1994. Proceedings of The Desert Tortoise Council 1987-1991:93-100.

METHODS OF AGE DETERMINATION OF THE DESERT TORTOISE, *GOPHERUS AGASSIZII*

David J. Germano and Thomas H. Fritts

Abstract. Methods previously used to determine ages of desert tortoises (*Gopherus agassizii*) have disadvantages because they are either 1) time consuming, 2) destructive, or 3) inaccurate. Repeated capture of chelonians marked as hatchlings gives accurate ages of individuals (Metcalf and Metcalf 1985), but requires much field effort over several years and is limited to only those individuals recaptured. Analysis of bone annuli is another accurate means of determining age (Zug et al. 1986; Zug 1991), but to date requires extraction of a long bone, which restricts uses to dead specimens. Shell-wear patterns roughly categorize all individuals in a population (Berry and Woodman 1984), but ages are only approximate and the amount of shell wear may be subject to a variety of environmental effects.

In contrast, the number of scute annuli counted on the shell accurately reflects the age of desert tortoises up until about 25 yr of age. Scute annuli not only allow for accurate age determination of individuals with only one handling, but also have the added advantage of yielding the growth history of each individual. Extending age determination of tortoises past 25 yr may be possible using thin sections of scute viewed under magnification. Scute annuli and laminations potentially provide an accurate means of aging all desert tortoises in a population.

INTRODUCTION

Determining ages of individuals is a necessary part of quantitative population analyses (Charlesworth 1980; Gibbons 1987). To accurately assess the status of desert tortoise populations, life-history traits such as juvenile survivorship, age-at-first-reproduction, adult survivorship, and fecundity must be determined. Fluctuations in these population traits can alter the viability of a population. Some life-history traits can only be determined by accurately aging each individual in a population. Several techniques have been used for determining ages in tortoises, but all have suffered from serious shortcomings that limit their use in living populations. We compare techniques used to determine ages of desert tortoises (*Gopherus agassizii*) and suggest the use of one technique that is accurate, non-destructive, and can be used with one handling of an individual.

Age Determination in Tortoises

Mark-and-Recapture - The most accurate method for determining ages of individual tortoises is to mark hatchlings or very young tortoises, for which ages at the time of first capture can be assigned accurately. These individuals are then recaptured over many years, and for each subsequent recapture the age is known (Medica et al. 1975; Gibbons 1987; Turner et al. 1987; Zug 1991). This technique yields exact ages of individuals, but it is time-consuming. Other disadvantages are that not all marked individuals will be recaptured, some die, and some disperse out of the area. The inability to find tortoises hampers recaptures of individuals, and this problem becomes more acute in areas of dense vegetation and in areas where dispersal is significant. Adult desert tortoises are fairly easily found in Mojave Desert habitats, but small tortoises are largely undetected (Berry and Turner 1986). All size classes become harder to detect as visibility becomes increasingly poorer in the dense vegetation of Sonoran and Sinaloan habitats (Germano et al. 1993).

Bone Analyses - This technique is accurate for young tortoises and can be used to determine ages of all individuals if corrections can be made for lost bone annuli due to reconstruction of bone in older individuals (Castanet et al. 1977; Castanet and Cheylan 1979; Zug et al. 1986; Zug 1991). The major drawback is the need for a long bone to be used in the analysis. To date, annual bone growth in tortoises has been detectable only in long bones such as humeri and femurs. Thus, only dead individuals can reasonably be used (Zug 1991).

Shell Wear - This technique has been devised for determining ages of individuals based on the size and relative wear of the shell (Berry and Woodman 1984). Using this method, individuals are placed into various size/age classes. Each individual is assigned to an approximate age class with only one handling and individuals are not killed. One disadvantage is that individuals are assigned to general age classes only, which are not very useful in quantitative population analyses. For example, if reproductively mature individuals are lumped together as adults, the population structure may seem skewed (Fig. 1). This could significantly alter a biologist's perception of the viability of a population. Another disadvantage is that although wear on shells is partly a function of age, it is also a function of differences in environmental factors, such as soil texture and habitat composition. Even within the same general area, two individuals of the same age may have different amounts of shell wear due to microsite differences. Desert tortoises exist in sandy valley habitats in the Mojave Desert as well as on rocky, boulder strewn hillsides in the Sonoran Desert (Germano et al. 1993).

Scute Rings - Many chelonians produce growth rings on their scutes that potentially can be used for age determination. Although the following list is not exhaustive, scute rings have been used for determining ages of young individuals of *Chrysemys picta* (Sexton 1959; Gibbons 1967, 1968a; Ernst 1971), *Chrysemys floridana* (Gibbons and Coker 1978), *Trachemys [Pseudemys] scripta* (Cagle 1946; Moll and Legler 1971), *Clemmys insculpta* (Lovich et al. 1990), *Clemmys guttata* (Ernst 1975), *Emydoidea blandingi* (Graham and Doyle 1977, Ross 1989; Congdon and van Loben Sels 1991), *Graptemys pseudogeographica* (Moll 1976), *Deirochelys reticularia* (Gibbons 1969), *Chelydra serpentina* (Gibbons 1968b; Galbraith and Brooks 1989), *Terrapene ornata* (Legler 1960; Blair 1976; Doroff and Keith 1990), *Terrapene carolina* (Ewing 1939; Stickel 1978; Stickel and Bunck 1989), *Kinosternon flavescens* (Long 1986), *Kinosternon subrubrum* (Ernst et al. 1973; Gibbons 1983), *Pyxis planicauda* (Kuchling and Bloxam 1988), *Chersina angulata* (Branch 1984), *Testudo hermanni* and *T. graeca* (Castanet and Cheylan 1979; Lambert 1982; Stubbs et al. 1984), *Geochelone gigantea* (Gaymer 1968; Grubb 1971; Bourn and Coe 1978; Swingland and Coe 1979), *Gopherus berlandieri* (Auffenberg and Weaver 1969; Judd and Rose 1983), *Gopherus flavomarginatus* (Gary Adest, pers. comm.), and *Gopherus polyphemus* (Landers et al. 1982). In addition, both Gibbons (1976) and Zug (1991) found that counts of scute rings were one of the best methods of estimating ages in chelonians.

The accuracy of counts of scute rings for determining ages of desert tortoises requires demonstrating a relationship between number of scute rings and actual age. This relationship was demonstrated for a population of desert tortoises at the Nevada Test Site in the northern Mojave Desert (Germano 1988). These tortoises ranged in age from 21-25 yr, and counts of scute rings were either exactly the age of the tortoise or were within 1-2 yr of the known age. In addition, counts of scute rings were exactly the same as age for six captive raised desert tortoises, and the number of bone rings counted from long bones were not significantly different from counts of scute rings for 16 dead desert tortoises (Germano 1988). Although this work demonstrated the utility of using scute rings to determine ages of desert tortoises, the use of easily seen scute rings limits aging animals to those < 20-25 yr of age. Sectioning the edges of scutes revealed the presence of additional layers in animals that seemed older than 25 yr (Germano 1992), tortoises for which scutes are beveled on the edges (Fig. 2). Only minimum estimates of age could be determined from this method because no tortoises of known age were available, but it is possible that this method actually reveals true ages of older individuals. Other methods of aging tortoises > 25 yr should be tested, such as bore cores taken from marginal scutes (P.A. Medica, pers. comm.).

DISCUSSION

Quantitative demographic analyses of desert tortoise populations require a knowledge of the age of living individuals. Mark-recapture, bone analysis, and the shell-wear technique have serious disadvantages that preclude their use in this type of analysis. Counts of scute rings are an accurate means of determining ages of desert tortoises less than 25 yr. Analysis of thinned sections of scute for tortoises older than 25 yr extended the ability to determine the age of any individual.

Criteria for using easily seen scute rings to age desert tortoises are that the number of rings is less than 20-25 and that the last ring shows a clean edge as it abuts the last ring of an adjacent scute (Fig. 2). Desert tortoises are likely older than the number of scute rings visible to the unaided eye when the last scute ring is not distinct and the edge of the scute is beveled (Fig. 2). For these individuals, we suggest cutting off a thin section of epidermal material from the edge of a carapace scute for further microscopic analysis. We have taken thin scute sections from living desert tortoises that appear older than 25 yr without significant harm to the individual.

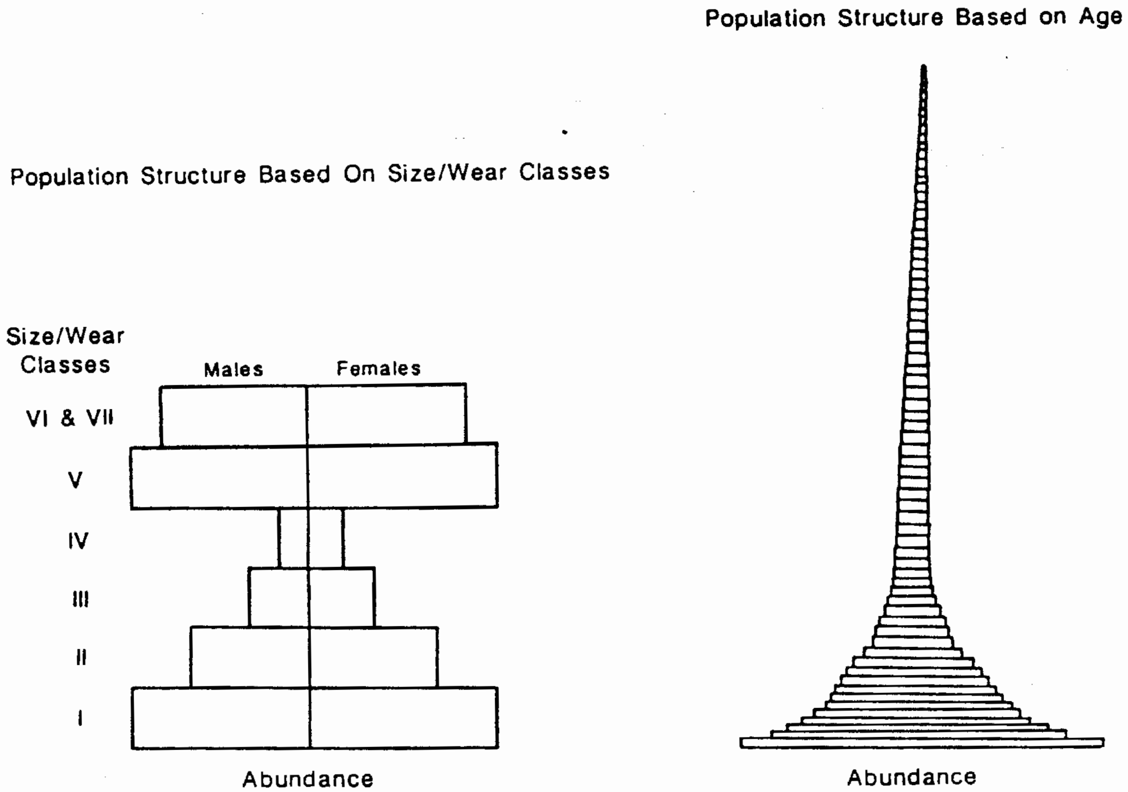

Figure 1. Biased age structure of desert tortoise populations may result from the inability to separate individuals into actual ages and due to the problem of not being able to find all individuals, particularly younger individuals. Hatchling and young tortoises may remain cryptic, which makes them under-represented in the age structure. For the hypothetical data we used here, lumping ages (left column) may over-represent adults, indicating that the population is declining. If tortoises were aged to the year (right column), the population structure might appear more typical of a growing or stable population.

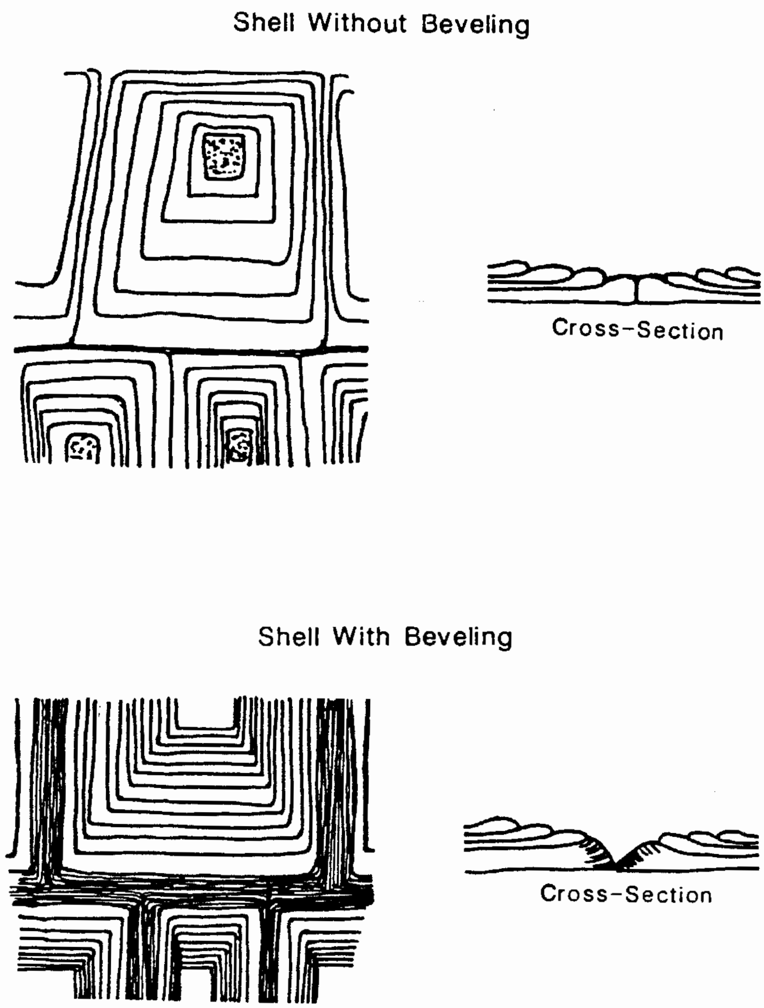

Figure 2. Above. A tortoise that is as old as the number of rings counted on a scute. The last ring of a scute abuts evenly with the last ring of an adjacent scute. **Below.** Beveling that can occur in a tortoise that is likely older than the number of rings that can be discerned easily. Adjacent scutes do not meet cleanly.

There is the potential to incorrectly age an individual by 1-2 yr if care is not taken to identify subannual rings from annual rings, i.e., some tortoises have two rings in one year. In addition, Berry (1987) has shown that a ring may not form in a very poor rainfall year. Individuals that do not grow appreciably for several years could be incorrectly aged, but careful inspection of scutes on the carapace and plastron should alert the investigator to this problem because a small chasm will likely form between normal rings due to the minimal growth. We also suggest the use of dental casts (Galbraith and Brooks 1987) to preserve a section of the carapace or plastron used in the growth analysis. This will speed data gathering in the field and provide a permanent record of growth. In our experience, the carapace is better for determining growth and age because it usually wears less than the plastron (Germano 1988).

The use of scute rings for determining the ages of desert tortoises seems to be applicable throughout their range. Based on the nearly 300 desert tortoises that we have examined from throughout their range, we have found that the general appearance and maximum number of rings on a scute is the same, subject only to variation in annual ring widths. In addition, all parts of the range of the desert tortoise experiences a period of adverse environmental conditions that slows tortoise growth and therefore produces an annual calibration. In the Mojave and Sonoran Deserts, cold winters cause tortoises to become dormant, and dryness from February to April causes dormancy in tortoises in Sinaloan habitats (Germano et al. 1993). Besides being useful for aging desert tortoises, scute rings have the additional benefit of providing a growth history of the first 25 yr of an individual (Germano 1988). Future population analyses of the desert tortoise can easily incorporate age determination of individuals, at least for those under 25 yr.

ACKNOWLEDGEMENTS

We thank R.B. Bury, N.J. Scott, M.A. Bogan, and M.A. Griffith for reading this manuscript and commenting on its content. We especially thank R. Chiovetti for his help with bone histological preparation and analysis of scute sections using scanning electron microscopy. We are grateful to F.B. Turner, P.A. Medica and R.B. Bury for providing access to specimens and data important to evaluating ring analyses.

LITERATURE CITED

Auffenberg, W. and W.G. Weaver. 1969. *Gopherus berlandieri* in southeastern Texas. Bull. Florida State Mus. Biol. Sci. 13:141-203.

Berry, K.H. 1987. Using growth ring counts to age wild juvenile desert tortoises. Paper presented at the 12th annual meeting of the Desert Tortoise Council. 28-29 March 1987, Las Vegas, Nevada.

Berry, K.H. and F.B. Turner. 1986. Spring activities and habits of juvenile desert tortoises, *Gopherus agassizii*, in California. Copeia 1986:1010-1012.

Berry, K.H. and A.P. Woodman. 1984. Preliminary investigations of shell wear in determining adult age groups in desert tortoises. Appendix 4 in The status of the desert tortoise (*Gopherus agassizi*) in the United States. Unpublished Report of the Desert Tortoise Council to U.S. Fish and Wildlife Service, Sacramento, California.

Blair, W.F. 1976. Some aspects of the biology of the ornate box turtle, *Terrapene ornata*. Southwestern Nat. 21:89-104.

Bourn, D., and M. Coe. 1978. The size, structure and distribution of the giant tortoise population of Aldabra. Phil. Trans. R. Soc. Lond. 282:139-175.

Branch, W.R. 1984. Preliminary observations on the ecology of the angulate tortoise (*Chersina angulata*) in the eastern Cape Province, South Africa. Amphibia-Reptilia 5:43-55.

Cagle, F.R. 1946. The growth of the slider turtle, *Pseudemys scripta elegans*. Am. Midl. Nat. 36:685-729.

Castanet, J. and M. Cheylan. 1979. Les marques de croissance des os et des ecailles comme indicateur de l'age chez *Testudo hermanni* et *Testudo graeca* (Reptilia, Chelonia, Testudinidae). Can. J. Zool. 57:1649-1665.

Castanet, J., F.J. Meunier and A. de Ricqles. 1977. L'enregistrement de la croissance cyclique par le tissu osseux chez les vertebres poikilotherms: donnees comparatives et essai de synthese. Bull. Biol. France Belgique 111:183-202.

Charlesworth, B. 1980. Evolution in age-structured populations. Cambridge Studies in Mathematical Biology: 1. Cambridge Univ. Press.

Congdon, J.D. and R.C. van Loben Sels. 1991. Growth and body size in Blanding's turtles (*Emydoidea blandingi*): relationships to reproduction. Can. J. Zool. 69:239-245.

Doroff, A.M. and L.B. Keith. 1990. Demography and ecology of the ornate box turtle (*Terrapene ornata*) in south-central Wisconsin. Copeia 1990:387-399.

Ernst, C.H. 1971. Growth of the painted turtle, *Chrysemys picta*, in southeastern Pennsylvania. Herpetologica 27:135-141.

________. 1975. Growth of the spotted turtle, *Clemmys guttata*. J. Herpetol. 9:313-318.

Ernst, C.H., R. W. Barbour, E.M. Ernst and J.R. Butler. 1973. Growth of the mud turtle, *Kinosternon subrubrum*, in Florida. Herpetologica 29:247-250.

Ewing, H.E. 1939. Growth in the eastern box-turtle, with special reference to the dermal shields of the carapace. Copeia 1939:87-92.

Galbraith, D.A. and R.J. Brooks. 1987. Photographs and dental casts as permanent records for age estimates and growth studies in turtles. Herpetol. Rev. 18:69-71.

Galbraith, D.A. and R.J. Brooks. 1989. Age estimates for snapping turtles. J. Wildl. Manag. 53:502-508.

Gaymer, R. 1968. The Indian Ocean giant tortoise *Testudo gigantea* on Aldabra. J. Zool. 154:341.

Germano, D.J. 1988. Age and growth histories of desert tortoises using scute annuli. Copeia 1988:914-920.

________. 1992. Longevity and age-size relationships of populations of desert tortoises. Copeia 1992:367-364.

Germano, D.J., R.B. Bury, T.C. Esque, T.H. Fritts and P.A. Medica. 1993. Range and habitats of the desert tortoise (*Gopherus agassizii*). *In*: R.B. Bury and D.J. Germano (eds.), Biology of North American tortoises. U.S. Fish and Wildlife Service, Fish and Wildlife Research. In Press.

Gibbons, J.W. 1967. Variation in growth rates in three populations of the painted turtle, *Chrysemys picta*. Herpetologica 23:296-303.

________. 1968a. Population structure and survivorship in the painted turtle, *Chrysemys picta*. Copeia 1968:260-268.

________. 1968b. Growth rates of the common snapping turtle, *Chelydra serpentina*, in a polluted river. Herpetologica 24:266-267.

________. 1969. Ecology and population dynamics of the chicken turtle, *Deirochelys reticularia*. Copeia 1969:669-676.

________. 1976. Aging phenomena in reptiles. pp. 453-475. *In*: M.F. Elias, B.E. Eleftheriou, and P.K. Elias, (eds.), Special Review of Experimental Aging Research. EAR, Inc. Bar Harbor, Maine.

________. 1983. Reproductive characteristics and ecology of the mud turtle, *Kinosternon subrubrum*, (Lacepede). Herpetologica 39:254-271.

_______. 1987. Why do turtles live so long? Bioscience 37:262-269.

Gibbons, J.W. and J. W. Coker. 1978. Ecology and life history aspects of the cooter, *Chrysemys floridana* (Le Conte). Herpetologica 33:29-33.

Graham, T.E. and T.S. Doyle. 1977. Growth and population characteristics of Blanding's turtle, *Emydoidea blandingii* in Massachusetts. Herpetologica 33:410-414.

Grubb, P. 1971. The growth, ecology and population structure of giant tortoises on Aldabra. Philos. Trans. R. Soc. London 260:327.

Judd, F.W. and F.L. Rose. 1983. Population structure, density and movements of the Texas tortoise *Gopherus berlandieri*. Southwest. Nat. 28:387-398.

Kuchling, G. and Q.M.C. Bloxam. 1988. Field-data on the madagascan flat tailed tortoise *Pyxis (Acinixys) planicauda*. Amphibia-Reptilia 9:181-187.

Lambert, M.R.K. 1982. Studies on the growth, structure and abundance of the Mediterranean spur-thighed tortoise, *Tesudo* [sic] *graeca* in field populations. J. Zool., London 1982(196):165-189.

Landers, J.L., W.A. McRae and J.A. Garner. 1982. Growth and maturity of the gopher tortoise in southwestern Georgia. Bull. Florida State Mus., Biol. Sci. 27:81-110.

Legler, J.M. 1960. Natural history of the ornate box turtle, *Terrapene ornata ornata* Agassiz. Univ. Kansas Publ., Mus. Nat. Hist. 11:527-669.

Long, D.R. 1986. Clutch formation in the turtle, *Kinosternon flavescens* (Testudines: Kinosternidae). Southwest. Nat. 13:1-8.

Lovich, J.E., C.H. Ernst and J.F. McBreen. 1990. Growth, maturity, and sexual dimorphism in the wood turtle, *Clemmys insculpta*. Can. J. Zool. 68:672-677.

Medica, P.A., R.B. Bury and F.B. Turner. 1975. Growth of the desert tortoise (*Gopherus agassizii*) in Nevada. Copeia 1975:639-643.

Metcalf, A.L. and E.L. Metcalf. 1985. Longevity in some ornate box turtles (*Terrapene ornata*) J. Herpetol. 19:157-158.

Moll, D. 1973. Environmental influence on growth rate in the Ouachita map turtle, *Graptemys pseudogeographica ouachitensis*. Herpetologica 32:439-443.

Moll, D. and J.M. Legler. 1971. The life history of a neotropical slider turtle, *Pseudemys scripta* (Schoepff), in Panama. Bull. Los Angeles Co. Mus. Nat. Hist., Sci. 11:1-102.

Ross, D. A. 1989. Population ecology of painted and Blanding's turtles (*Chrysemys picta* and *Emydoidea blandingi*) in central Wisconsin. Wisconsin Acad. Sci., Arts, Letters. 77:77-84.

Sexton, O.J. 1959. A method of estimating the age of painted turtles for use in demographic studies. Ecology 40:716-718.

Stickel, L.F. 1978. Changes in a box turtle population during three decades. Copeia 1978:221-225.

Stickel, L.F. and C.M. Bunck. 1989. Growth and morphometrics of the box turtle, *Terrapene c. carolina*. Herpetologica 23:216-223.

Stubbs, D., A. Hailey, E. Pulford and W. Tyler. 1984. Population ecology of European tortoises: review of field techniques. Amphibia-Reptilia 5:57-68.

Swingland, I.R. and M.J. Coe. 1979. The natural regulation of giant tortoise populations on the Aldabra atoll: recruitment. Phil. Trans. R. Soc. Lond. 286:177-188.

Turner, F.B., P.A. Medica and R.B. Bury. 1987. Growth and age in the desert tortoise at the Nevada Test Site. Copeia 1987:974-979.

Zug, G.R. 1991. Age determination in turtles. Herpetol. Circ. No. 20. 28 pp.

Zug, G.R., A.H. Wynn and C. Ruckdeschel. 1986. Age determination of loggerhead sea turtles, *Caretta caretta*, by incremental growth marks in the skeleton. Smithsonian Contrib. Zool. No. 427.