

SKELETOCHRONOLOGY AS A METHOD OF AGING OLIGOCENE *Gopherus laticuneus* AND *Styemys nebrascensis*, USING *Gopherus polyphemus* AS A MODERN ANALOG

By

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Abstract of Thesis Presented to the Graduate School  
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SKELETOCHRONOLOGY AS A METHOD OF AGING OLIGOCENE *Gopherus*  
*laticuneus* AND *Styemys nebrascensis*, USING *Gopherus polyphemus* AS A MODERN  
ANALOG

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The use of skeletochronology in many reptile groups has become a common method of incremental growth analysis over the last 20 years. With the exception of sea turtles, this method has been largely overlooked as a feasible alternative to scute annuli counts or carapace lengths in turtles and tortoises. Incremental growth layers in sea turtles have been correlated to annual growth cycles. In thin bone sections, a light, wide band represents a season of rapid growth; and a thin, dark band represents a season of slow growth or stasis, making up a single year's growth. Growth layers are analyzed by taking determined-thickness thin sections from humeral shafts of specimens.

The discovery of an unusually rich assemblage of fossil tortoises in northwestern Nebraska warranted study of the skeletochronology at this site. Incremental growth rings are a viable option in this case for individual age determination, as carapace lengths are not preserved well in the fossil record. The two species in question, *Gopherus laticuneus* and *Styemys nebrascensis*, were all collected within the White River Group of the central

United States. Individual bones were prepared, and thin-sections were prepared to estimate the tortoises' ages. Before thin sectioning fossil materials, a modern analog (*Gopherus polyphemus*) was tested to determine the validity of methods used. This modern example shows enough similarities in size and presumed environmental conditions to provide a good comparative analog.

Data collected from *G. laticuneus* and *S. nebrascensis* is applied to determine age structure of the populations at the site. Information gathered from *G. polyphemus* will provide the groundwork for further exploration of the technique of skeletochronology and its use with other groups of organisms.

## CHAPTER 1 INTRODUCTION

The task of aging long-lived chelonian species has been a continuing problem for scientists as long as the species have been studied. Numerous techniques have been devised to accurately estimate the probable age of wild and captive raised individuals. While some of these methods have been found to be more successful than others, no method has been able to consistently predict accurate age estimates for individuals. More popular methods cited by other researchers include mark-release-recapture, scute annuli counts, carapace/and or plastron measurements, scute wear assessments, and skeletal changes (Zug 1991). However, without known-aged individuals as a reference, no method of age estimation is precise.

Another way to estimate age for some reptilian and amphibian species is skeletochronology. This method (by which incremental marks of skeletal growth (MSG) can be counted in a cross-section of a long bone) has been proven to be a reliable age indicator in some species. While this technique has been used extensively in other taxa, it has never been used in *Gopherus polyphemus* or any fossil chelonian species.

It is my intent to evaluate the use of skeletochronology as a viable technique to estimate the ages of the fossil tortoise species *Gopherus laticuneus* (Cope), *Stylemys nebrascensis* (Leidy) and the extant tortoise species *Gopherus polyphemus* (Daudin). Skeletochronology data collected in this study were also cross-referenced with two other aging techniques, to compare similarities and differences in the methods. Finally,

plausibility and the extent to which skeletochronology may be implemented were also addressed.

*Gopherus laticuneus* and *Styemys nebrascensis* are common in the White River Group badlands of the North American High Plains. Both species have a geologic range spanning the Chadronian/Orellan (Eocene/Oligocene) boundary interval (Hutchinson 1992, 1998; Prothero and Swisher 1992). They were chosen because of the excellent preservation of fossil materials and the abundance of available specimens.

*Gopherus polyphemus* represents the modern analog. It is found exclusively in the southeastern United States, ranging from southern South Carolina south to Dade County, Florida; and west to the eastern portion of Louisiana (Auffenberg and Franz 1982; Franz and Quitmyer In press). This species was chosen because of its close relationship to the fossil *Gopherus* species, the relative similarity between the modern tortoise's environment and the proposed paleoenvironment of the fossil species, and the relative abundance and accessibility of materials.

### **Importance of this Research**

The objective of this project is to test the validity of skeletochronology as an accurate measure for aging tortoises, both fossil and extant. While this method has been used in some amphibians, reptiles, dinosaurs, mammals and even birds, skeletochronology has been largely overlooked for aging tortoises. The few published studies using tortoises mainly reveal positive results (Grubb 1971; Castanet and Cheylan 1979; Germano 1988). Having an accurate method for aging tortoises is extremely important in demographic studies. Information on age at sexual maturity, maximum age in the wild, and growth differences between sexes can all be deduced using skeletochronology.

Another aspect that could be examined in the fossil record is why *Stylemys* went extinct and why *Gopherus* evolved at this time interval. As previously discussed, the climate and the environment changed distinctly over the Eocene-Oligocene boundary. The fossil record also shows a decrease in the genus *Stylemys* shortly after the boundary and an increase in the number of *Gopherus* specimens present. Skeletochronology may lend assistance in determining why this change in genera occurred.

### Overview of the Nebraska Badlands

The White River Group is made up mainly of volcanoclastic, fluvial, eolian and lacustrine sediments that have accumulated across the mid-continent of North America from the late Eocene to the early Miocene (37 to 29 million years ago.) Outcrops of these stratigraphic units are most commonly seen in Nebraska, South Dakota, Colorado, Montana, and Wyoming (Terry et al. 1998).

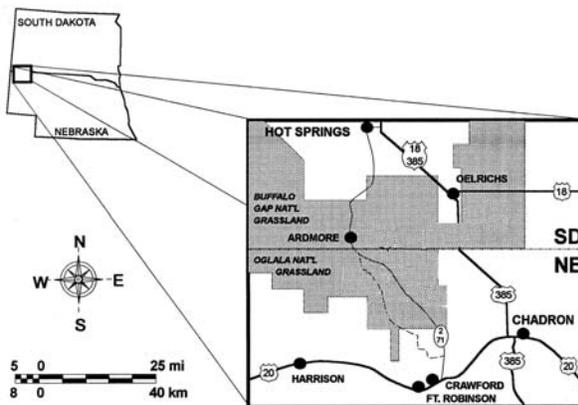


Figure 1-1. Map of northwestern Nebraska field area (Terry and LaGarry 1998; Figure 4 on page 124).

The Chadronian/Orellan boundary, which coincides with the Eocene-Oligocene transition, has been placed at  $33.59 \pm 0.02$  Ma (Prothero 1994; Obradovich et al. 1995; Terry 1998). The type section for the Orella member lies in outcrops near Toadstool Park in Sioux and Dawes counties of northwestern Nebraska. The outcrops form the base of

the Pine Ridge escarpment throughout the area, and are a result of regional uplift during the Laramide Orogeny. Subsequent uplift during the Cretaceous resulted in the retreat of the Cretaceous Interior Seaway, exposing and weathering Cretaceous-age sediments. The altered late Cretaceous Pierre Shales occur at the base of the White River Group, and are unconformably overlain by the Chadron and Brule formations (the latter consisting of the Orellan, and Whitneyan members). In northwestern Nebraska, the Arikaree Group overlies the Whitney member. In most exposed areas, the White River Group is overlain by more-resistant brown siltstone and sandstone layers, which form buttes and tables, or steep sloping spirals across the outcrop (Terry 1998).

The Chadron Formation of northwestern Nebraska is divided into two distinct layers based on lithology, color, and erosional surfaces. The lower unit, known as the Peanut Peak Member, is predominantly composed of a bluish-green to gray hummocky mudstone. The smectite-rich layer can be up to 8.65 m. thick, and weathers into haystack like hills that have a characteristic popcorn-like surface (Terry 1998).

The upper layer of the Chadron is composed of variegated silty claystone, siltstone, and isolated channels of sandstone bodies (Terry and LaGarry 1994; Terry 1995, 1998; Terry et al. 1995). The Big Cottonwood Creek Member, as named by Terry and LaGarry (1998), contains various purplish-white layers that are composed of gypsum, volcanic ash, and limestone (Schultz and Stout 1955). The uppermost portion of the Big Cottonwood Creek Member also coincides with the Eocene-Oligocene boundary at approximately 34 million years ago.

The division between the Chadron and the overlying Brule formations can be marked by a change in lithology, and has been dated by  $^{40}\text{Argon} / ^{39}\text{Argon}$  dating and can

be seen in Figure 1-2 (Swisher and Prothero 1990). Sediments change from a variegated silty mudstone and claystone; to a tan and brown clayey siltstone, siltstone, sheet sandstone, and channel sandstone complex. Schultz and Stout (1955) originally separated the Chadron and Brule formations by the “Persistent White Layer” or “Purplish White Layer” (PWL), which is composed of volcanic ash, or tuff. After more careful analysis, the actual separation occurs 10-15 meters above the PWL (Swisher and Prothero 1990; LaGarry 1998).

Dating of ash layers has provided accurate ages for the different members of the Brule Formation. A detailed geochronology of the Eocene-Oligocene boundary was decided upon at a special meeting at Gubbio and Massignano, Italy, in the late 1980s. Based on the decisions made at that meeting, Swisher and Prothero (1990) were able to publish an agreed upon date of the Oligocene ash layers. Previously  $^{40}\text{Ar}/^{39}\text{Ar}$  dating of biotite extracted from the PWL yielded an age of  $33.59 \pm 0.02$  million years ago (mya). The correct Eocene-Oligocene boundary is now located 10 to 15 m. above PWL and has been recalibrated at 33.7 mya. The Orellan-Whitneyan boundary has been shifted to 32.0 million years ago, and the Whitneyan-Arikarean boundary has been placed at 20.0 mya (Prothero and Whittlesay 1998).

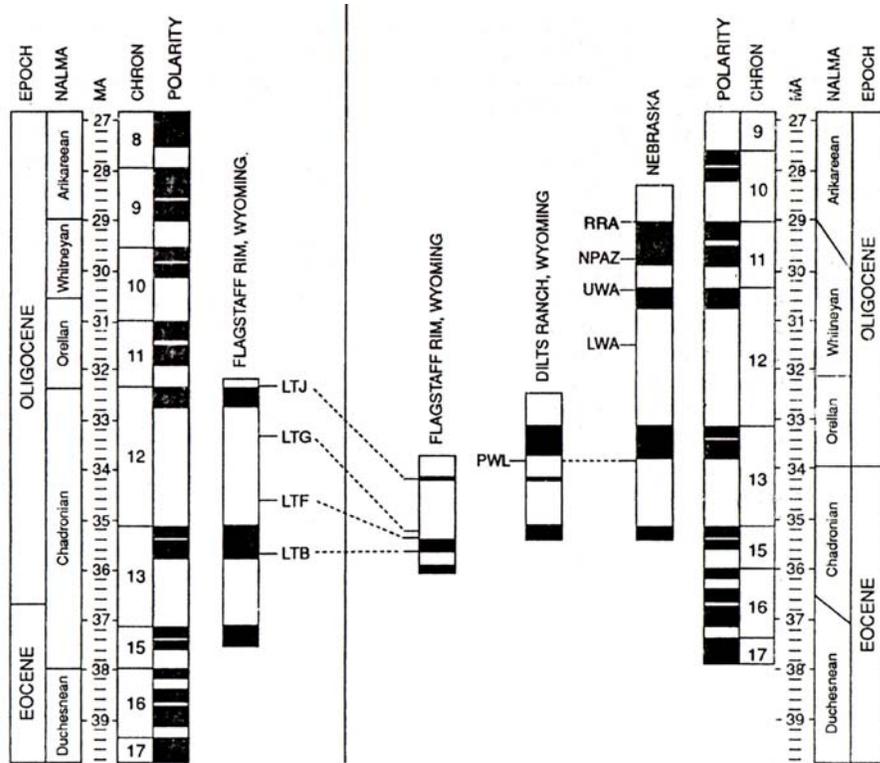


Figure 1-2.  $^{40}\text{Ar}/^{39}\text{Ar}$  Dating of the White River Group (Swisher and Prothero 1990; Figure 1 on page 761).

The lower unit of the Brule Formation in northwestern Nebraska is formally known as the Orellan member. Two distinct zones can be distinguished across the exposed outcrop. The lower layer contains brown-orange and brown volcaniclastic clayey siltstones and silty claystones, sheet sandstones, and a distinct volcanic ash (known as the serendipity ash.) The upper layer consists of single and multistoried channel sandstones (LaGarry 1998).

The upper unit of the Brule Formation is named the Whitney member. Like the Orellan member, the Whitney is also divided into two distinct layers, both of which are composed mainly of sandstones and siltstones. The upper layer contains the upper and lower Whitney ashes. The lower ash layer is above the boundary between the Whitneyan and Orellan. This boundary is characterized by intertonguing ash except where the

Whitneyan channel cuts dip down into the upper Orellan, which can be seen in Figure 1-3 (LaGarry 1998).

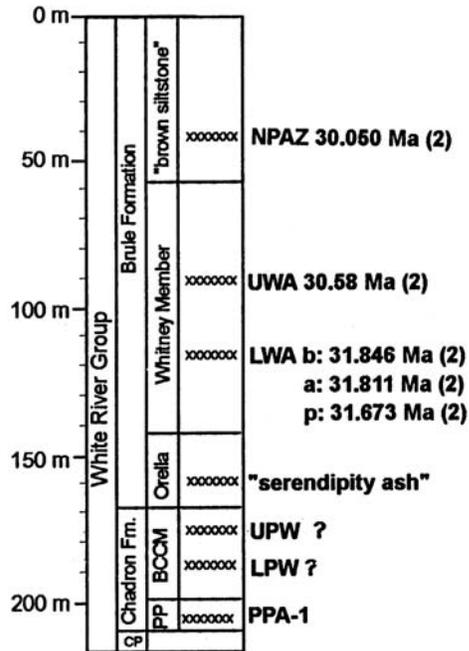


Figure 1-3. Stratigraphy of the White River Group at Toadstool Park, NE (Terry and LaGarry 1998; Figure 9 on page 133).

Based on paleosols studied from Badlands National Park, South Dakota, the White River Group represents an extensive alluvial plain (Retallack 1983). Reddish-brown paleosols found in the late Eocene are thought to be a result of sediment accumulations along the banks of rivers during this period. Most paleosols are eroded, redeposited materials that were reworked by an extensive alluvial system that was present in this area. More sedimentation is thought to have occurred in areas that were sparsely vegetated as opposed to heavily wooded areas surrounding streams. Retallack figured that sparse vegetation allowed for more sedimentation/erosion to occur, with wooded areas being more stable.

Fossil root and seed traces during this period represent a habitat dominated by herbaceous vegetation. As drying continued throughout the Orella and Whitney members, shrub dominated plains became more prevalent with woodlands becoming more concentrated around streams. Woody plants indicative of this time period include the hackberry (*Celtis hatcheri*), for which fossilized seeds have been recovered. (Retallack 1983).

### **Modern *Gopherus polyphemus* Samples**

I used samples for thesis work, from gopher tortoises only found in the north-central portion of Florida. The northern-most samples were collected in High Springs, Florida and the southern most samples were collected near Melbourne, Florida. Samples were also collected from Rattlesnake Island in the Fort Matanzas National Park near St. Augustine, Florida. Traditionally, these tortoises are found in sandy upland areas of pine (*Pinus* spp.) and oak (*Quercus* spp.) with an under story of wiregrass (*Aristida* spp.), beach scrub, oak hammocks, or pine flatwoods (Auffenberg and Franz 1982; Ernst et al. 1994). Annual precipitation levels over the range of *G. polyphemus* are between 1162-1593 mm. (Germano 1994).

*Gopherus polyphemus* is an avid burrower and may keep several burrows active at any given time. Active and abandoned burrows are used, not only by the tortoise, but also by a host of other vertebrate and invertebrate species. In Florida, tortoises are active most of the year, retreating to their burrows at night and only coming out for portions of the day. When they are above ground, *G. polyphemus* spends most of its time basking and searching for food or feeding (Smith 1992; Ernst et al. 1994). Although there is little evidence on the longevity of *G. polyphemus*, captive raised *G. berlandieri* (the Texas

tortoise) have been documented at ages exceeding 52 years of age (Judd and McQueen 1982).

### **Fossil Tortoise Background**

Tortoises do not have a very long fossil record when compared to other chelonian groups. Tortoise origins probably lie in the mid Eocene (50 mya) with the fossil genus *Hadrianus*. Specimens have been found across North America and Europe. The genus is probably very closely related to the modern genus of *Manouria* (Auffenberg 1974; de Broin 1977; Hutchinson 1980; McCord 2002). While turtles in general are relatively abundant in the fossil record, complete specimens (including skulls, shells, and limbs) that allow proper identification are extremely rare. Therefore, the precise taxonomic placement for *Hadrianus* is unknown. This genus was probably a subtropical group that originated in Asia and dispersed to North America. By the late Eocene, the genus *Styemys* and possibly *Gopherus* split from *Hadrianus* and begins a radiation that continues for millions of years.

The genus *Styemys* encompasses a number of species that span from the late Eocene through the Miocene (40 to 10 mya; McCord 2002). *Styemys nebrascensis* is one of the most common turtle fossils in North America. It was the first fossil turtle described in the United States by Joseph Leidy in 1851 (Hay 1908, Hutchinson 1996). Specimens have been found throughout North and South Dakota, Wyoming, Colorado, and Nebraska for over 150 years. The geologic range of the species occurs in the Chadron Formation through the Orellan member of the Brule Formation, which samples a period of 4 to 5 million years between the late Eocene and early Oligocene.

*Styemys* is defined by a number of diagnostic characteristics, which can be observed in all relatively complete specimens. Instead of listing all of the characteristics

that are species specific, I will only use those that aided me in identification of species.

Distinguishing Features: The normal neural formula for the species = 4(6(6(6(6(6(6(6 or 4(8)4(6(6(6(6(6. The number after the parentheses is indicative of the number of sides per neural bone starting from the first neural. In all specimens the posterior epiplastral excavation is shallow or absent (Auffenberg 1964). The nuchal scale is longer than it is wide. The anterior lobe of the plastron is wider than it is long. Also, the shape of the humeral head in *Stylemys* is compressed dorso-ventrally in adults (Auffenberg 1964). A proportionately thicker and more rounded shell is diagnostic of the species (Hay 1908; Hutchinson 1996). The carapace of these individuals may reach or exceed lengths of 530 mm. Finally, the square, boxed-off shape of the gular projection is extremely different from that of *Gopherus*.

The genus *Gopherus* first appears in the fossil record during the late Eocene (about 34 mya) with *Gopherus laticuneus*. Although it is unknown if the genus is descended from *Stylemys* or from an as-yet determined ancestor, the genus did overlap with *Stylemys nebrascensis* and they do share a number of similar characteristics (Hay 1908; Auffenberg 1964). Traditionally, both species have been very closely linked because both have the shared-derived character of having a premaxillary ridge in their upper jaw. Unfortunately, as stated earlier, tortoise skulls are quite rare in the fossil record and a more comprehensive and conclusive studies need to be undertaken. While the presence of a premaxillary ridge is a derived character in most tortoises it may be a primitive character in North American tortoises (Crumly 1994; McCord 2002). *Gopherus laticuneus* is the most primitive species of the genus, which includes four extant species (*G. polyphemus*, *G. flavomarginatus*, *G. berlandieri*, and *G. agassizii*). It has been

placed as a separate subgenus *Oligopherus* (Hutchinson 1996). McCord (2002), however, feels that this change needs to be tested before this arrangement is accepted. Relationships of this new clade have not been tested and the assignment is based on a lack of characters rather than a presence of characters. More traditional views of the species place it as a more basal form to all other species of the genus that proceed in time (Bramble 1971; McCord 2002).

The following characteristics that define *Gopherus laticuneus* can easily distinguish specimens from those of *S. nebrascensis*. Distinguishing features: The normal neural formula found = 6)6)4(6(6(6(6(6 or 4(8)4(6(6(6(6(6. The posterior epiplastron excavation is relatively shallow (Hutchinson 1996). In contrast to *Styemys*, the nuchal scale of *G. laticuneus* is short and rather wide and the shell is generally much thinner. Another suite of characteristics, that are found in most specimens are overly pronounced and toothed epiplastral extensions (or beaks) as well as extended and toothed xiphiplatra in the plastron.

### **Paleoenvironment and Paleoclimate**

The use of skeletochronology relies on seasonal cycles to preserve marks of skeletal growth (MSG). Therefore, information on the paleoclimate during the Eocene-Oligocene transition is important to my thesis. Seasonal variation is very important for production and definition of MSG (Castanet and Smirina 1990). The Eocene-Oligocene transition throughout the geologic record shows a major shift in climatic conditions. Work completed on paleosols from the Big Cottonwood Creek member (late Chadronian) show a gradual change from humid, forested conditions to more seasonal, semi-arid conditions occurring in the early Oligocene (Terry 2001). This change was originally interpreted as the “Terminal Eocene Event” when it was first detected. After redefinition

of the Eocene-Oligocene boundary, the event has now been termed the “Early Oligocene Event” (Prothero and Heaton 1996; Terry 2001).

Marine sediments from this period present a large shift in the oxygen isotope record, which may reflect an overall cooling period in the early Oligocene (Shackleton and Kennett 1975; Miller 1992; Zachos et al 1992; Prothero 1994; Zachos et al. 2001). A positive shift in the oxygen isotope record of 1.3 parts per million (ppm.) has been recorded in benthic foraminifers. Miller (1992) has deduced that 0.3-0.4 ppm. of the shift may be due to an increase in Antarctic ice at this time, with the other 1.0 ppm. of change being a result of a decrease in the global temperature. It is speculated that the temperature of the badlands region in the early Oligocene around 16 °C, which is a decrease from an Eocene greenhouse (Berggren and Prothero 1992). Ice was present on Antarctica in the early Oligocene as determined by ODP ice cores that were taken on the continent in the late 1980s. Although the amount global ice volume is still debatable during the Oligocene, there was ice in the region as early as 33 million years ago (Zachos et al. 1992).

Carbon isotopes during this period also show evidence of deep-ocean circulation patterns in the early Oligocene. Miller (1992) has shown that pulses of cold, nutrient-depleted water began circulating toward the south from the Arctic and cold, nutrient-rich Antarctic water began circulating toward the north from the south. These deep-water flow patterns have also been indicated by unconformities at the Eocene-Oligocene transition in marine geologic records (Prothero 1994). All of these changes in ocean circulation, ice, and resulting changes in sea level drastically affected the climate globally, as can be demonstrated in the fossil record of North America.

Land plants during this period have been used to demonstrate the continental temperature shift across the Eocene-Oligocene period. Using a leaf margin index, Wolfe (1992) shows a significant temperature drop occurring in the early Oligocene. Fossil leaf impressions after the cooling tend to be a lot smaller than pre-cooling leaves, post-cooling leaves tend to have jagged margins rather than the smooth margins of earlier samples, and a distinct change from tropical to more deciduous trees has been noted. Work by Wolfe (1978, 1992), suggest a mean annual temperature shift in North America of 8-12 degrees Celsius in less than a million years (Prothero 1994).

Reptile and amphibian records show a decline in the number of species at this time due to the cooling and drying trends. Hutchinson (1982, 1992, 1996) has shown that the diversity of most aquatic reptiles and amphibians (including freshwater turtles, crocodiles, and salamanders) drop off severely at the end of the Eocene. Tortoises, however, being more adapted for life on land and drier climates, continue to be commonplace in the early Oligocene. As mentioned previously, *Styemys nebrascensis* evolved during the middle to late Eocene although it does not become common until the Eocene-Oligocene boundary. *Gopherus*, on the other hand, did not evolve until the latest Eocene and can be found in the upper Chadron and lower Brule Formations. Bramble (1971) and McCord (2002) suggest that *Styemys* is a more mesic-adapted species, while *Gopherus* is a more xeric-adapted species.

Retallack (1983, 1992) has taken the work of Wolfe a step farther and has used paleosols from the badlands of South Dakota and Nebraska to speculate on the paleoenvironment of the early Oligocene. Paleosols from the Chadron Formation of the late Eocene represent dense canopies of trees and an annual rainfall ranging from 500-

900 mm. per year (See Figure 1-4). After the Oligocene deterioration, however, paleosols of the Brule Formation are more representative of open woodlands and show annual rainfalls of less than 500 mm. per year (See Figure 1-5) (Prothero 1994).

Root traces also indicate a thinning of the forests during this period. Whereas the badlands area was a dense forest in the late Chadronian, then became more open, shrub dominated plains in the early Oligocene. Retallack (1983, 1992) has reconstructed an early Oligocene that was mainly dry, open savanna woodland cut by a number of braided streams. Trees were scattered and separated by large areas of shrubs.

### **Skeletochronology**

Skeletochronology is a method by which an estimate of the age of an individual can be determined from cyclic skeletal growth. As a skeletal element grows throughout the life of an individual, new layers of bone are added onto its outer surface. Knowing that these periosteal layers are annual, they can then be counted to estimate the age of the individual in question (G. Erickson, pers comm). Layers can be broken into new growth zones (MSG) and lines of arrested growth (LAG).

In many ways this technique can be compared with dendrochronology, where rings are counted in the trunk of a tree to determine its age. The drawback with skeletochronology, however, is the ability of bone to resorb and redeposit layers from the core of the bone outward. This leaves the researcher with an age minimum if resorption is not accounted for (Parham and Zug 1997). Thus, it is extremely difficult to age long-lived individuals unless one accounts for resorption. Refer to Francillon-Viellet et al. (1990) for a more complete review of the mineralization of skeletal tissues and skeletochronology.



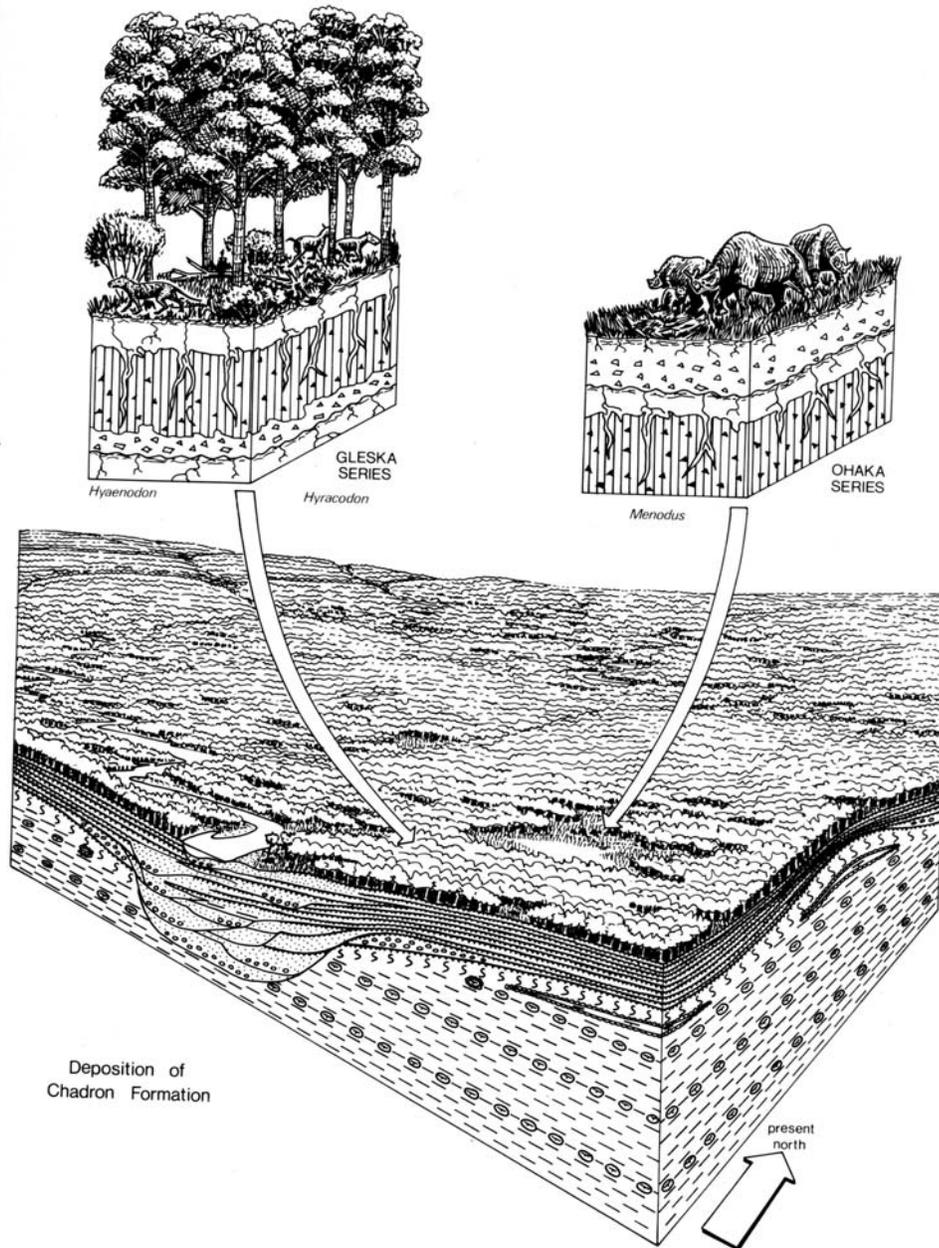


Figure 1-5. Paleoenvironment of Early Oligocene badlands of South Dakota (Retallack 1983; Figure 42 on page 51).

## CHAPTER 2 THE USE OF *GOPHERUS POLYPHEMUS* FOR BASELINE DATA

Baseline data were collected from extant tortoises before fossil specimens were sectioned for this project. The purpose for using modern *Gopherus polyphemus* was to determine if skeletochronology would be a viable method before cutting rare fossil specimens. *Gopherus polyphemus* was chosen as a modern analog for the following reasons: 1) although they are a species of special concern in Florida, carcasses are still relatively abundant, 2) they are closely related to the fossil species used in this project, and, 3) in a previous study, a closely related species (*Gopherus agassizii*) provided positive results for age determination (Germano 1988).

Some authors have recommended the use of long bones (i.e., humerus and/or femur) for skeletochronology while others have suggested using vertebrae, sclerotic rings, teeth (etc.) (Zug 1991). Therefore, I attempted to use a number of different skeletal elements to determine which would be the most advantageous to my research. It should also be noted that bones were taken from both the left and right sides of the individuals. Consistency is important to research and ideally individual bones from a given side of the body should be used, however most specimens were incomplete and alternating bones were used. This will not hinder my analysis, as all bones in the animals' body will grow at a steady rate (G. Erickson pers. comm). I also tested this notion on one of my specimens; for DJE-2002-3, both left and right humeri were sectioned and both consistently showed the same number of growth marks. Sets of bones from *G. polyphemus* specimens were chosen for sectioning. Humeri, femora, scapulae, ilia,

and vertebrae were collected, sectioned and examined for marks of growth. Initially, I predicted that bones from the pelvic or shoulder girdle might prove better for my research because those elements are preserved more regularly in fossil specimens. Articulated girdles tend to remain within the shell of dead specimens and provide for a better chance for preservation (personal observation). Upon examination of the scapula and ilium, they did have growth marks but, samples were found to have undergone more resorption and remodeling than other bones. Vertebrae were also highly remodeled and a majority of the growth marks were partially, or not visible. The humerus and femur proved the most effective in maintaining growth marks with the least amount of resorption and remodeling.

Based on the results of the initial evaluation of MSG, the humerus became the focus for this project. The decision between the humerus and the femur was based on the availability of material. In both modern and fossil samples, there were more humeri than femora available. Nevertheless, counts of growth marks in all bones that were recorded will be included below.

In addition to the use of modern *G. polyphemus* materials, I was also given a single sample of a known age *Geochelone elegans*, Indian Star tortoise, which had been raised in captivity by Ray Ashton. It was my hope that this known-age specimen could verify skeletochronological counts in other specimens. The humerus was sent along with the rest of the *G. polyphemus* specimens to be sampled.

Another advantage in using the modern gopher tortoise for this project is that there are more possibilities for parallel age estimates based on other methods of age calculation (Halliday and Verrell 1988). The most beneficial specimens would have been known-age

individuals (Castanet and Smirina 1990), unfortunately no such samples of *G. polyphemus* could be located and I had to rely on other techniques.

Scute ring counts have been used quite extensively in research involving chelonians. They are thought to be annual in most species and can be easily seen and counted. The drawbacks, however, include: loss of rings due to excessive wear, false rings being counted as annuli, and older individuals become difficult to age because of the closer spaces of the rings on the scute (Germano 1988). For this study, scute annuli provided me with a method to evaluate and cross-compare the age estimations I have made based on skeletal growth marks.

Scute annuli counts were accurate for most of the specimens, however one individual could not be aged using this technique. One large, gravid female proved to be too old and its scutes were too worn to count. Due to this problem, I decided to also evaluate age estimates with another method that is used regularly in studies of chelonians. Measuring straight-line carapace and/or plastron length has been used by a number of investigators to correlate size and age (Landers et al. 1982; Mushinsky et al. 1994). This can also be a reliable method of aging turtles given the proper circumstances. There are a number of drawbacks and restrictions however, that should be addressed.

Given that chelonians are ectotherms, size can vary based on the environment experienced by different populations. Average temperature, rainfall, vegetation levels, nutrition, etc. can all influence the growth rates of individuals (Gibbons 1976). Therefore, carapace and plastron lengths will vary from population to population throughout a given range. Plastron lengths in gopher tortoises are very limiting for another reason. The epiplastral extension (beak) of gopher tortoises is a highly variable

feature on an individual basis. Males tend to have a longer projection than females, although this is not a rule. Plastron length can be measured but its use in estimating age is suspect (Mushinsky et. al. 1994).

Carapace lengths tend to be more precise than plastron length for age reconstruction however, estimates are still relative and not absolute. Straight-line carapace length is a popular method among researchers. However, the drawbacks mentioned previously should be kept in mind and individuals from different populations should not be compared to one another unless they share a common geographic range/ or environment.

#### **Testing *Gopherus laticuneus* and *Styemys nebrascensis* for Marks of Skeletal Growth**

Based on results from work done with *G. polyphemus*, the humerus was chosen for the fossil research. I prepared the fossil specimens in the Vertebrate Paleontology prep lab at the Florida Museum of Natural History (FLMNH). Table 2-1 shows all the samples collected and the localities from which they came. After samples were embedded, cut, and polished they were examined for marks of skeletal growth. The fossil specimens showed growth marks similar to those documented in *G. polyphemus*. The growth marks analyzed within the long bones of the fossils were compared to age estimates based on plastron and carapace lengths. Scutes do not preserve in the fossil record because they are keratinous. As such, scute annuli counts are not an option when working with fossil species. Measurements of shell dimensions can be compared; however, there are no known shell length-age classes for these species. The lengths recorded and compared in this study are the first documented comparison of shell lengths and age in these fossil tortoise species.

In the *G. laticuneus* and *S. nebrascensis* specimens the plastron of individuals tends to preserve better in the fossil record than the carapace. Deformities in the fossilization process tend to compress and misshape the carapace in fossil tortoises (personal observation). In many cases, shell length estimates had to be addressed. In the fossil specimens, available shell fragments were compared with specimens that were better preserved. Therefore, a majority of fossil tortoise shell estimates of length are not exact, but still provide valuable information.

Table 2-1. Fossil tortoise specimens, identifications, and locality information.

Specimen	Species	Locality
DE-2002-1	<i>G. laticuneus</i>	Horse Hill Low
DE-2002-2	Unknown	Horse Hill Low
DE-2002-3	<i>G. laticuneus</i>	Horse Hill Low
DE-2002-4	<i>S. nebrascensis</i>	Horse Hill Low
DE-2002-5	<i>S. nebrascensis</i>	Horse Hill Low
DE-2002-6	Unknown	Horse Hill Low
DE-2002-7	<i>S. nebrascensis</i>	Bald Knob High
DE-2002-8	<i>G. laticuneus</i>	Bald Knob High
DE-2002-9	Unknown	Sagebrush Flats
DE-2002-10	<i>G. laticuneus c.f.</i>	Sagebrush Flats
DE-2002-11	<i>G. laticuneus</i>	Sagebrush Flats
DE-2002-12	<i>S. nebrascensis</i>	Sagebrush Flats
DE-2002-13	<i>S. nebrascensis</i>	Sagebrush Flats
DE-2001-14	<i>S. nebrascensis</i>	Turkeyfoot East High
DE-2002-15	<i>S. nebrascensis</i>	Orellan Pasture 33B low
DE-2001-16	<i>S. nebrascensis</i>	II #2 Pasture 33B low
DE-2001-17	<i>G. laticuneus c.f.</i>	Pasture 33B low
UF 20975	<i>G. laticuneus</i>	Unknown
UF 191470	<i>S. nebrascensis</i>	Turkeyfoot East High
UF 201906	<i>S. nebrascensis</i>	Sagebrush Flats #2
RF-NE-Orel-37	<i>S. nebrascensis</i>	Turkey Foot
RF-NE-Orel-74	<i>G. laticuneus</i>	Pettipiece west in Basin
RF-NE-Orel-39	Unknown	Turkey Foot above PWL
RF-NE-Orel-42	<i>G. laticuneus</i>	Turkey Foot
RF-NE-Orel-66	<i>S. nebrascensis</i>	Turkey Foot East
RF-NE-Orel-12	<i>S. nebrascensis</i>	Bald Knob East Butte (N. face)

### CHAPTER 3 COLLECTION OF *GOPHERUS POLYPHEMUS* DATA

The remains of *Gopherus polyphemus* specimens came from private collections as well as carcasses that were collected by Richard Franz and myself. The FLMNH collections were not available for study due to the destructive nature of the skeletochronology process. As a result, under the FLMNH Florida Fish and Wildlife Conservation Commission collection permit #WS01058 I was able to salvage carcasses that were either, mortally wounded on roads, killed by predators, or burned in wildfires.

Two adult males and a hatchling were loaned from the Chelonian Research Institute in Oviedo, Florida, under the direction of Peter Pritchard. One female and one juvenile were collected by Karen Frutchey, a graduate student at University of Central Florida, in the National Archie Carr Reserve near Melbourne, Florida. Franz and I collected three road kill females on roads in Alachua County. Boyd Blihovde (a park ranger from Wekiwa Springs, near Orlando, Florida) donated another road kill female. Dick Franz and I collected the remaining carcasses on Rattlesnake Island within the Ft. Matanzas National Park at St. Augustine, Florida. This was in conjunction with Dave Parker, a ranger at the park, and Federal Permit #FOMA-2002-SCI-0001. Ray Ashton also donated one specimen of a known-age *Geochelone elegans*.

Before bones could be measured and sectioned all individuals needed to be prepared. Some of the carcasses collected were skeletonized either naturally or by them in screen cages outside for a period of 6 weeks. Individuals were then rinsed in a

weak solution of industrial strength soap or bleach and scrubbed with a soft brush to remove any remaining tissue. Bones were then dried under a heat lamp.

All individual bones were then measured for length and width. The diameter of the diaphyseal shaft of the humerus is especially important when estimating resorption of growth lines in thin section. However, there is no correlation between individual bone size or length and age (Castanet and Cheylan 1979).

For preparation of the thin sections, all samples were sent to Matson's Laboratory, LLC of Milltown, Montana. Following standard procedures, bones were cut, embedded in paraffin, and injected with hematoxylin dye (Castanet and Cheylan 1979; Zug et al. 1986; Chinsamy and Raath 1992). The dye is an important aide in making the annual growth marks more distinguishable. The sections were then embedded in plastic and mounted on petrographic slides. For further discussion of the technique see the Matson's website at [www.MatsonsLab.com](http://www.MatsonsLab.com). Before the slides were returned, Gary Matson determined age estimates for all specimens by skeletochronology. As for the *G. elegans* specimen, the actual age was withheld from both Mr. Matson and me until it could be analyzed microscopically. This information was withheld to test the validity of skeletochronology.

Upon receiving the sectioned specimens I independently determined individual age estimates for all bones. The marks of skeletal growth were identified using a compound microscope and were counted on two separate occasions. The average of the two counts provided the age estimates for all individuals. It should be noted that no set of growth mark counts varied by more than 1-2 lines in between counts, meaning that counts were consistent. In previous studies, the phenomenon of double rest lines has been observed

(Castanet and Smirina 1990). These non-periodic lines, which can be a result of a double annual growth cycle, were not found in specimens analyzed during this study. In addition to counting the number of growth marks, I measured the widths of all increments from the center of the bone.

In order to measure the growth marks and account for resorption, two separate protocols were employed. First, the method published by Parham and Zug (1997), based on work done with fish otoliths, was tested. All counts and measurements used growth marks found on the ventral side of the bone. Due to resorption, growth layers are not equally spaced around the circumference of the humerus (Parham and Zug 1997). When looking at a bone in thin section, the growth marks persist longer on the dorsal and ventral sides of the bone (also known as the short axis). Therefore, I recorded the radius of the humerus as half the diameter of the resorption core plus the sum of the growth marks on the ventral half of the bone.

Resorption of growth marks (= periosteal layers) lost into the resorption core of the bone is a major problem in age estimation. The Parham regression protocol, which is used by fish specialists, assumes that growth layer width declines with age, and the slope of a regression curve for the declining growth rate can be identified by using the radius or diameters of the element at different consecutive ages and the subsequent growth layer widths from each of these radii (Ralston and Miyamoto 1983; Parham and Zug 1997). To determine the number of lost layers, the radius of the resorption core is substituted with the regression equation Eq. 3-1:  $\text{Radius} = \text{hatchling radius} + [(\text{slope}) * (\text{number of lost layers})]$  and the number of lost layers =  $(\text{radius} - \text{hatchling radius}) / \text{slope}$ . The number of lost layers is then added to the number of observed layers to derive an age estimate or

total number of layers. In bone growth, early periosteal layers tend to be tightly packed followed by a number of layers that are more widely spaced. These widely spaced layers continue until the animal reaches sexual maturity, at which point they tend to become closely spaced again (G. Erickson, pers. comm). The variation in growth line width may lead to an exaggeration in the number of total growth marks (both present and reabsorbed) when the mean width is calculated. Therefore, I have found that this method tends to overestimate age much more so than the second protocol known as the average layer thickness (Castanet) protocol.

The second protocol is the most commonly used model for estimating loss of growth marks in reptile and amphibian studies. The average layer-thickness (Castanet) protocol uses the mean width of the three existing innermost layers, which is then divided into the radius of the bone's short axis, which provides an estimate of the number of resorbed marks (Castanet and Cheylan 1979; Zug et al. 1986; Castanet and Smirina 1990; Parham and Zug 1997; Erickson and Tumanova 2000). While my findings show this to be a more appropriate protocol, Parham and Zug (1997) caution that it may yield an overestimate of growth mark counts because they were evidently not aware of the closeness of young rings and its impact in their equation.

Scute ring measurements were also collected and correlated with skeletochronology age estimates. On the shells of many chelonians, concentric rings form on each individual scute. Many researchers have found that these rings (=annuli) are annual in some species and can be positively correlated with age up until a given point, usually 20 years of age (Cagle 1946; Sexton 1959; Castanet and Cheylan 1979; Judd and Rose 1983; Galbraith and Brooks 1987; Germano 1988). The second costal scute of the carapace was

chosen to count rings following Germano (1988). This scute was chosen for two reasons: the carapace receives much less wear than the plastron allowing for preservation of scute annuli and the second costal is much squarer than others, making the annuli easier to distinguish.

True rings were distinguished from false rings based on descriptions by Legler (1960) and Landers et al. (1982). Annual rings were counted if they formed a deep groove around the entire scute, as shown in Fig. 3-1. I made counts on two separate occasions and the average of the two counts was used as the age estimate. These results were then matched with the growth mark counts taken from long bones.

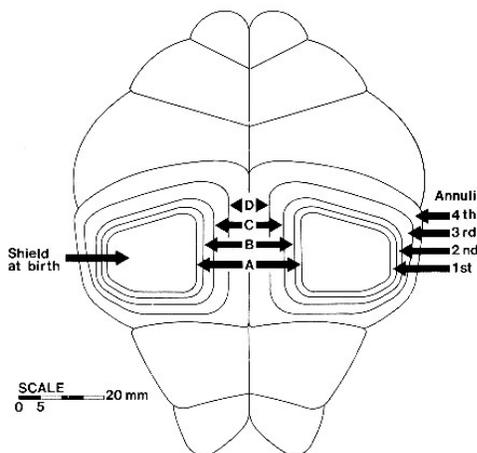


Figure 3-1. Measurement of scute annuli on the plastron (Landers et al. 1982; Figure 1 on page 84).

The other set of measurements taken from *G. polyphemus* specimens includes shell dimensions from all of the samples. Measurements taken include: straight-line carapace length (SCL), straight-line plastron length along suture (PL), and the length of hyoplastron at the suture. Some studies have shown that carapace and/or plastron length are suitable for age/size relationships (Landers et al. 1982; Mushinsky et al. 1994). The latter measurement (hyoplastron) was taken in order to check the accuracy of calculating

carapace length based on plastron elements as published in Franz and Quitmyer (In press).

Straight-line carapace lengths were recorded by placing large calipers at either end of the tortoise's shell at the midline. This measurement was taken instead of the alternative, which is an over-the-shell measurement using a measuring tape, because of the increased chance of error in broken or misshapen shells. Plastron lengths were also recorded in a similar fashion. Large calipers were used to take a straight-line measurement down the midline suture of the plastron including the gular extension. Small calipers were used to take measurements of the hyoplastron. Lengths of these elements were taken down the midline suture as per Franz and Quitmyer (In press).

#### **Collection of *Gopher laticuneus* and *Styemys nebrascensis* Data**

I collected the fossil tortoise specimens in the summer of 2001 with the aide of Bruce MacFadden and volunteers that were on the annual Pony Express trip to the Nebraska badlands. Specimens were recovered from the property being leased by Barbara and Reed Toomey and on Forest Service land near Toadstool Park outside of Crawford, Nebraska (see Figure 1-1). Specimens designated with the field code RF-NeOrel are on loan from the FLMNH (Nebraska) collection maintained by Richard Franz. The main sites of collection for the summer 2001 collections include: Horse Hill Low, Turkey Foot East High, Sagebrush Flats, and Bald Knob High and the Pettipiece family ranch.

As mentioned previously, all fossil materials are from the Chadronian and Brule Formations of the White River Group. For verification in the field, the upper purplish white layer (PWL) was located and only materials above it were collected. Most samples come from the "turtle-oreodont" zone in the boundary area between the Chadron

Formation and the Orellan member of the Brule Formation. Fossils were all well below the Whitneyan-Orellan boundary. This transition is very obvious as there is a distinct change in sedimentology at the boundary. GPS coordinates were also taken at the site of each fossil discovery for reference.

Specimens were either bagged or jacketed in the field and transported back to the FLMNH. Some were photographed in the field, for verification, before collection. I prepared all specimens to recover humeri, took shell measurements, and identified individuals to species. Preparations were performed using a dremel tool, dental picks, an air scribe, and various adhesives in the prep lab at FLMNH with the help of Russ McCarty.

Before sectioning, fossil bones were measured in the same way that modern samples were. Specifically, the lengths and widths of bones (or the remaining portions of bones) were recorded. No discrimination was made as to whether the right or left humerus was used due to the extreme rarity of fossil tortoise limb bones. Rough cuts were made on a rock saw prior to embedding to get a clean surface in the mid diaphyseal shaft (See Figure 3-2). Sections are cut from the mid-shaft to avoid remodeling that may occur near the proximal or distal end of the long bone (Parham and Zug 1997). Humeri were then put into molds and embedded in Pour-a-Cast clear plastic. Samples were then allowed to cure for a few days to ensure hardening.

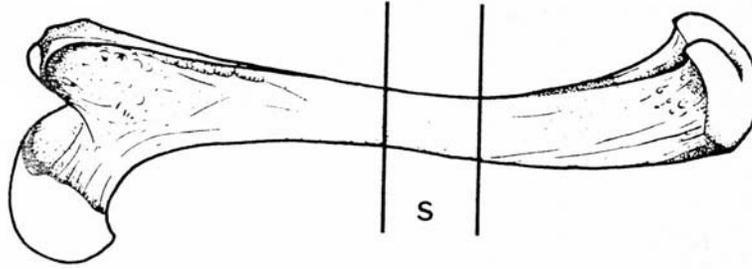


Figure 3-2. A mid-diaphyseal cut is made on the humerus (Castanet and Cheylan 1979; Figure 1 on page 1651).

A slow speed Isomet saw made by Buehler was used to cut 1-3 mm sections from the humeri samples. The plastics used in the embedding process did not allow for finer cuts, as section warping was visible in thinner samples. Sections were then mounted on petrographic slides using a two-part epoxy manufactured by Logitech. Slides were then allowed to cure for a few days before grinding.

Slide grinding was performed at the laboratory of Gregory Erickson at Florida State University under his guidance. Slides were sanded on tabletop grinders using different grits in a fining up sequence. A coarser paper (600 grit) was used initially to remove excess material, and finer papers (800-1200 grit) were used in preceding succession to remove any coarse grooves or imperfections left behind. For fossil slides, most were sanded down to a thickness around 100 micrometers or less. Slides were then viewed under a compound microscope to count and measure growth marks. Measurements were taken in an identical manner to those described for *G. polyphemus*.

In addition to the measurements taken from the humeri of the fossil tortoises, plastron and carapace lengths were also recorded. Plastron lengths were more easily obtained due to better preservation of the flat elements. Carapace shape and structure

was not preserved in a number of samples. Therefore, most shell lengths had to be estimated. Preserved materials were compared to complete specimens in order to estimate the size of the shells in question.

Both extant and fossil tortoise annual growth mark counts were correlated and analyzed. Modern *G. polyphemus* growth mark estimates were matched with scute ring counts in order to test the validity of both methods. Plastron and carapace lengths were also matched with growth mark estimates in both fossil and extant species to compare size-age relationships. Published size-age correlations were used to estimate ages of individuals in this study (Landers et al. 1982; Mushinsky et al. 1994).

CHAPTER 4  
*GOPHERUS POLYPHEMUS* BONE TESTING

To test the validity of different bones and because of the constraints of available materials, I decided to test a number of skeletal elements. A selection of bones, including the humerus, femur, scapula, ilium, and vertebra, was chosen to represent all different aspects of the skeleton. Results show that the humerus and femur are the best bones for gopher tortoise skeletochronology. Table 4-1 shows all counts of visible skeletal growth marks made in the different bones of *G. polyphemus* that were sent to Matson's Laboratory for sectioning.

Table 4-1 also shows the sex of each individual, although juveniles cannot be sexed. A series of *Gopherus polyphemus* specimens including: four adult males, four adult females, five juveniles, and a hatchling were sectioned to test the viability of skeletochronology in a tortoise population. Under each element heading, the number of visible growth marks are shown and the side from which the skeletal element is identified. It should also be noted that in specimen DJE-2002-3, I sampled both the left and right humeri to test the variability between left and right sides. In this specimen, both the left and right humerus showed the same number of visible growth marks.

While Matson's provided interpretations of growth marks in vertebrae, I was unwilling to commit to those counts after a personal inspection. There is a very high degree of resorption and remodeling in the tortoise vertebrae that is unparalleled in other elements. I assert that vertebrae are not good indicators when performing

skeletochronology in *Gopherus polyphemus*, which also probably holds in other chelonian species.

The one sample of *G. elegans* was also included in Table 4-1. Skeletochronology counts and scute annuli were both compared. Unfortunately, due to the climatic conditions in which this specimen was held, the results were not at all meaningful. The individual was kept in an artificial summer-like climate year round to enhance growth, thereby excluding any seasonal cyclity.

Table 4-1. *Gopherus polyphemus* identification number, sex, visible MSG counts for each bone, and side tested.

Specimen	Sex*	Humerus/Side	Femur/Side	Scapula/Side	Ilium /Side	Vertebra
DJE-2002-1	F	18 left	21 right	17 right	21 left	9-11 rings
DJE-2002-2	M	10 right	11 right	7 right	8 right	10-12 rings
DJE-2002-3A	F	9 left			6 left	7-9 rings
DJE-2002-3B		9 right	13 right	6 right		
DJE-2002-5	M	7 right	6 right	7 right	7 right	5-7rings
DJE-2002-6	J	3 right	0 right	0 right	0 right	0-1rings
DJE-2003-9	J	6 right	7 left	6 right	8 right	N/A
DJE-2003-10	J	6 right	8 left	4 right	7 right	N/A
DJE-2003-12	F	8 right	7 left	5 right	7 right	3-5 rings
DJE-2003-14	J	7 left	N/A	6 right	7 right	8-10 rings
DJE-2003-15	F	7 right	6 right	6 right	5 right	N/A
DJE-2003-16	J	10 right	7 right	5 right	4 left	N/A
PPC 6669	M	12 right	14 right	15 right	14 right	N/A
PPC 6674	M	17 right	21 right	18 right	21 right	15-17 rings
PPC 3510	H	0 yrs	N/A	N/A	N/A	N/A
<i>G. elegans</i>	J	0 right	0 right	0 right	0 right	N/A

\* M=Male, F=Female, J=Juvenile and H=Hatchling

### Humerus Growth Mark Counts and Distance Measurements

In all samples, the scapula and ilium were inconsistent with respect to the number of growth marks found in the humerus and femur. The shapes and function of these bones lead to highly variable remodeling and resorption (Gary Matson pers. comm). Long bones, such as the humerus and femur, with their more cylindrical shafts tend to

have slower and steadier rates of remodeling and resorption (Klinger and Musik 1992). Therefore, I recommend using either the humerus or femur elements while studying skeletochronology in tortoises. I chose the humerus because of the relative abundance (availability) of bones in both modern and fossil specimens.

The next step in the process is to account for resorption of growth marks within each bone. Tables 4-2 and 4-3 list the specimens, and display visible growth mark counts, distances of each mark as specified by the equation, and the number of resorbed rings calculated per the two different methods. It should be noted that all growth mark counts are  $\pm 1$  growth line. This range is to account for the partial year in which the animal died (G. Erickson pers. comm).

Table 4-2. *Gopherus polyphemus* age estimates based on the protocol published by Castanet and Cheylan (1979).

Specimen	Radius (mm)	Distance of Growth Marks from the Center of the Bone (mm)																		Avg Width of Marks 1-3 (mm.)	Resorbed Marks	Age (yrs.)
		2.51	2.65	2.74	2.82	2.89	3.03	3.4	3.5	3.56	3.61	3.64	3.66	3.69	3.7	3.73	3.76	3.79	3.82			
DJE-2002-1	3.875	2.51	2.65	2.74	2.82	2.89	3.03	3.4	3.5	3.56	3.61	3.64	3.66	3.69	3.7	3.73	3.76	3.79	3.82	0.103	18	42
DJE-2002-2	3.16	0.64	0.84	1.28	1.67	1.99	2.39	2.55	2.62	2.79	2.91									0.35	1	11
DJE-2002-3A	3.65	1.28	1.8	1.98	2.18	2.25	3.08	3.38	3.5	3.62										0.3	4	13
DJE-2002-3B	3.81	1	1.21	1.36	1.68	1.83	2.06	2.86	3.3	3.49	37.4									0.22	4	14
DJE-2002-5	3.65	0.9	1.8	2.4	2.75	3.02	3.44	3.6												0.1	1	8
DJE-2002-6	1.865	1.44	1.58	1.74																N/R	N/A	3
DJE-2003-9	1.55	0.89	1	1.29	1.36	1.4	1.42													N/R	N/A	6
DJE-2003-10	2.17	1.11	1.32	1.55	1.74	2.1	2.14													N/R	N/A	6
DJE-2003-12	3.49	2.1	2.64	2.7	3.06	3.08	3.35	3.43	3.48											0.34	6	14
DJE-2003-14	2.25	1.4	1.73	1.92	2.12	2.18	2.22	22.4												N/R	N/A	7
DJE-2003-15	3.47	1.58	1.86	2.15	2.63	2.92	3.12	3.42												0.35	4	11
DJE-2003-16	3.30	1.34	1.48	1.64	2.04	2.32	2.59	2.64	2.75	2.99	3.1									0.23	5	15
PPC-6669	3.16	1.3	1.68	2	2.08	2.41	2.67	2.71	2.82	2.96	2.98	3.1	3.2							0.26	5	17
PPC-6674	3.735	1.35	1.52	1.67	1.82	2.11	2.29	2.85	3.25	3.33	3.45	3.52	3.74	3.78	3.88	4	4.12	4.14		0.136	8	25

Table 4-3. *Gopherus polyphemus* growth estimates using the resorption protocol suggested by Parham and Zug (1997).

Specimen	Distance of Growth Marks from Center of Resorption Core (mm.)															Slope	Absorbed rings	Visible Growth Marks	Total (yrs.)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
DJE-2002-1	3.160	4.110	4.530	4.630	4.780	4.860	4.910	4.940	4.980	5.030	5.050	5.060	5.080	5.090		0.434	10.002	14.0	24.0
DJE-2002-2	1.280	1.660	2.090	2.350	2.400	2.430	2.460	2.510	2.580	2.660	2.690					0.225	8.607	11.0	19.0
DJE-2002-3A	2.850	2.880	3.160	3.200	3.850	4.180	4.240	4.250	4.260							0.504	6.971	9.0	16.0
DJE-2002-3B	2.390	2.640	2.900	3.080	3.130	3.340	3.480	3.600	3.690							0.411	7.162	9.0	16.0
DJE-2002-5	4.140	4.200	4.660	4.840	5.240	5.280										0.967	4.684	6.0	10.0
DJE-2002-6	0.000																0.000	0.0	0.0
DJE-2002-9	1.310	1.480	1.700	1.890	1.950	1.990	2.010	2.050								0.206	6.302	8.0	14.0
DJE-2002-10	1.930	1.980	2.250	2.610	2.910											0.480	4.504	5.0	9.0
DJE-2002-12	2.100	2.410	2.450	2.790	2.860	3.330	3.530	3.610								0.423	6.761	8.0	14.0
DJE-2002-14	1.360	1.910	2.190	2.500	2.560	2.600	2.640	2.660								0.308	6.193	8.0	14.0
DJE-2002-15	2.013	2.213	2.263	2.325	2.538	2.713	2.950	3.175								0.573	3.426	8.0	14.0
DJE-2002-16	3.213	3.500	3.588	3.700	3.913	4.200	4.250									0.654	5.275	7.0	12.0
PPC-6669	2.240	2.690	3.200	3.490	3.760	3.950	4.050	4.100	4.930	5.130	5.190	5.230	5.280	5.130		0.406	10.788	14.0	24.0
PPC-6674	2.440	2.590	2.910	3.010	3.080	3.730	4.190	4.250	4.390	4.510	4.540	4.660	4.913	5.010	5.040	0.380	11.222	15.0	26.0
PPC-3510	0.000															0.000	0.000	0.0	0.0

### Scute Annuli and Shell Length Assessment

Table 4-4 shows each *G. polyphemus* specimen, the number of growth marks calculated using the Castanet and the Parham methods, and the number of scute annuli counted from individual scutes. Checking the correspondence (covariance) of the two resorption methods required determination of  $R^2$  values to compare the number of scute annuli and the number of growth lines per each method (See Figures 4-1 and 4-2). These values are listed in separate columns after each of the two growth mark estimates. While scute annuli can be a good age indicator for some specimens, given the one individual that was too worn to age, I used another method to validate my estimates (Halliday and Verrell 1988).

Table 4-4. *Gopherus polyphemus* age estimates based on scute annuli counts and both skeletochronology protocols employed.

Specimen	Scute Annuli Counts	Parham Growth Counts (yrs.)	Castanet Growth Counts (yrs.)
DJE-2002-1	worn smooth	24.0	42.0
DJE-2002-2	11.0	19.0	11.0
DJE-2002-3A	12.0	16.0	13.0
DJE-2002-3B		16.0	14.0
DJE-2002-5	9.0	10.0	8.0
DJE-2002-6	5.0	0.0	3.0
DJE-2003-9	7.0	14.0	6.0
DJE-2003-10	10.0	9.0	6.0
DJE-2003-12	8.0	14.0	14.0
DJE-2003-14	10.0	14.0	7.0
DJE-2003-15	13.0	14.0	11.0
DJE-2003-16	12.0	12.0	15.0
PPC-6669	11.0	24.0	17.0
PPC-6674	16.0	26.0	25.0
PPC-3510	0.0	0.0	0.0
<i>G. elegans</i>	5.0	0.0	0.0

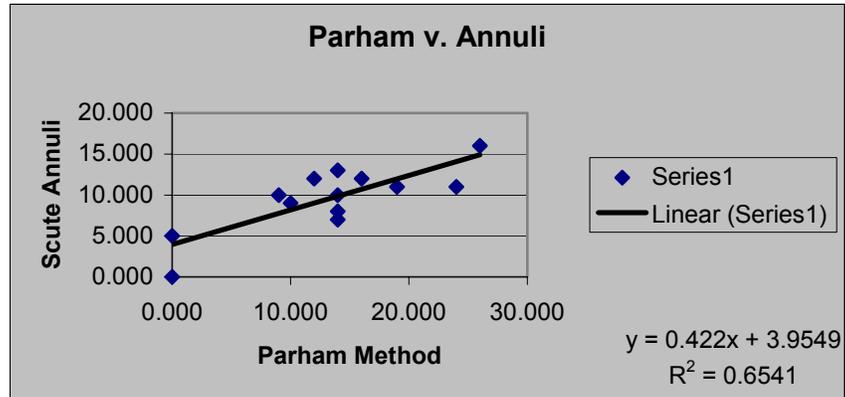


Figure 4-1. Parham growth estimations plotted against annuli counts.

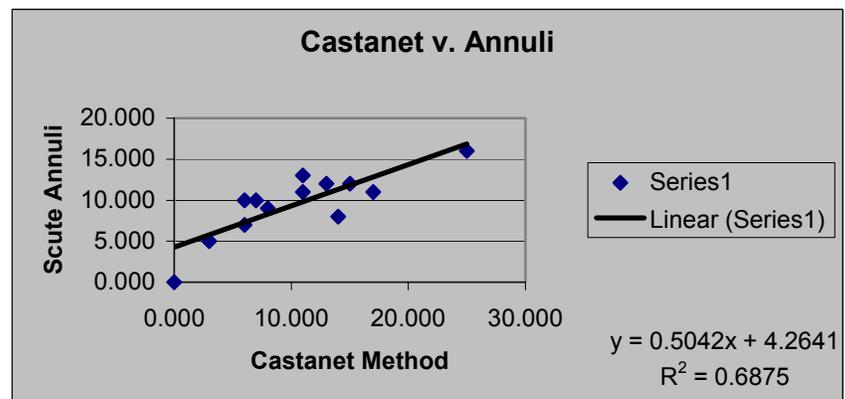


Figure 4-2. Castanet growth estimates plotted against annuli counts.

The determinations of carapace and plastron lengths are other methods that have been employed to estimate ages in wild caught and captive raised chelonians (Landers et al. 1982; Mushinsky et. al. 1994). While both measurements can be useful tools, they also have drawbacks that have been previously discussed. Landers et al. (1982) published a classic study linking plastron suture lengths to age in *G. polyphemus* (See Figure 4-3). Although their tortoise population was from southern Georgia, the climate is similar enough to north central Florida to allow for comparison with the specimens studied here. Therefore, for individuals where accurate plastron measurements are available, Table 4-5 shows the specimens' plastron lengths along with both the Castanet and the Parham growth mark counts. Again, to find the best fit, the  $R^2$  values for plastron

length vs. growth mark count for both methods have been calculated and are found in Figures 4-4 and 4-5. As stated above, plastron measurements are not as accurate in members of the genus *Gopherus* as other tortoise genera. Sexual dimorphism, in the form of epiplastral extensions tend to exaggerate plastron lengths in many specimens (Mushinsky et al. 1994).

Some turtle and tortoise studies rely on carapace measurements as another method of aging individuals, although these lengths can be misleading (Zug 1991). Length measurements can vary based on quality of habitat and caution should be used when comparing disjunct populations (Mushinsky et. al 1994). These measurements, however, were recorded for most of the specimens used as a gauge in this study. Unfortunately, many skeletons, when collected, were disarticulated and exact straight-line carapace lengths therefore are estimates. To estimate carapace lengths, the calculation methods of Franz and Quitmyer (In press) were implemented. They found that the hyoplastron bone length along the suture scales allometrically to body size. This allometric relationship can be described using a straight-line regression that they derived Eq. 4-1:  $\text{Log } y = a + b(\text{log } X)$ .

Where  $b$  = the slope of the line

$a$  = the y intercept

$x$  = the independent variable (Hyoplastron length along suture)

$y$  = the dependent variable (estimated body size/ carapace length)

Based on this formula, Franz and Quitmyer they found that  $a = 1$  and a slope of the line ( $b$ ) = 0.75 in modern gopher tortoises can be used. Using their equation, I was able to obtain straight-line carapace estimations for those tortoises whose shells were beyond

repair. Table 4-7 shows the actual and estimated straight-line carapace lengths and the actual hyoplastron lengths for all of the *G. polyphemus* specimens in this project.

Based on the actual and estimated straight-line carapace lengths, I was able to compare age relationships between skeletochronology estimates and shell lengths (Table 4-6). To determine ages based on carapace lengths, I used the size classes published by Mushinsky et al. (1994) (See Figure 4-6). These classes were based on studies of tortoise populations in central Florida and are close enough to my populations to be considered appropriate correlations. Table 4-6 shows the estimated ages of my specimens using the length-age comparisons of Mushinsky et al. (1994), both the Parham and Castanet growth mark counts. The  $R^2$  values of the Mushinsky carapace lengths vs. age counts for both methods can be seen in Figures 4-7 and 4-8. All of these other age correlations provide firm support for the skeletochronology age estimates that have been found in this project.

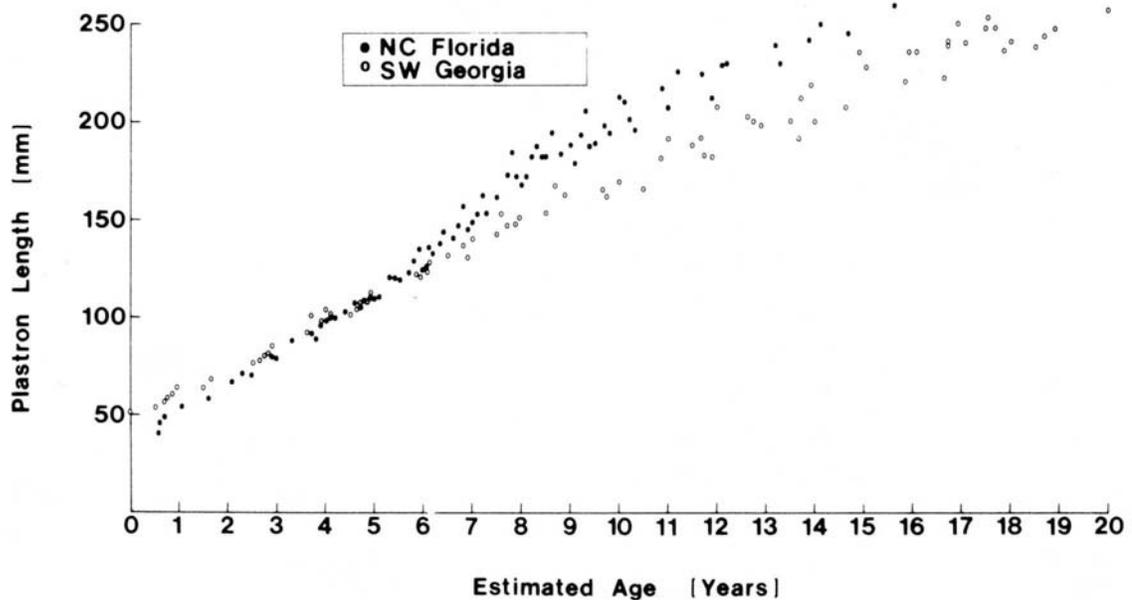


Figure 4-3. *Gopherus polyphemus* age estimations based on plastron lengths by Landers et al. (1982; Figure 13 on page 101).

Table 4-5. *Gopherus polyphemus* age estimates based on Landers et al. (1982) plastron measurements compared with skeletochronology estimates.

Specimen	Ages estimates plastron measurements (yrs.)	Parham Growth Counts (yrs.)	Castanet Growth Counts (yrs.)
DJE-2002-1	15.0	24.0	42.0
DJE-2002-2	9.0	19.0	11.0
DJE-2002-3A	13.0	16.0	13.0
DJE-2002-3B		16.0	14.0
DJE-2002-5	11.0	10.0	8.0
DJE-2002-6	6.0	0.0	3.0
DJE-2003-9	5.0	14.0	6.0
DJE-2003-10	8.0	9.0	6.0
DJE-2003-12	10.0	14.0	14.0
DJE-2003-14	7.5	14.0	7.0
DJE-2003-15	12.0	14.0	11.0
DJE-2003-16	N/A	12.0	15.0
PPC-6669	10.5	24.0	17.0
PPC-6674	10.5	26.0	25.0
PPC-3510	0.0	0.0	0.0

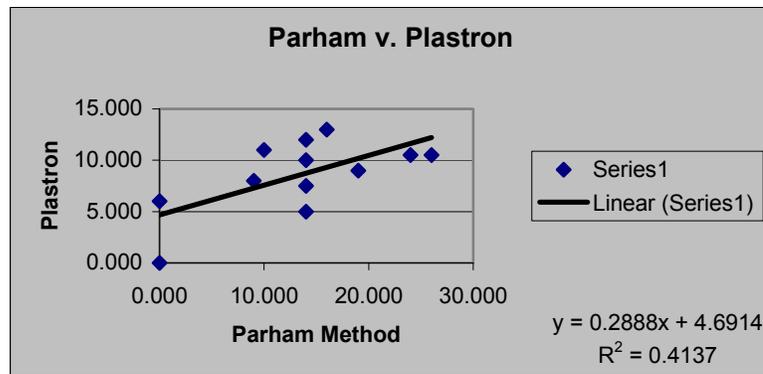


Figure 4-4. Parham age estimates plotted against plastron lengths.

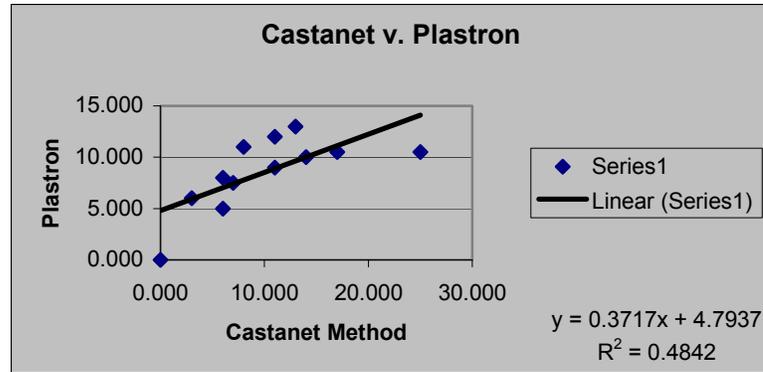


Figure 4-5. Castanet age estimates plotted against plastron lengths.

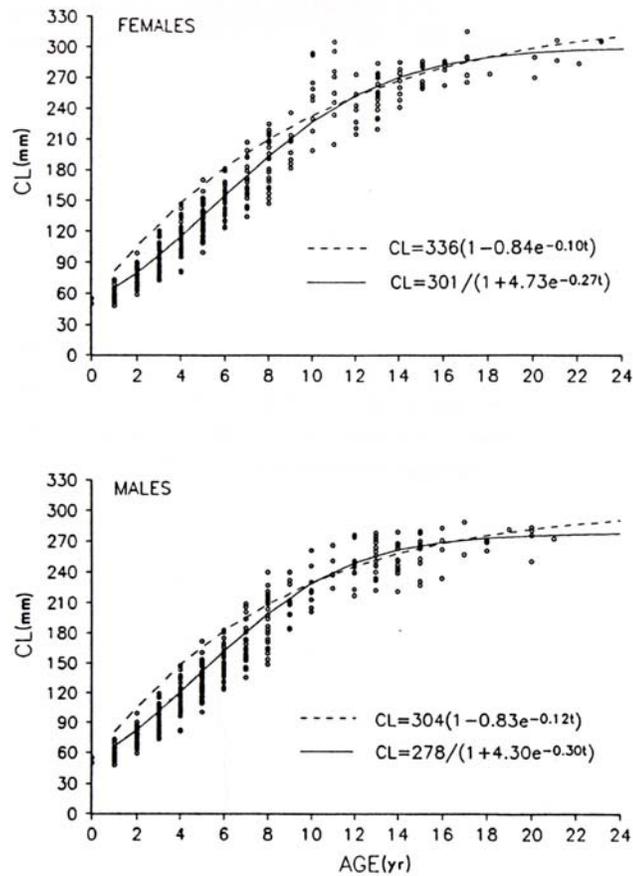


Figure 4-6. *Gopherus polyphemus* age estimates based on carapace lengths (Mushinsky et al. 1994; Figure 1 on page 122).

Table 4-6. *Gopherus polyphemus* age estimates based on Mushinsky et al. (1994) carapace lengths compared with skeletochronology estimates.

Specimen	Ages estimates from carapace (yrs.)	Parham Growth Counts (yrs.)	Castanet Growth Counts (yrs.)
DJE-2002-1	16.0	24.0	42.0
DJE-2002-2	9.0	19.0	11.0
DJE-2002-3A	13.0	16.0	13.0
DJE-2002-3B		16.0	14.0
DJE-2002-5	14.0	10.0	8.0
DJE-2002-6	5.0	0.0	3.0
DJE-2003-9	5.0	14.0	6.0
DJE-2003-10	7.0	9.0	6.0
DJE-2003-12	10.0	14.0	14.0
DJE-2003-14	8.0	14.0	7.0
DJE-2003-15	11.0	14.0	11.0
DJE-2003-16	12.0	12.0	15.0
PPC-6669	12.0	24.0	17.0
PPC-6674	11.0	26.0	25.0
PPC-3510	0.0	0.0	0.0

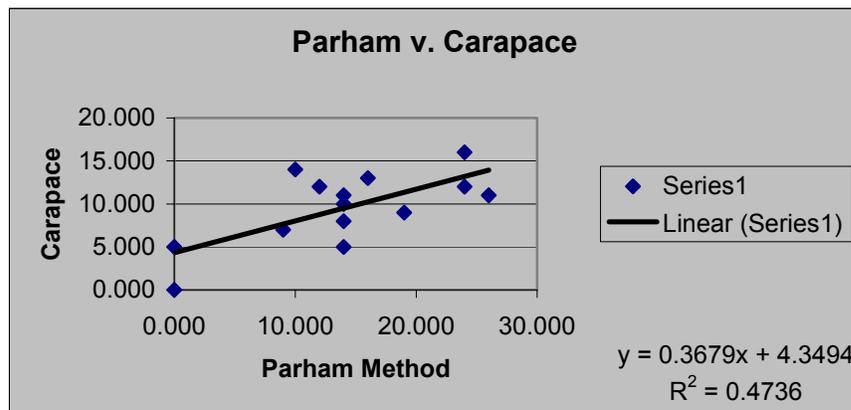


Figure 4-7. Parham age estimates plotted against carapace lengths.

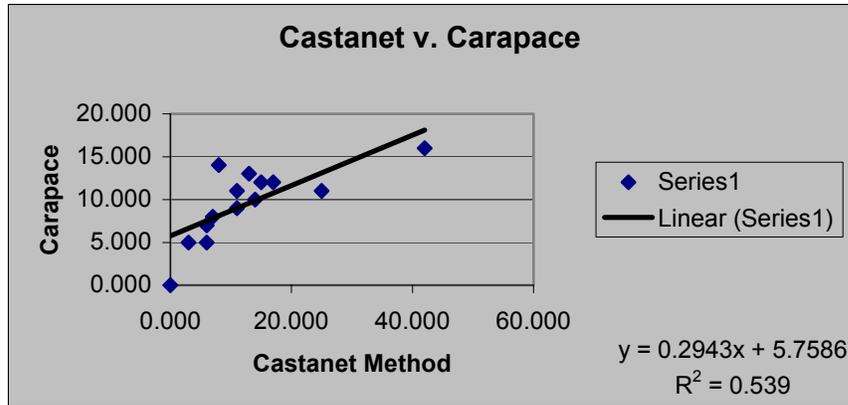


Figure 4-8. Castanet age estimates plotted against carapace lengths.

Table 4-7. *Gopherus polyphemus* shell dimensions and the estimates based on the findings of Franz and Quitmyer (In press).

Specimen	Carapace (mm.)	Hyoplastron (mm.)	Carapace Estimates (mm.)	Plastron (mm.)
DJE-2002-1	278.00	63.70	225.50	266.00
DJE-2002-2	224.00	60.10	215.85	210.00
DJE-2002-3A	265.00	65.40	229.98	244.00
DJE-2002-3B				
DJE-2002-5	258.00	64.80	228.39	227.00
DJE-2002-6	150.00	30.80	130.74	128.00
DJE-2003-9	130.00	33.70	139.87	116.00
DJE-2003-10	174.00	42.00	164.98	167.00
DJE-2003-12	218.00	60.10	215.85	199.00
DJE-2003-14	187.00	47.80	181.79	169.00
DJE-2003-15	220.00	49.30	186.05	210.00
DJE-2003-16	242.00	N/A	N/A	N/A
PPC-6669	244.00	65.10	229.18	223.00
PPC-6674	234.00	59.30	213.69	224.00
PPC-3510	45.50	N/A	N/A	46.50

### Fossil Humerus Growth Line Counts and Distances

The number of visible growth lines and the number of resorbed growth lines in the bones of *S. nebrascensis* and *G. laticuneus* were counted. Unlike the extant species, which were analyzed by Matson's Lab, I prepared, counted, and measured all fossil

specimens. A list of all fossil specimens, their localities, and the species identification can be seen in Table 2-1.

Marks of skeletal growth were more difficult to discern in fossil specimens due to the effectiveness of staining in fossils and also due to the required increased thickness of the prepared thin-sections. Mineral replacement has also destroyed some bone microstructure, which made counts difficult.

Table 4-8. Fossil tortoise age estimates determined using the Castanet resorption model

Specimen	Humerus Diameter (mm.)	Radius (mm.)	Avg Width of Rings 1-3 (mm.)	Resorbed Rings	Age (yrs.)
DE-2002-1	21.45	10.7	0.46	6	31
DE-2002-2	4.2	2.1		NR	8
DE-2002-3	16.32	8.16	0.296	7	29
DE-2002-4	11.76	5.88	0.28	9	19
DE-2002-7	7.73	3.86		NR	8
DE-2002-8	22.9	11.5	0.328	12	41
DE-2002-10	1.92	0.96		NR	0
DE-2002-11	10.4	5.23	0.32	2	17
DE-2002-12	8.83	4.41		NR	8
DE-2002-13	20.4	10.2	0.224	10	40
DE-2001-16	5.568	2.78		NR	9
DE-2001-17	30.1	15.1		N/A	too remodeled
UF 191470	3.4	1.7		NR	5
UF 201906	4.84	2.42		NR	8
UF 209750	11.96	5.98	0.188	11	28
RF-NEOREL-12	4.8	2.4		NR	8
RF-NEOREL-37	13.74	6.86	0.21	2	17
RF-NEOREL-39	15.17	7.58	0.41	6	17
RF-NEOREL-42	15.79	7.89	0.216	9	25
RF-NEOREL-66	5.88	2.94		NR	8
RF-NEOREL-74	15.6	7.8	0.53	1	30

Based on the results of the study of *G. polyphemus*, it was decided that the Castanet method for accounting for resorption was more accurate than the Parham method.

Therefore, for fossil specimens, resorption was addressed only using the Castanet method. Table 4-8 shows the specimen number, number of visible growth lines, average distance of the inner growth lines, number of resorbed growth lines and the total

estimated age for each individual. Again, all age estimates include a range of  $\pm 1$  year based on the season of death. Skeletochronology estimates were compared to carapace and plastron lengths, although there are no published reports of age classes in fossil chelonians. Scutes are composed of keratin and unfortunately, are not preserved in the fossil record, precluding their use in comparing annuli counts.

### **Fossil Tortoise Shell Measurements**

Whenever possible, the straight-line carapace and plastron lengths of fossil specimens were compared to the number of skeletal growth marks. The same drawbacks that hold true for *G. polyphemus* also hold true for fossil samples. In most cases, the carapaces of fossil individuals were completely disarticulated or misshapen due to the fossilization process. Efforts were made to estimate the straight-line carapace lengths in most individuals; however, most carapace lengths are estimates. The allometric model derived by Franz and Quitmyer (In press) was tested as an option however, fossil *S. nebrascensis* and *G. laticuneus* obviously had different growth curves and grew to larger sizes than modern *Gopherus*, thus making their growth model impractical. Therefore, carapace lengths are based on comparisons between articulated shells and portions of disarticulated shells of my specimens. As this is the first study of fossil tortoise growth, there were no relevant comparisons to be made. Fossil tortoise specimens and their estimated carapace lengths are presented in Table 4-9.

I also encountered similar problems using fossil plastra. Many plastra were not intact due to the fossilization process and disarticulation, so estimations have to be made. Accurate length measurements for plastra were obtained since they are much flatter than the carapace. Along with measurements for fossil carapace and plastron lengths, the

estimated age of the individuals has also been included. This information is provided to gauge the size vs. age of fossil individuals and can be seen in Table 4-9.

Table 4-9. Carapace and plastron lengths of fossil tortoises compared with skeletochronology age estimates.

Specimen	Carapace (mm.)	Plastron (mm.)	Age Estimates
DE-2002-1	~560	~480	31 yrs.
DE-2002-2	~95	~80	8 yrs.
DE-2002-3	~530	~450	29 yrs.
DE-2002-4	~260	~240	19 yrs.
DE-2002-7	~230	~210	8 yrs.
DE-2002-8	562	481.5	41 yrs.
DE-2002-10	~85	~90	0 yrs.
DE-2002-11	~307	~282	17 yrs.
DE-2002-12	240	~210	8 yrs.
DE-2002-13	566	500	40 yrs.
DE-2001-16	~165	~145	9 yrs.
DE-2001-17	~600	~530	too remodeled
UF 191470	97.9	82.0	5 yrs.
UF 201906	124	111.5	8 yrs.
UF 209750	366	360	28 yrs.
RF-NEOREL-12	158	~142	8 yrs.
RF-NEOREL-37	~350	~340	17 yrs.
RF-NEOREL-39	~425	~405	17 yrs.
RF-NEOREL-42	~425	~405	25 yrs.
RF-NEOREL-66	156	132	8 yrs.
RF-NEOREL-74	~430	~410	30 yrs.

## CHAPTER 5 USE OF DIFFERENT BONES IN SKELETOCHRONOLOGY

A number of different skeletal elements were taken from *G. polyphemus* specimens for sectioning. The humerus and femur (from the front and hind limbs respectively), scapula (from the shoulder girdle), ilium (from the pelvis), and one vertebra from the thoracic region of each individual were sectioned. The visible counts from those sections are shown in Table 4-1.

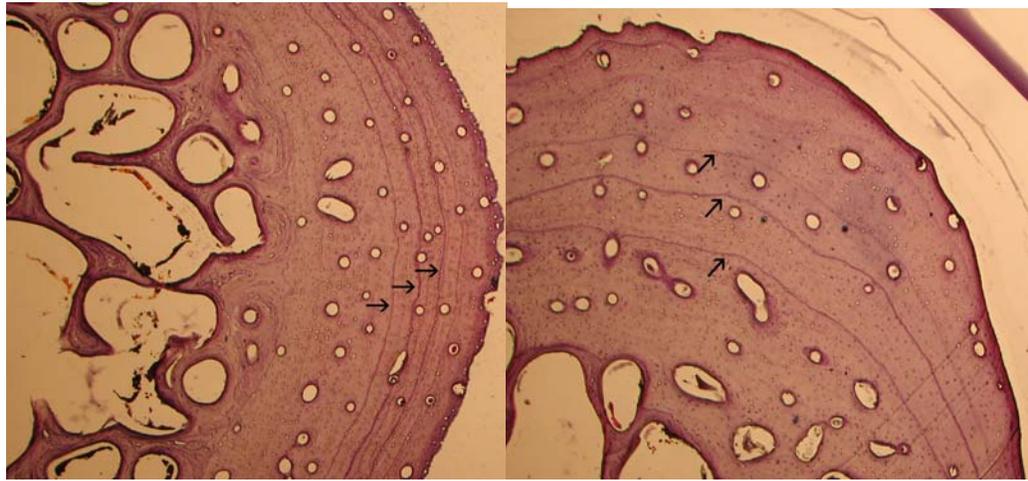


Figure 5-1. Slides depicting ilium (left) and scapula (right) cross-sections. Arrows point to visible MSG.

While most sampled elements showed similar visible growth mark counts, there were a number of contributing factors that resulted in using the humerus for this study. The humerus, femur, and girdles are more often found than other parts of the skeleton. Long bones, in particular, tend to undergo the slowest and steadiest rate of remodeling and resorption. Both of these long bones have shafts that are much more cylindrical in shape than the other skeletal elements tested. The longer cylindrical shafts, and the

absence of bone processes for muscle attachment limit the amount of resorption and remodeling in bone (G. Erickson pers. comm). Also, the humerus and femur have been most prominently used in skeletochronology studies dealing with reptiles and amphibians, and therefore, the techniques applied for accounting for resorbed rings apply to these bones (Klinger and Musik 1992).

It was originally speculated that bones of the pelvic or shoulder girdle might be the best elements based on availability in both modern and fossil specimens. This is due to the fact that fused girdles tend to remain trapped inside tortoise shells long after death. While this is true in most cases, the problems encountered when dealing with resorption outweigh the positives of a larger specimen count. Sample size was not an issue with modern *G. polyphemus* due to the relative abundance of materials. Fossil specimens, however, were much harder to obtain. As suspected, most individuals retained remains of the pelvic and shoulder girdles while very few had limb elements preserved. Nevertheless specimens preserving limb bones were a high priority for field collecting. Despite being somewhat rare, sufficient individuals were collected that preserved long bones.

Finally, the decision was narrowed down between the humerus and femur of the fore and hind limbs. The humerus was chosen based solely on the relative abundance of elements in the fossil collection. The relative abundance of humeri as opposed to femora seems to result from the protection offered by the tortoises' shells. When a tortoise tucks into its shell, the front limbs can be tucked more completely and tightly into shell. This protection may allow for preservation of the front limbs while many of the hind limbs

have been left unprotected and are lost. Therefore, the humerus was the best choice for use in my skeletochronological research.

### **Comparison between Skeletochronology and Other Techniques for Aging**

Skeletochronology has been a widely accepted technique for aging reptiles and amphibians for over 20 years (Castanet and Cheylan 1979; Zug et al. 1986; Halliday and Verrell 1988; Castanet and Smirina 1990; Zug 1991; Germano 1992; Erickson and Tuminova 2000). And while it is a partially destructive method, the results are much more consistent than other methods discussed in this project. Obviously, skeletochronology cannot be used in all situations, such as short-term field studies or studies that involve extremely rare groups (Gibbons 1976). The method still provides an important opportunity for scientists to learn important demographic information about tortoise populations. A purpose of skeletochronology is to gain insight into a population from aging individual specimens.

To account for resorption of MSG, the Parham method and the Castanet methods were both tested in *G. polyphemus*. These estimates were then compared with other methods of aging chelonians in order to determine the accuracy of the two protocols. The Castanet method is the more common method and has been employed for over 20 years (Castanet and Cheylan 1979; Castanet and Smirina 1990; Klinger and Musik 1992), whereas the Parham method is more traditionally used in aging fish (Parham and Zug 1997).

Comparing  $R^2$  values, I found the Castanet method to be the more consistent resorption model. Comparing scute annuli counts versus the two models for resorption, one can see that the values are quite similar (Parham model:  $R^2 = 0.65$ ; Castanet model:  $R^2 = 0.69$ ) but slightly higher for the Castanet model. While this is not an overly high

correlation, it should be noted that scute annuli counts are not consistent after the animal reaches its early twenties (Germano 1988, 1992; Mushinsky et al. 1994; Aresco and Guyer 1998). After this point in life of the tortoise, scute wear and condensation of rings makes for less reliable annuli counts.

Comparing skeletochronology age counts to plastron lengths resulted in similar findings. When plotting both age count models versus plastron length, the Castanet model again shows higher  $R^2$  values. The Parham method provided a correlation of  $R^2 = 0.41$ , while the Castanet method provided a correlation of  $R^2 = 0.48$ . Again, these correlations are not overly different, but significant enough to accept the Castanet model as the more consistent method. These  $R^2$  values are low because of the problems associated with the use of plastron measurements when aging members of the genus *Gopherus*. The epiplastral extension may be misleading when aging individuals, therefore I would not advise using this measurement for aging *G. polyphemus* (Mushinsky et al. 1994).

Finally, age estimates involving carapace lengths were also correlated with resorption age estimates. The Parham method provided a correlation of  $R^2 = 0.47$ , whereas the Castanet method provided a correlation of  $R^2 = 0.54$ . In this instance, we see that the Castanet method has a much better fit over the Parham method when it comes to modeling for resorption. Based on the higher statistical significance of the Castanet method, resorption of fossil MSG was calculated using this method.

DJE-2002-1 deserves some special consideration based on the advanced age that was produced using the Castanet method. While shell dimensions yielded ages around 15-16 years, and the Parham method yielded an age of 24 years, the Castanet method

provided an age of 42 years. There are a number of conditions that should be addressed when discussing this individual. It was an extremely old female, which was killed by a motor vehicle. The tortoise was gravid and at least two eggs were observed when the remains were collected. In addition, the scutes on the shell were worn smooth, and no annuli could be counted. It has been reported in a number of reptiles and other animals that gravid females may utilize mineral deposits from bone to produce eggshell (Wink and Elsey 1986; Wink et al. 1987). After reproduction and egg-laying, the deposits are restored with remodeled bone, which leaves the bone highly altered. Thin-sections of this specimen revealed large, open vacuities that may have been a result of calcium and phosphorous utilization. In any case, these data were included in all computations to be scientifically accurate. Future researchers should note that potentially gravid females should be regarded with caution, and examined for mineral utilization as suggested in Wink and Elsey (1986) and Wink et al. (1987).

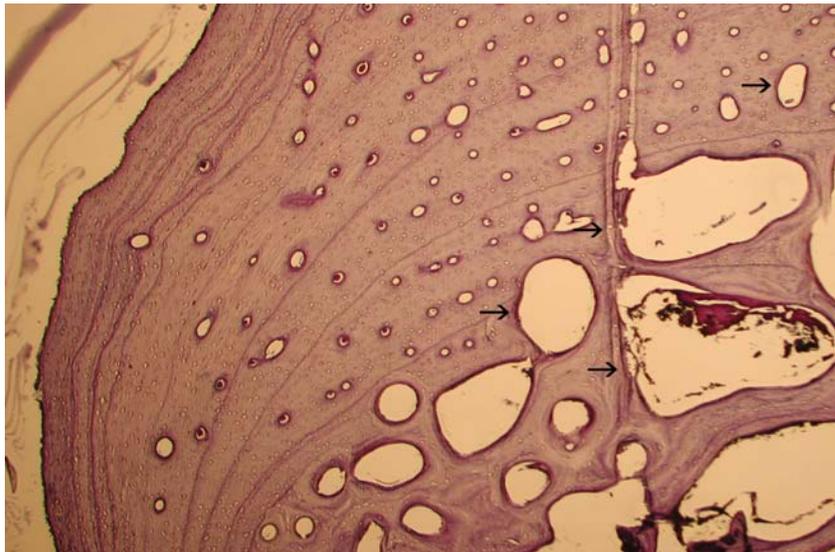


Figure 5-2. DJE-2002-1 humerus thin section with arrows showing open vacuities.

Caution should also be used when comparing shell dimensions from different populations. Separate populations across a given species range may have different body

dimensions due to changes in habitat, food availability, and climatic conditions (Gibbons 1976; Mushinsky et al. 1994). To determine ages of individuals from carapace and plastron lengths in this project, I used previously published growth trajectories based on two different populations. Plastron data were taken from Landers et al. (1982), who studied populations in southern Georgia, and carapace data were taken from Mushinsky et al. (1994), who studied populations in central Florida. These data are still viable for this study because of the intermediate proximity of my *G. polyphemus* specimens to both populations. With any given age estimates, an uncertainty of  $\pm 1$  year should always be included, due to season of death.

#### **Skeletochronology in the Known Age Sample of *Geochelone elegans***

The three-year-old individual that was donated to this study by Ray Ashton did not positively correlate skeletochronology with the actual age or scute annuli counts. While at first this came as quite a surprise, after discussing the living conditions of this animal with Ashton, the results are not disturbing. The bone was highly remodeled, showing no outwardly visible signs of growth marks. This phenomenon can be explained by the fact that this individual was kept in a controlled environment for its entire life. Therefore, the individual had no seasonal changes and did not go into torpor (hibernation). Marks of skeletal growth are constrained by marks of arrested growth, which naturally occur during torpor (Castanet and Smirina 1990). In this case, the animal grew continuously throughout the year.

#### **Fossil Tortoise Humerus Counts and Shell Measurements**

Marks of skeletal growth in *S. nebrascensis* and *G. laticuneus* were visible in most specimens despite the lack of staining and increased slide thickness. Calcite veins filled in cracks in many humeri, making growth mark counts more difficult in fossils. In many

cases, growth marks needed to be traced around the entire circumference of the bone to get accurate counts.

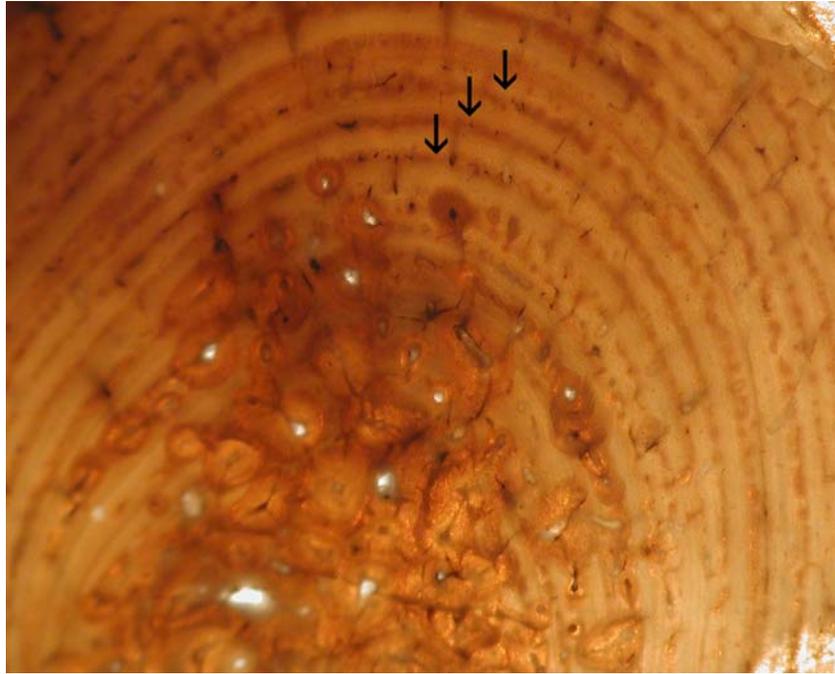


Figure 5-3. RF-NeOrel-74 humerus thin section with arrows showing well-defined MSG.

The fossil tortoise samples collected ranged in age from a hatchling up to an individual that had well passed the point of senescence. Hatchling bone, DE-2002-10, is easily discerned from older juveniles by its highly porous tissue and relatively small size. At the other extreme, DE-2001-17, had reached its maximum growth (= senescence), which occurs in extremely old amphibians and reptiles with indeterminate growth. This specimen had extremely large limbs and a very high amount of remodeling. After an individual reaches maximum size, the skeletal tissue becomes highly remodeled, which makes it impossible to age. It can be safely assumed, based on findings from the other individuals that this individual was probably over 50 years in age.



Figure 5-4. DE-2001-17 humerus thin section showing high level of remodeling. Arrows show section of MSG that has not been destroyed.

With a larger sample size, growth rates are attainable for the two fossil tortoise species reviewed in this study. In order to predict growth rates, a growth series including specimens ranging from hatchlings and juveniles up to large adults is needed. For this study, complete growth series for the two fossil species identified were not collected. Instead, a composite growth series involving individuals from two separate species is represented. This enabled me to increase the sample size but does not provide enough data points for growth rate predictions. However, based on specimen sizes and ages, growth rates were probably very similar for both species. Based on shell measurements, it seems that both species attained adult sizes over  $\frac{1}{2}$  of a meter in length in many cases and could have taken as long as 40 years to attain this size.

Size classes based on age are difficult to attempt at this stage. There are two different species being examined, and while they appear to be very close in size and age,

there are not enough specimens to construct complete growth sequences for both. It is documented (Erickson and Tumanova 2000) that a complete growth series is important in determining age classes. Future work including, collection of additional specimens and comparisons with other museum collections will make identification of age classes possible. Until that time, loose affiliations between size and age have been constructed.

CHAPTER 6  
SKELETOCHRONOLOGY AS AN ACCEPTABLE TECHNIQUE OF AGING  
MODERN AND FOSSIL TORTOISES

In this study, skeletochronology has been tested and validated as a reliable method for aging modern *Gopherus polyphemus* as well as *Styemys nebrascensis* and *Gopherus laticuneus*. Previously this method has been tested on sea turtles, freshwater turtles and other species of tortoises with mixed results (Castanet and Cheylan 1979; Germano 1992; Parham and Zug 1997; Bjorndal et al 1998). For wild-raised tortoises, skeletochronology can be an invaluable asset to any population study. This statement is also extended to the two fossil tortoise species that are studied.

Even though skeletochronology is a destructive technique, it is nonetheless an important tool that should be utilized in population studies whenever possible. When bone growth mark counts are coupled with scute annuli counts and shell dimensions, the resulting information is meaningful. Age-size classes can be determined, age at sexual maturity, mean population age, and maximum age of a population are all examples of data that can be ascertained using this method. As stated earlier, however, there were not enough specimens in this study to make these deductions. Skeletochronology is used to extract individual ages of specimens and compile them in order to describe the population being studied. With that in mind, outliers should not be discredited or removed from a data set, on the other hand, they should be included in studies. This information will aide in the overall description of a given population. Use of fossil tortoise material for skeletochronology is also an acceptable method for studying the paleoecology of extinct

species. The positive results that were produced in this study are due, in part, to the excellent preservation of fossil materials. Historically, the badlands of South Dakota and Nebraska have been known for the excellent preserved fossils found in that region, and the fossil tortoise specimens studied here were no exception. In other areas where fossil preservation is not as pristine, researchers should know the animal, something about the proposed environment, and the associated fauna.

The results obtained from this study on fossil tortoises have laid the groundwork for future studies. Demographic information including: sizes of hatchlings, loosely set age classes, maximum size and probable age restraints have been determined. To increase the amount of information that can be gathered, more specimens of younger and older individuals should be collected and studied. Looking at the Results section, one can see that most specimens fell in or about the same age class. This artifact is a result of two factors: 1) smaller individuals do not preserve well in the fossil record and 2) larger individuals are more difficult to collect and transport. Future collecting should focus on these two size groups.

The demographic information gathered is important for examining the shift in genera during the Eocene-Oligocene transition. As discussed previously, the climatic conditions and habitat began to change. Also the genus *Styemys* was in decline and *Gopherus* was beginning to become more prevalent in the fossil record. The information gathered from this study will provide insight into why this change occurred. Obviously *Gopherus* was more suitably adapted, but why? With the collection of more specimens from both genera, this information can be determined.

### **Skeletal Elements Used in Skeletochronology**

The use of different skeletal elements in skeletochronology has been subject to much debate in the literature (Zug 1990). Historically, long bones found in the front or hind limbs have been the most widely tested elements. For this study elements of the shoulder and/or pelvic girdle or vertebrae might also be useful given that they are commonly found in fossil specimens. Upon microscopic inspection of modern specimens, however, the amount of remodeling and resorption in these specimens were not conducive for skeletochronology studies. Vertebrae in particular are not usable for studies involving tortoises.

The humerus and femur, however, are valuable elements for skeletochronology studies. Both bones showed optimal results when sectioned and viewed under a compound microscope. For tortoise studies, I feel that either element could be used to obtain similar results. The humerus was chosen for this study due to the relative abundance of specimens, particularly fossils. This abundance is due to the idea that front limbs have a greater chance for preservation in tortoises. Either side of the skeleton can be used, and interchanging sides between specimens is acceptable (as seen in DJE-2002-3), however a concerted effort should be made to maintain a level of consistency.

### **The Use of Captive-Raised Individuals and Skeletochronology**

Extreme caution should be used when dealing with individuals that have been captive-raised. Alteration of habitat, seasonal cycles and food supplies can greatly influence the bone remodeling in individuals. This is not to say that annual growth marks are not present, they cannot be seen due to high levels of remodeling. Growth marks will inherently be laid down in individuals' bones regardless of climatic conditions, whether they can be observed, however, is a different matter (G. Erickson pers. comm).

The sample of *G. elegans* that was used in this study clearly defines the inherent problems that can hinder proper aging techniques. Natural size, scute annuli, and growth marks in bone can be highly skewed when the organisms' habitat is adjusted. This holds true not just for tortoises, but also for most animal species. Unnaturally increasing growth rates will lead to data that is not truly representative of a given species. Therefore, I would approach data based on captive-raised individuals with caution.

A new study is now being conducted under the direction of Ray Ashton to determine the validity of skeletochronology in *G. polyphemus*. This work will involve injecting tetracycline dyes into live individuals to mark rates of skeletal growth. These individuals are being raised in outdoor pens and will eventually be sacrificed. It is believed that these test individuals will lend further credence to the use of skeletochronology as an invaluable method for aging tortoises.

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## BIOGRAPHICAL SKETCH

Dana J. Ehret was born on July 31, 1979 in Spring Lake Heights, New Jersey and lived with his parents and sister until entering college. He attended Spring Lake Heights Elementary and Manasquan High School. Upon graduating high school, he studied marine biology at the Richard Stockton College of New Jersey. He received his Bachelor of Science degree in 2001, under the guidance of Dr. Roger Wood. He was also involved with Dr. Wood and his Diamondback terrapin conservation efforts at the Wetlands Institute, Stone Harbor, New Jersey during the 2000 and 2001 summer field seasons. In August of 2001 he entered the University of Florida Department of Geological Sciences and began work on his master's thesis, studying modern and fossil tortoises from North America. Upon completion, he will continue his studies of *Gopherus polyphemus* in the School of Natural Resources and Interdisciplinary Ecology at the University of Florida starting in the Fall of 2004. Conservation and ecology of turtle and tortoise species are his primary areas of concern, and he hopes to devote his life to chelonian preservation.