on non-critical samples prior to sectioning the research material. Most skeletochronological studies of reptiles have cut 15–16 μm sections, although Frazier (1982) reported satisfactory results at 30 μm and Zug et al. (1986) obtained satisfactory results at 6–8 μm. Mayer's hematoxylin preparation is the most widely used stain in skeletochronological studies, but Ehrlich's and Harris' also give good results.

Once sectioned and mounted on a slide, data collection can begin. The bone shows an alternating pattern of light and dark staining layers (Fig. 5) The lightly stained layers (zones) were produced during times of rapid growth, the darkly stained layers during periods of slow or no growth. The latter are narrow and are called lines of arrested growth (LAG). Together, a zone and a LAG comprise one complete growth cycle and are called mark of skeletal growth (MSG). For estimating ages, the MSGs are counted and often measured, because each growth cycle is assumed to represent a specific, nonvarying interval of time, e.g., 1 yr for temperate-zone turtles. Only the periosteal MSGs are counted and measured; endosteal layers are produced as the bone is remodeled and their production is acyclic.

Evaluation. — The assumptions of skeletochronology are similar to those for estimating age by counting scute layers/marks. First, skeletal elements possess a regular cyclic growth pattern, and this growth pattern leaves discrete marks within the bone. Second, the cyclic growth is directly associated with regular climatic events so that each layer or set of layers represents a specific interval of time. Third, the number of growth layers lost through reabsorption and remodeling can be estimated from the number and size of the remaining MSGs.

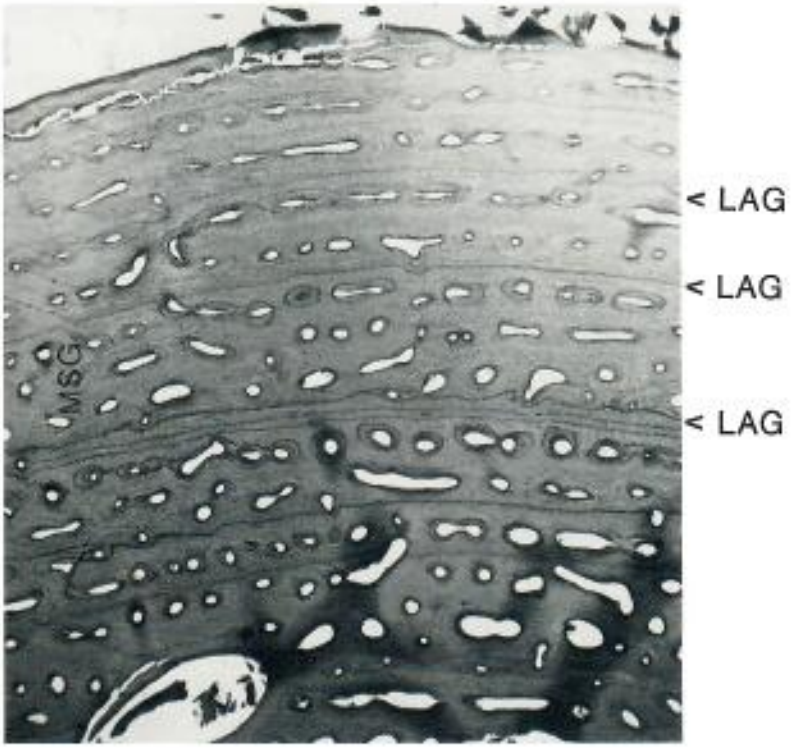

Figure 5. Cross-section of *Caretta caretta* humerus. Abbreviations: LAG, line of arrested growth; MSG, mark of skeletal growth.

Turtle bone does show distinct periosteal layers, and each layer appears to be associated with a single growth season. However, not all skeletal elements possess distinct MSGs that can be counted and measured. Distinct layers are commonly most evident in the long bones, humerus and femur, (*Testudo hermanni* and *T. graeca*, Castanet and Cheylan, 1979; *Emys orbicularis*, Castanet, 1985; *Chelonia mydas* and *Eretmochelys imbricata*, Frazier, 1982; *Chelydra serpentina*, Hammer, 1969; *Caretta caretta*, Zug et al., 1986) and in sclerotic ossicles (*Dermochelys coriacea*, Zug, unpublished). Growth layers are irregular, indistinct or absent in peripheral bones of the carapace, dentary, cervical vertebral centra, penultimate phalanges and ulna (*Caretta caretta*, Zug et al., 1986), dentary and vertebrae (*Macroclemys temminckii*, Dobie, 1971). Even in the humerus and femur, problems arise that make counting difficult. The problems include resorption and remodeling, accessory LAGs, no visible growth layers, discontinuous LAGs, irregular LAGs and MSGs, and compaction of MSGs.

Hemelaar (1985) developed a technique for determining a toad's age (*Bufo bufo*) even when earlier MSGs have been lost through resorption. The technique requires the estimation of the minimum and maximum diameters of each year's MSGs, then the calculation of 95% confidence limits for each year's MSG diameters. With these data, the age of the smallest remaining MSG can be estimated and added to the number of remaining MSGs to estimate the toad's actual age. This method is most precise with the younger, actively growing animals and, as Hemelaar notes, the MSG diameter confidence limits must be developed for each population studied and for each sex as well. A less precise method of estimating the total number of MSGs measures the widths of remaining MSGs, calculates an average MSG width, and divides this width into the radius of the skeletal element (minus its radius at hatching). The resulting value is likely to be an overestimate, because the outer MSGs are deposited when the turtle is older and growing slower, hence the average width will be narrower than if the average included the MSGs produced during the early periods of most rapid growth (Zug et al., 1986).

The major assumption of this technique is an annual production of each MSG. The data supporting the "one-MSG=one-year" hypothesis is indicative but not entirely unequivocal (reviewed in Castanet, 1985, and Zug et al., 1986). A high correlation between number of MSGs and scute layers in a number of turtle species, and a match between actual age and number of MSGs in small samples of European species raised in semi-natural conditions or in a laboratory with an annually fluctuating environment support the hypothesis. Consensus opinion supports the hypothesis for temperate-zone populations; no tropical turtles have been examined relative to this question. Skeletochronological age estimates should not be used as ages for individual turtles but as statistical values to provide means, ranges or confidence intervals for various ecological or physiological classes of animals.

Examples. — Cited above.

AGE ESTIMATION THROUGH STRUCTURAL MODIFICATION

Scute Polishing

Principle. — In species of turtles that do not shed their scutes, the scutes are subjected to constant abrasion and wear. This abrasion causes a gradual lost of the earlier scutes and an eventual erasure of all growth marks on the scutes. The amount of wear can indicate the relative age of turtles.

Practice. — Old testudinids and terrestrial emydids can be easily recognized by their smoothly polished shells. Only recently, however, has anyone attempted to quantify the degree of wear and to associate wear with age classes (*Gopherus agassizii*; Berry and Woodman, 1983). Seven wear classes

were recognized on the basis of: 1) wear of hatchling (first) scutes and subsequent scute growth zones; 2) chipping, flaking, peeling and thickness of scute laminae; 3) osteoporosis of the carapace; 4) growth pattern determined from scute growth zones; 5) carapace size. The wear classes proposed for *Gopherus agassizii* are not discrete age classes; for example, the early wear classes contain juvenile, immature and subadult tortoises.

Evaluation. — Scute wear data permit only the most coarse interpretation of a population's demography; however, for initial conservation and management decisions, these data do reveal whether a population shows recruitment or a concentration of older individuals. The potential variation associated with shell wear (e.g., diet, soil type, terrain, individual activity patterns) would not recommend this age determination technique for long-term population studies.

Examples. — Cited above.

Skeletal Changes

Principle. — Each skeletal element or skeletal unit changes in shape, structure or relative size as the animal grows larger and older. Specific morphologies in this sequence of change can often be linked to physiological/endocrinological events, e.g., attainment of sexual maturity, and be used as estimates of relative age.

Practice. — With the exception of periosteal layering, changes in skeletal morphology have not been examined as characteristics for determining age. Three aspects are potentially useful: 1) porosity of bone; 2) closing and relative position of canals and foramina on bones; 3) size and sequence of closing of shell fontanelles. All these features show some age-specific differences, but none have been calibrated against known aged specimens.

Bone porosity may be best studied by obtaining some measure, either absolute or relative, of bone density. Bone cores (biopsies) from posterior peripheral bones could be examined for volume or mass and these data used directly or proportionally to some standard body dimension. If dealing with skeletal preparations, the volume or mass of a specific element compared to another dimension of that element would permit comparison of porosity in different size/age individuals.

At hatching, the carapace and plastron are loosely articulated and possess numerous open areas (fontanelles) where the bony plates do not meet. The size, shape and sequence of closure of these fontanelles is likely species specific and may serve as indicators of an individual's age. However, there are no published observations detailing the ontogenetic changes in fontanelles and the association of these changes with age (actual or estimated). In general, shell fontanelles grow shut with maturity or shortly thereafter.

Comments on the use of foramina and canals in cranial and postcranial skeletons match those for shell fontanelles, because there is a similar absence of studies on their ontogeny. A clear pattern of ontogenetic change occurs in the ectepicondylar foramen of the humerus (*Caretta*; Zug et al. 1986). This foramen is an open canal in hatchlings, and as the turtle grows, the canal deepens, is eventually closed over to form a foramen and continuing growth even after maturity shifts the foramen proximally (Fig. 6). Other foramina or canals may show similar ontogenetic changes that could be used as aging criteria.

Evaluation. — Bone porosity may prove to be most difficult to quantify accurately. Further, our present knowledge of the sequence of porosity change during a turtle's life is largely anecdotal and confined to the assumption that old (= large with well worn shells) turtles show the greatest porosity. There is but a single study on the ontogeny of skeletal mass relative to body mass in turtles (*Chrysemys picta*; Iverson, 1982); this study shows a steady increase in relative skeletal mass from hatching to maturity; thereafter, relative mass remains stable. This stability of adult skeletal mass (relative) appears characteristic for most turtles (Iverson, 1984) and indicates that porosity (bone density) does not change with age in adults, hence is unsuitable for age determination of adults. A further difficulty may be a female's mobilization of calcium for egg shell production and the corresponding increase in bone porosity during this period (*Sternotherus odoratus*; Edgren, 1960). Iverson's ontogenetic data indicates decreasing porosity (increasing density) with maturation, but the two other mass factors, i.e., closing of fontanelles and increase in bone thickness, may contribute more to the increasing mass than does a change in porosity.

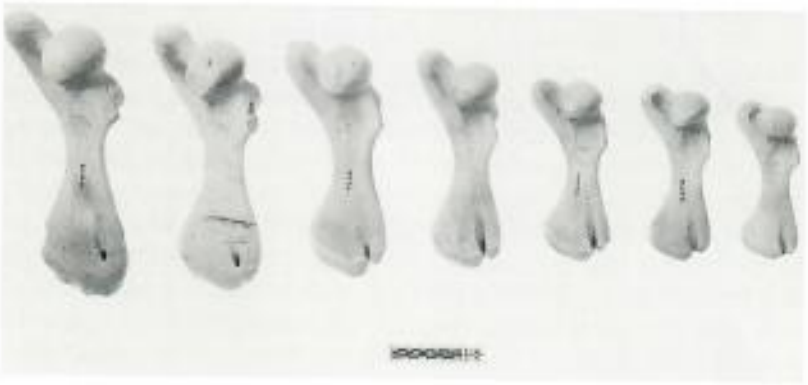

Figure 6. Dorsal view of a series of *Caretta caretta* humeri showing the closure of the ectepicondylar foramina.

Ontogenetic changes in foramina and shell fontanelles offer potentially good criteria for aging turtles once the sequence of change relative to age is documented. The method requires access to the skeletons (dead turtles) and will likely prove most useful in situations where there has been a mass die-off and an estimate of the population structure of the dead turtles is desired. In this respect, this method has potential use for examining the age composition of turtles in archeological middens and fossil assemblages.

Examples. — Cited above.

Coloration Changes

Principle. — Color and color pattern often possess distinct shades and patterns in different life stages of turtles.

Practice. — Several classes of color and color pattern changes are observed in turtles and each offers different information for age and sex determination. As yet, none of these specific changes have been associated with age data; however, changes have been linked with sex and size class data. Color and pattern changes occur usually when individuals are reproductively active, for example the brightening of head and carapace colors in male *Batagur baska* (Moll, 1980). In other cases, both sexes show pattern differences when they reach sexual maturity whether or not they are reproductively active (e.g., *Trionyx muticus*; Ernst and Barbour, 1972).

Published color or pattern changes discriminate only between juveniles and adults; perhaps when examined closely, they will provide criteria for aging juveniles during the transition to adult color patterns. The development of a melanistic plastron in *Trachemys scripta* (and some other emydid turtles) begins in juveniles and continues in adults (Barbour and Carr, 1940), thereby providing a means of aging juveniles and adults. Eye and head color in *Terrapene carolina triunguis* used in combination permit the recognition of sex and three broad age classes (Schwartz, Schwartz and Kiester, 1984).

Evaluation. — At this time, difference in color and pattern can be used at only a coarse level to identify juveniles, subadults, adults and old adults. Pattern and color changes need to be better documented and calibrated with age data (actual or estimated). Once calibrated, color data may prove useful in aging living turtles and adults that have lost other external indicators of age. Color data may be population specific or at least geographically variable in sequence and/or rate of change (McCoy, 1968), so standards will need to be developed for each population. Further, coloration may be associated directly with size and not age, e.g., *Chelonia mydas* (Balazs, 1986). Samples for calibration must be sufficiently large to include fast and slow growing individuals.

Examples. — Cited above.

COMPARISON OF TECHNIQUES

Of all the age determination techniques, only ages derived from mark-release-recapture studies, and only from individuals marked as emerging hatchlings, are actual (true) ages. These actual ages are necessary to calibrate and/or verify the ages from all other techniques. Known age data are limited to the accurate calibration of age estimates derived from the same population of animals. Turtles in different populations experience different types and amounts of food and are subjected to different microenvironments; thus, the correlation of actual age with the parameters for estimating age may also differ and comparison outside the actual-aged population requires caution.

Age estimates derived from scute growth zones have proved to be reliable and accurate in numerous turtle species from temperate climates. This technique is also the most convenient, since the turtles are not sacrificed and the counts/measurements can be taken at the site of capture and the turtles released immediately and unharmed. When verified and combined with known-aged data, scute age estimates nearly match actual age data in accuracy.

In those species lacking distinct scute growth zones, skeletochronology and (potentially) lens masses can provide age estimates, if some animals can be sacrificed. These age estimates are best used as statistical samples to provide age means and confidence intervals for various classes of turtles and not as specific ages for individual turtles.

Size class data are useful as "age" estimates only in those situations where scute growth zones and/or the population can be sampled briefly or it is inadvisable to sacrifice any of the turtles, e.g., endangered species. The other techniques are largely untested and may prove useful when the aforementioned techniques cannot be used.

ACKNOWLEDGEMENTS

The organization of this review is patterned after Morris' review (1972) of age determination in mammals. I found his manner of presentation useful for a rapid introduction to the techniques of age determination and hope to mimic his successful conveyance of information. My interest in age determination in amphibians and reptiles has been encouraged and supported by numerous individuals and organizations to all of whom I am grateful, but I especially wish to thank A. Wynn, W. R. Heyer, H. Wimer, Department of Vertebrate Zoology (National Museum of Natural History), and the Smithsonian's Research Opportunities Fund. I also thank C. H. Ernst, W. R. Heyer, J. B. Iverson, A. S. Rand, and I. R. Swingland for their critical evaluation of drafts of this review; however, I remain responsible for errors, misinterpretations, and omissions.

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