RADIOGRAPHIC ANALYSIS OF THE OSTEOLOGICAL DEVELOPMENT IN THE MANUS OF THE FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*) AS AN AGE-ESTIMATING TECHNIQUE

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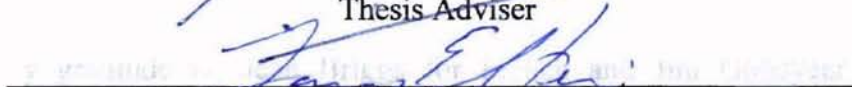
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Chapter V

ABBREVIATIONS

C1	carpal bone one
C2	carpal bone two
C3	carpal bone three
C4	carpal bone four
d.e.	distal epiphysis
dist.	distal
F	female
GLG	growth-layer-group
i	intermedium
M	male
m	meter
Mc	metacarpal
p.e.	proximal epiphysis
P1	first phalanx
P2	second phalanx
P3	third phalanx
prox.	proximal
r	radiale
R	radius
TL	total body length
U	ulna
u	ulnare

Introduction and Objectives for the Radiographic Analysis of the
Osteological Development in the Manus of the Florida Manatee,
Trichechus manatus latirostris, as an Age-Estimating Technique

INTRODUCTION

The purpose of this study was to investigate the postnatal developmental sequence of the

ossification centers in the manus of the endangered Florida manatee (*Trichechus manatus*

latirostris) (U. S. Fish and Wildlife Service 1994) and to develop an age-estimating

technique based on flipper radiographs.

Manatees and dugongs are the only two living genera in the mammalian order Sirenia.

They are unique as they are the only herbivorous marine mammals, and live in rivers,

estuaries and coastal waters (Reynolds and Odell 1991). The dugong (*Dugong dugon*) is

exclusively marine and lives in the western Pacific and Indian oceans (Nishiwaki and Marsh

1985). There are three species of manatees. The West African manatee (*Trichechus*

senegalensis) lives on the west coast and in the rivers of Africa, while the Amazonian

manatee (*Trichechus inunguis*) inhabits rivers of the Amazon basin in South America

(Caldwell and Caldwell 1985). The West Indian manatee (*Trichechus manatus*) is

classified into two subspecies based on population distributions and variations in cranial

morphometrics (Domning and Hayek 1986, O'Shea and Ludlow 1992): *Trichechus*

manatus manatus or the Antillean manatee lives in the West Indies, northern South

America and Central America; and *Trichechus manatus latirostris* or the Florida manatee

inhabits the coasts and inland waters of Florida, and the southeastern seaboard of North America (Ronald *et al.* 1978, Caldwell and Caldwell 1985), and is the focus of this study.

Florida manatees are one meter (m) in total body length (TL) at birth and are weaned between one and two years of age at approximately 1.5 m (Bonde 1983 unpublished data). Males and females attain puberty and first reproduction as early as two years of age (2.4 m) (Hernandez *et al.* 1995) and three years of age (2.5 - 3.0 m) (Marmontel 1995), respectively. Successful rearing of a calf, however, may not take place until six years of age or older (O'Shea and Ludlow 1992). With a 12-14 month gestation, female manatees give birth every 2-3 years during favorable conditions and may reproduce for over twenty years (O'Shea 1994). Although analysis of growth-layer-groups in the petrous temporal bone has been successfully used to age manatee skeletons (Marmontel *et al.* 1996), no reliable technique has been developed to estimate the age of living manatees. Manatees are known to live to 60 years of age (O'Shea 1994) and grow to over 3.8 m in length (O'Shea and Ludlow 1992). The Florida manatee is federally listed as an endangered species (U.S. Fish and Wildlife Service 1994) with an estimated 2,600 individuals still surviving in the wild in the United States of America (B. Ackerman pers. comm. 1996). Being large, long-lived, slow moving aquatic mammals they are susceptible to human encroachment and exploitation. The continued human impact on Florida's natural resources, including habitat alteration and destruction as well as increasing mortalities resulting from collisions with boats, leaves an uncertain future for the Florida manatee (O'Shea 1994). Full implementation of an effective management

plan (U.S. Fish and Wildlife Service 1989) is required to protect this endangered species from further decline. The success of such conservation strategies are dependent upon reliable scientific information on the life history and biology of the manatee. A key component, which this thesis seeks to accomplish, is the ability to estimate age in the Florida manatee.

OBJECTIVES

The specific objectives of this research were to:

1) review the literature on sirenian manus osteology and mammalian age-estimation techniques (Chapter 2);

2) identify the separate ossification centers in the manus (Chapter 3);

3) determine when each ossification center "first appeared" postnatally as related to total body length (TL) (Chapter 3);

4) determine the sequence of development of these ossification centers as related to TL (Chapter 3);

5) determine when each manus epiphyseal ossification center was "first fused" and "consistently fully fused" to its respective diaphysis and when each carpal bone reached the adult shape as related to TL (Chapter 4);

6) correlate ossification events with chronological age by comparing TL of radiographed manatees with TL of aged manatees (included in Chapters 3 and 4);

7) examine the unusual developmental ossification pattern of the fifth digit phalanges (Chapter 5).

The osteological development of the Florida manatee manus was studied primarily by examination of radiographs of a postnatal series of 179 flippers. Anatomical terminology follows Nomina Anatomica Veterinaria 1994 (Frewein *et al.* 1994) unless otherwise stated.

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CHAPTER II

Literature Review of Sirenian Manus Osteology and
Techniques for Age Estimation in Mammals

INTRODUCTION

The distal thoracic limb, or flipper, of the Florida manatee (*Trichechus manatus latirostris*) displays the basic mammalian plan of five digits and is enclosed in a single covering of skin. The only external demarcation of these digits is the three to four nails (Watson and Bonde 1986) on the distocaudal edge of the dorsal surface of the flipper. The flippers are used for walking on the bottom of its aquatic habitat, bringing food to and cleaning its mouth, scratching and social caressing, and as an aid in turning, steering, and propulsion (Hartman 1979). Traditionally, the skeleton of the manus is composed of the bones of the carpus, the metacarpus, and the digits (Flower 1885). For the purpose of this thesis, however, the distal radius and ulna have been included in the description of the manus. Throughout the thesis, anatomical terminology, unless otherwise noted, follows Nomina Anatomica Veterinaria 1994 (Frewein *et al.* 1994), in which the carpal bone terminology follows that originally proposed by Gegenbaur (1864).

In the adult manatee manus (Fig. 2.1), the carpus typically consists of six roughly cuboidal short bones arranged in two transversely orientated rows: a proximal row composed of three bones in cranial to caudal order - the radiale, the intermedium, and the ulnare; and a distal row of three bones in cranial to caudal order - carpals one and two

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(fused), carpal three, and carpal four (Flower 1885). The metacarpus skeleton consists of five small long bones, numbered cranial to caudal, I-V. The five digits consist of one to three stout long bone phalanges per digit, represented by the phalangeal formula: I_{1-2}, II_3, III_3, IV_3, V_{2-3}. Because of the orientation of the flipper in the manatee, the metacarpus and digits are numbered from cranial (I) to caudal (V), which topographically corresponds to terrestrial mammals medial (I) to lateral (V) orientation. This atavistic preservation of the embryonic orientation of the thoracic limb (Romer and Parsons 1977) in postnatal manatees necessitates the use of the cranial/caudal terminology for the topography of the manus. Along with this orientation, the dorsopalmar reference axis is retained in the manatee manus.

SIRENIAN MANUS OSTEOLOGY

Distal radius and ulna:

An early 19th century account based on two museum skeletons of manatees, *Trichechus sp?*, from the Brazilian region reports that the distal one-third of the radius and ulna are fused to each other in adult manatees (Cuvier 1809), although each bone has a separate diaphysis and distal bony epiphysis in immature manatees (Dart 1974). Similar findings are recorded for mature and immature dugongs (Anderson 1898). This fusion is atypical in generalized terrestrial mammals (Flower 1885), and more recent accounts also noted this fusion between the distal ends of the radius and ulna in manatees (Quiring and Harlan 1953, Sukhanov *et al.* 1986, Watson and Bonde 1986) and in the dugong (Freund 1904, Kaiser 1974). Examination of museum skeletons of manatees reveal that the distal

epiphyses of the radius and ulna fuse together (craniocaudally) prior to these epiphyses fusing with their respective diaphyses (proximally) (Dart 1974), whereas in dugongs, the distal radial and ulnar epiphyses fuse first to the diaphyses and then with each other (Dart 1974). Reports have not been found describing whether the radial and ulnar distal epiphyses develop as two separate cartilage models each with their respective ossification center, or as one common cartilage mass with two ossification centers.

Carpus:

The adult manatee has a flattened, broad carpus including six short bones arranged in two transverse rows (Flower 1885) (Fig. 2.1); a separate accessory carpal bone is not represented (Cuvier 1809, Flower 1885, Howell 1930) although others suggest that it is incorporated into either the ulnare (Owen 1866) or the ulnare and the fifth carpal bone (Bahrdt 1933, Sukhanov *et al.* 1986). Three carpal bones are in the proximal row (cranial to caudal): the radiale is a cuboidal bone that articulates proximally with the cranial half of the distal radial epiphysis; the intermedium is dorsopalmarly elongated with rounded ends and articulates proximally with the caudal half of the distal radial epiphysis; and the rectangular ulnare is the largest carpal bone, it articulates proximally with the entire distal ulnar epiphysis, and supports the fifth metacarpal bone distocaudally. Three carpal bones are in the distal row: the angular carpal bone one has a pea-sized projection on its dorsal surface that is considered to represent carpal bone two (Flower 1885) and this composite bone articulates proximally with the radiale and intermedium and distally with metacarpals one and two (Cuvier 1809, Owen 1866); carpal bone three is rounded and

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proximally articulates solely with the intermedium and distally with metacarpals two and three; the caudal-most bone in the distal row is large and somewhat spherical and is considered to represent either carpal bone four (Quiring and Harlan 1953, Sukhanov et al. 1986), or the fusion of the fourth and fifth carpal bones (Cuvier 1809). It articulates proximally with the ulnare, caudally with metacarpal five and distally with metacarpals three and four.

Fusion variations in the carpus:

In the manatee, the first and second carpal bones are sometimes seen as separate bones and thus the carpus has seven bones (Flower 1885). When the first and second carpal bones are fused in mature animals and considered one bone, six carpal bones are reported (Cuvier 1809, Owen 1866, Anderson 1898, Bahrdt 1933, Sukhanov et al. 1986, Watson and Bonde 1986). In two individuals, the radiale and intermedium were fused and carpals one and two were also fused, to result in a carpus of five bones (Quiring and Harlan 1953). Fusion of the ulnare and the intermedium has been noted in one flipper from a bilateral ectrodactylous Florida manatee (Watson and Bonde 1986).

Ulnare composition:

The ulnar carpal bone of the adult manatee is considered to represent one of three different developmental scenarios: exclusively the ulnare (Cuvier 1809, Flower 1885, Anderson 1898, Quiring and Harlan 1953); the fusion of the ulnare and the accessory carpal bone (Vrolik 1851, Owen 1866, Bahrdt 1933); or the combination of the ulnare,

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accessory and the fifth carpal bones (Bahrdt 1933, Sukhanov *et al.* 1986). In none of these cases, each based on few specimens, were multiple separate skeletal elements found to verify any of these scenarios. The verification of a multi-element composition of the manatee ulnare could be investigated with a developmental series of prenatal and neonatal flippers.

Metacarpals:

The four living sirenians all have five metacarpal bones (Anderson 1898). In postnatal immature sirenians the metacarpal bones have separate ossification centers for both the proximal and distal epiphyses (Todd and Todd 1938). This developmental pattern differs from typical terrestrial mammals (Evans 1993) and humans (Greulich and Pyle 1959) in which there is a separate ossified epiphysis for only one end of each metacarpal bone. The metacarpal epiphyseal pattern seen in the manatee manus is similar to that found in cetaceans, where both mysticetes and odontocetes have proximal and distal epiphyses of their metacarpals and phalanges (Ogden *et al.* 1981, Rommel 1990). The manatee's styliform first metacarpal bone is the most diminutive metacarpal (Owen 1866). Progressing caudally, each metacarpal elongates and becomes more robust. Metacarpal five is the longest and broadest metacarpal and displays an asymmetrically expanded distal epiphysis.

Phalanges:

Sirenians have been reported with varying numbers of phalanges (Table 2.1). Throughout this thesis, the phalanges will be referred to as first, second and third phalanges which is equivalent to proximal, middle and distal phalanges of Nomina Anatomica Veterinaria 1994 (Frewein *et al.* 1994). The somewhat elongated and dorsopalmarly flattened phalanges also exhibit separate proximal and distal ossified epiphyses (Todd and Todd 1938) like the metacarpal bones. These epiphyses develop on the first and second phalanges but are variable in development on the third phalanges (Dart 1974). In the manatee, digit I supports one (Home 1821, Vrolik 1852) and occasionally two (Murie 1872) phalanges. Digits II, III and IV each have three phalanges (Cuvier 1809, Bahrdt 1933, Watson and Bonde 1986) but have also been recorded as having only two each (Anderson 1898, Quiring and Harlan 1953). Some authors have referred to the third phalanx as a "nail-like structure" (Quiring and Harlan 1953) or have concluded that the second and third phalanges have fused in the second and fourth digits (Anderson 1898). The phalanges of the fifth digit are quite different from those in other digits in their unusual shape and development and are the subject of Chapter V of this thesis.

Reported variations in phalangeal counts could result from the loss of small bones during skeletal preparation. Occasionally, discrepancies occur between the text and figures, e.g., Home (1821), in which the text states the phalangeal formula of a West Indian manatee as 2,3,4,3,2, while an accompanying figure illustrates the phalangeal formula as 1,2,3,2,1.

Perhaps the inclusion of the metacarpals gives the higher phalangeal formula. Anatomical studies are performed on bones of an individual in which the skeletal maturity is measured at a fixed point in time and will therefore have only one of many stages of ossification development. Differences in maturity stages are best observed and documented through a developmental series of animals using radiography and dissection.

Developmental osteology:

Manatees, like whales and dolphins, have proximal and distal ossified epiphyses on both ends of the long bones in the manus. In addition, manatee bones are peculiar in their internal structure. Long bones of a typical terrestrial mammal manus have one ossified epiphysis and are composed of a compact, cortical bone periphery surrounding a hollow medullary cavity in the middle of the diaphysis and cancellous bone in the metaphyses and epiphyses (Dyce *et al.* 1996). In contrast, the long (and other) bones of the manatee are extremely dense and lack a central medullary cavity and the cancellous bone becomes so dense that it grossly appears indistinguishable from the thick outer-layer of cortical bone (Fawcett 1942). This is the result of a relatively slower resorption of bone with a scarcity of osteoclasts, but a continued deposition of bone. The unusually dense structure of sirenian bone is referred to as osteosclerotic as the bones are both grossly thickened and the medullary space is replaced with compact bone, similar to that in the cortices (Domning and de Buffrénil 1991). When radiographically examined, the dense manatee bones reveal a solid radiopaque image with little or no structural detail, especially in skeletally mature animals (Kaiser 1976).

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The estimation of age of individuals in a wild population is critical to understanding the life-history of a species, including manatees (O'Shea and Ackerman 1995). Age-related life-history stages of the Florida manatee such as reproductive viability and longevity are vital in establishing sound management and conservation policies (Marine Mammal Commission 1992). Various techniques for the estimation of age in wild and domestic terrestrial mammals have been reviewed (Morris 1972). These include body weight and length, baculum dimensions, eye lens weight, pelage color, tooth succession and wear, secondary sexual characteristics, skull proportions and suture fusion, fusion of epiphyses in limb bones, and the incremental lines seen in the teeth, bones, horns, and claws. One commonly used and reliable technique is that based on the layering pattern of bones and teeth (Klevezal' and Kleinenberg 1969).

Bone and Teeth Layering:

Seasonal growth cycles result in layering of bone and dental tissues in all mammals (Klevezal' 1980). During the growing season the animal normally has optimal nutrition with good body growth and deposits a relatively broad layer of bone; during the season with less food, migration, or hibernation, body growth slows and it deposits a relatively narrower, denser layer of bone. Physiological stresses, environmental changes or unusual seasonal conditions may also result in reduced bone deposition (Klevezal' and Kleinenberg 1969). Under normal growing conditions, one broad layer of bone (summer deposition) and one narrow band (winter) represents one annual growing cycle and is

termed a growth-layer-group (GLG) (Klevezal' and Kleinenberg 1969). Determination and analyses of these growth layers has become an often-used criterion for estimating age in mammals (Laws 1952, Klevezal' 1980), particularly those based on correlations with known-age and tetracycline marked individuals (Myrick 1980). This GLG technique uses skeletal parts that have been extracted from live animals (teeth) or can be recovered from carcasses (teeth and bones). The skeletal structures used in estimating age must be a permanent part of the animals' anatomy and should grow continuously throughout life without substantial remodeling, and thus minimal loss of growth layers (Odell 1977). Counting the cementum and dentine layers in teeth has become the standard age-estimation technique in pinnipeds (Laws 1953) and toothed cetaceans (Perrin and Myrick 1980).

Bones of mature manatees undergo resorption due to remodeling (Odell 1977) and thus may obliterate GLGs in older individuals. This resorption, the extreme density of the bones, and the lack of known-age specimens has been a challenge in developing a technique for age estimation using already proven methods of GLG counting (Marmontel 1993). In manatees, GLGs have been counted in ribs (Fawcett 1942, Domning and Myrick 1980), mandibles (Odell 1977) and in the petrous temporal bones (Marmontel *et al.* 1996). Growth-layer-groups must be correlated with chronological age in known-age specimens to obtain age and to validate age-dependent life-history data from unknown-aged specimens.

Layering of skeletal tissues has also been investigated in dugongs and manatees although the limited availability of known-age manatees has restricted the calibration of any age-estimation technique in this species. The dugong has a pair of tusk-like incisors, which are the only teeth present throughout their lives, and they have only six cheek teeth in each jaw quadrant. These tusks have annually deposited GLGs and can therefore be used for aging individuals (Marsh 1980). Unlike dugongs, manatees do not have incisors and continually replace their 6-8 cheek teeth several times over their lifetime (Domning and Hayek 1984). Consequently, other aging techniques have been investigated for manatees. The ages of 45 perinatal Florida manatees (1.0 - 1.5 m TL) were estimated based on tooth eruption patterns and then related to total body length (Odell and Clark 1993). Perinatal calves had three fully erupted molars in each jaw and almost all of these calves lacked postnatal dentine deposition. Annual layering of bone in a rib has been reported for one tetracycline injected Amazonian manatee (Domning and Myrick 1980). More recently, in an extensive study of 1,196 Florida manatees, GLGs were counted in histological sections of the petrous temporal bone (periotic bone as per Marmontel) (Marmontel 1993, Marmontel et al. 1996). This study concluded that one GLG was deposited annually in the petrous temporal bone and that the number of GLGs was consistent with chronological age. Growth-layer-group counts were validated as annuli by analysis of growth layers in 17 known-age manatees. Bone resorption begins interfering with age estimation at 15 to 20 years of age and in manatees longer than 3.0 m TL (Marmontel 1995). This technique is now considered the most accurate way of aging manatee carcasses (O'Shea and Ackerman 1995).

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In terrestrial mammals, skeletal maturity (the cessation of longitudinal bone growth, closure of epiphyseal cartilage growth plates, and fusion of the bony epiphysis to the diaphysis) and sexual maturity occur at approximately the same time (Greulich and Pyle 1959, Evans 1993). The long bones of pinnipeds are similar to terrestrial mammals in their bone structure and in the timing of skeletal and sexual maturity (Watson *et al.* 1985). In cetaceans (Ogden *et al.* 1981) and sirenians (Marmontel *et al.* 1996), however, skeletal maturity is attained years after sexual maturity.

Radiographic Studies:

Radiography allows us to look at the normal *in situ* topography of bony elements. It is valuable for revealing the initial appearance of ossification centers, the growth of ossification centers, and the fusion of epiphyses to diaphyses. A significant benefit of radiography is that it is non-invasive and, as a result, can be used on live animals as well as on carcasses. Radiography can be repeated many times on the same individual without injury, and is therefore a useful technique for longitudinal studies of skeletal growth and the sequence of development of bones.

Radiographic techniques are well established to study the development and maturation of the skeleton, especially the thoracic limb, in man (Greulich and Pyle 1959), domestic dog (Hare 1960), domestic horse (Myers and Emmerson 1966), and wild terrestrial mammals (Morris 1972). For example, the radiographic state of development of epiphyses of the distal radius, ulna and long bones of the manus have been used for age estimation of red

foxes, *Vulpes fulva* (Sullivan and Haugen 1956, Harris 1978), grey foxes, *Urocyon cinereoargenteus* (Sullivan and Haugen 1956), black bears, *Ursus americanus* (Marks and Erickson 1966), and hedgehogs, *Erinaceus europaeus* (Morris 1971). In these species, age was estimated by either comparison with known-age material, by the counting of dental GLGs, or by known season of birth. On the other hand, there are few radiographic studies of thoracic limb bone development in marine mammals. In pinnipeds, the first appearance, sequence of development, and fusion of distal antebrachial and manus ossification centers were correlated with total body length of young California sea lions, *Zalophus californianus* (Watson *et al.* 1985). The application of this data was to discriminate between premature pups and dead full term or neonatal pups. In cetaceans, scores were assigned for the number of ossification and fusion sites to evaluate metacarpal and phalangeal development compared with other morphological data for physical and sexual maturation in common dolphins, *Delphinus delphis* (Hui 1979). Six stages of appearance, development, and fusion of the distal epiphyses of the radius and ulna were determined for Dall's porpoises, *Phocoenoides dalli dalli*, and short-finned pilot whales, *Globicephala macrorhynchus* (Ogden *et al.* 1981). These radiographic studies were conducted on both live animals and salvaged carcasses.

In sirenians, one of the first analyses of thoracic limb bone development based on radiographs was of the dugong, *Dugong dugon* (Freund 1904). This study examined seven dugong flippers from Torres Strait - three pairs dissected from maturing animals and one whole flipper preserved in formalin of a young dugong. The radiographs show

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small diaphyseal ossification centers in the youngest dugong while the other flippers have ossified epiphyseal centers. The fifth digits contained either two, three, or four phalanges. More recently, comparative radiographs of cleaned, museum skeletal specimens were published of all four living sirenians (*D. dugon, Trichechus manatus, T. inunguis*, and *T. senegalensis*) (Kaiser 1974). Radiographs of one living West African manatee (*T. senegalensis*) were included. The density of the different bones, or parts of bones, in the thoracic limb as well as distinguishing separate and fused manus ossification centers were illustrated. In the manatees separate epiphyseal ossification centers are seen in most phalanges and the number of phalanges per digit varies. This author apparently reversed the digits since he notes that the thumb is large in *Trichechus* and the fifth digit is "weakly developed and displays only one phalanx".

For the Florida manatee, *Trichechus manatus latirostris*, preliminary studies on the radiographic development of the manus from salvaged flippers have been briefly reported: 37 manatees (Black and Giep 1980); 125 manatees with a range of total body lengths from 1.0 - 3.5 m (Watson and Hensen 1985); and 161 flippers from 151 manatees with a total body length range of 1.0 - 3.5 m (Goodyear and Watson 1995). These three studies used many of the same manatees, 106 of which have had their ages estimated in Marmontel's study on petrous temporal bone growth-layer-groups (Marmontel 1993 - Appendix B). Thus, these radiographed individuals, which have been aged (or are of known age) create the basis for potential correlations for age estimation of manatees based on flipper radiographs.

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In the Amazonian manatee, *Trichechus inunguis*, a longitudinal study was conducted on 15 captive manatees in which flippers were radiographed every six months to chart the development of the manus bones against body weight, TL, and chronological age (de Assis Ribeiro and Best 1984). This preliminary study found that a known-age female at ten years of age had not yet completed the development and fusion of epiphyseal ossification centers of the flipper bones. This suggests that the extended length of time that bone development and maturation occurs in manatees allows a radiographic technique to be used throughout much of the manatee's life, including many years after sexual maturity. An extensive annotated bibliography on the Sirenia is now available and contains approximately 4600 references spanning the years 1494 to 1994 (Domning 1996).

SUMMARY

Age estimation in wild and domestic terrestrial mammals is reliably accomplished by examination of non-skeletal and skeletal specimens. Estimating the age of marine mammals has been investigated and is now used successfully in pinnipeds, odontocetes and dugongs. Manatees, however, lack permanent teeth and have a unique histological development and maturation of their bones which presents challenges in developing an age-estimating technique. A key to estimating the age of wild manatees is that the data be correlated with known-age individuals. An age-estimating technique for the endangered Florida manatee would be most valuable if it could accurately estimate ages

and be performed on live animals. The objective of this study was to examine the development of the bones in the manus of the Florida manatee using radiographs of flippers from salvaged animals. These findings are correlated with radiographic data from known-age manatees to establish a base for a viable age-estimation technique to use on living animals.

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TABLE 2.1 - Variations of Manatee Phalangeal Formulae

Author	**Species**	**Specimen**	**Phalangeal Formula**
Cuvier 1809	*Trichechus manatus* or *T. inunguis*	1.9 m TL	0,3,3,3,3
	T. manatus or *T. inunguis*	2.3 m TL	?
Home 1821	*T. m. manatus*	Female adult	2,3,4,3,2
			1,2,3,2,1
Vrolik 1852	*T. manatus*	1.4 m TL	1,3,3,3,3
		2.5 m TL	1,3,3,3,2
Owen 1866	*Trichechus sp?*	?	1,3,3,3,3
Murie 1872	*T. m. manatus*	Female 1.65 m TL	2,3,3,3,2
	T. m. manatus or *T. inunguis*	Male 1.22 m TL	2,3,3,3,3
Flower 1885	*T. manatus*	?	"never increased ... beyond the limit usual in the Mammalia"
Anderson 1898	*T. manatus*	?	_,2,_,2,_
Bahrdt 1933	*T. manatus latirostris*	?	1,3,3,3,2
Quiring & Harlan 1953	*T. manatus latirostris*	Female 3.25 m TL	1,2,2,2,2
		Male 2.96 m TL	1,3,3,3,2
Dart 1974	*Trichechus sp?*	5 specimens/1 literature	1-2,3,3,3,2-3
Kaiser 1974	*Trichechus sp?*	?	"large individual variation"
Watson & Hensen 1985	*T. manatus latirostris*	125 individuals 1.03-3.55 m	2,3,3,3,2
Sukhanov *et al.* 1986	*T. manatus*	?	1,3,3,3,2
Watson & Bonde 1986	*T. manatus*	150 flippers	1,3,3,3,2

Figure 2.1

Outline sketch of an adult Florida manatee showing the skeletal elements. Dorsal view of left flipper. Carpal bones, proximal row - radiale (r), intermedium (i), ulnare (u); carpal bones, distal row - carpals one and two fused (C1 + C2), carpal three (C3), carpal four (C4); first through fifth metacarpal bones (Mc I-V); first phalanx (P1); second phalanx (P2); third phalanx (P3); phalanges two and three fused (P2 + P3).

Proximal

Radius   Ulna

r   i   u

C1 + C2   C3   C4

Cranial   Caudal

Mc I

Mc II

Mc III

Mc IV

Mc V

P1

P1

P1

P1

P1

P2

P2

P2

P2 + P3

P3

P2

P3

P3

Distal
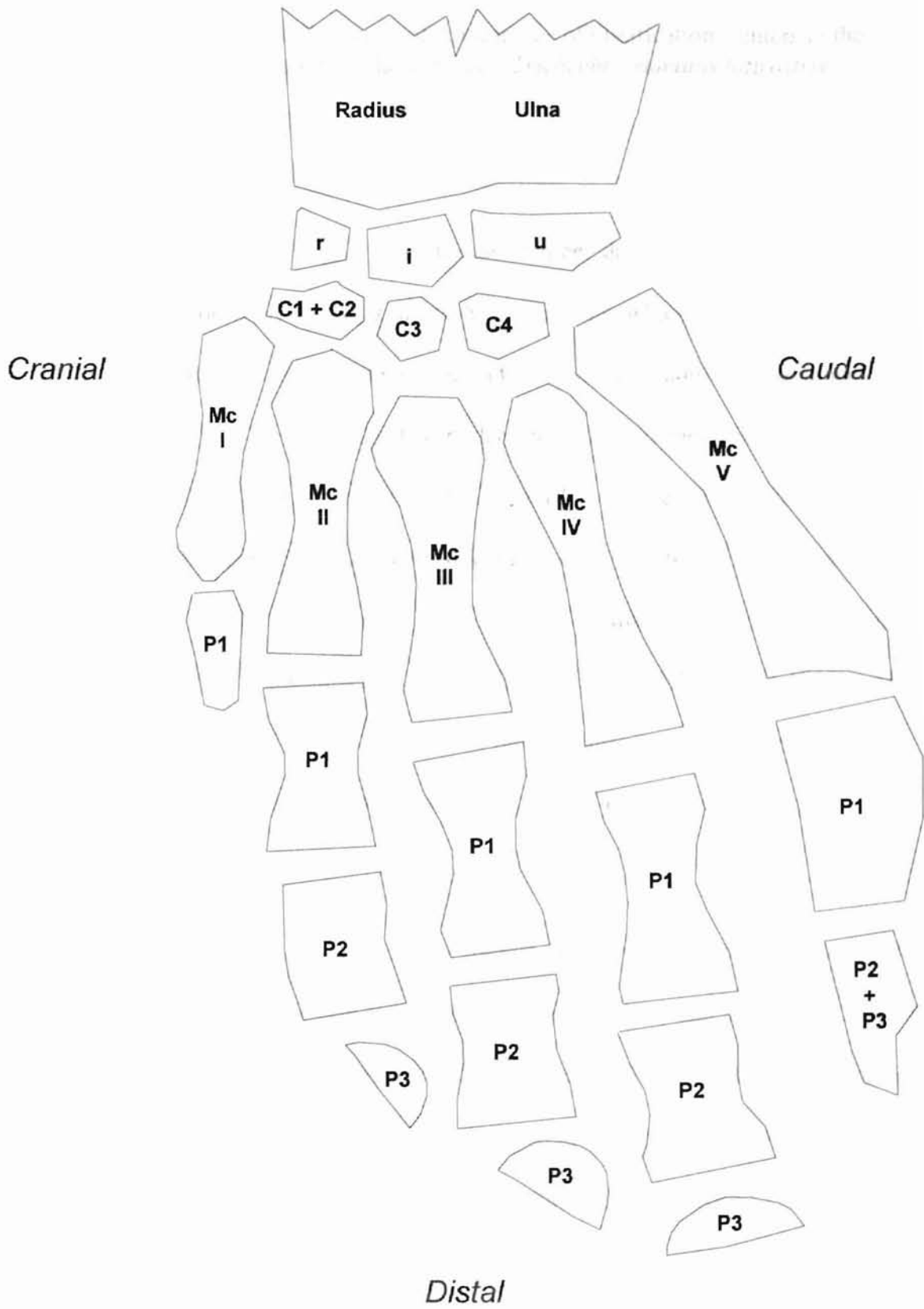
First Appearance and Sequence of Appearance of Ossification Centers in the
Manus of Postnatal Florida Manatees, *Trichechus manatus latirostris*

INTRODUCTION

Early accounts of the osteology of the manatee flipper date from the beginning of the

19th century with detailed drawings of museum skeletons, which included findings such

as the fusion of the distal third of the radius and ulna (Cuvier 1809) not previously found

in marine mammals. The phalangeal formulae and carpal elements were described by

other comparative anatomists (Home 1821, Owen 1866, Flower 1885). Murie's (1872)

landmark anatomical study of *Trichechus* documented the number of phalanges per digit

whereas others investigated the variation and fusion of cartilage and bony elements in the

carpus, metacarpals and phalanges (Vrolik 1852, Anderson 1898, Bahrdt 1933). These

early reports, being based on few individuals, provide little information on the sequence

of development and maturation of the ossification centers in the manatee flipper.

Radiographic examination of the postnatal osteological development of the mammalian

skeleton has been studied in wild (see review by Morris 1972) and domestic (Chapman

1965) terrestrial and marine mammals (Ogden *et al.* 1981). In the early twentieth

century, radiographs depicting developmental stages of skeletal elements gave new

insight into the skeletal development of two immature dugongs (Freund 1904), and there

are limited preliminary studies on the development and number of ossification centers in

the manus of immature and adult Florida manatees (Black 1980, Black and Giep 1980, Watson and Hensen 1985). These studies were all based on radiographs (37 to 125 individuals) although none recorded the total body length or chronological age at which each ossification center first appeared. In studying flipper anomalies in the Florida manatee, three individuals with skeletal abnormalities in the manus were found (Watson and Bonde 1986). To better understand the development and maturation of the bones in the manatee manus it would be desirable to have many specimens from different age groups to describe a sequence of the first appearance of each postnatal ossification center.

The future of the Florida manatee is dependent upon effective conservation and management plans which necessarily must be based on accurate life-history information. For this reason, manatee flippers were collected and radiographed to evaluate bone development at different stages of maturation and to establish an age-estimation technique.

The objectives of this study of the Florida manatee manus were to: 1) identify the separate ossification centers; 2) determine when each ossification center first appeared postnatally as related to total body length (TL); 3) determine the sequence of development of these ossification centers as related to TL; and 4) correlate ossification events with chronological age by comparing TL of radiographed manatees with TL of aged manatees (Marmontel 1993).

Examination and analysis of radiographs of 179 flippers revealed that at birth (1.0 - 1.5 m TL) the diaphyses of the radius, ulna, metacarpals I-V and phalanges (I-1, II-3, III-3, IV-3, V-1-3) were well ossified. At 1.9 m carpal bones and bony epiphyses began to appear. Other epiphyseal ossification centers appeared, and continued developing after sexual maturity (2.8+ m). These specific ranges of TL for each postnatal manus ossification center establish the sequential development of the manatee manus bones and form the basis for a possible method to estimate the age of manatees. This is an expansion of an abstract of preliminary results (Goodyear and Watson 1995).

MATERIALS AND METHODS

SPECIMENS

Disarticulated flippers were collected and frozen from 167 Florida manatee carcasses, *Trichechus manatus latirostris*, salvaged from the coast and waterways of Florida (1976-1984, 1996). Salvage records from the National Biological Survey, Sirenia Project (Gainesville, FL) provided field number, total body length (TL), sex, side of body of flipper, salvage location and date, and descriptive comments for each individual. The sample consisted of flippers from 92 male and 75 female manatees that ranged in TL from a 1.0 m neonate to a 3.6 m adult (Appendix A). Recorded field measurements of TL were used with a 95% level of confidence and therefore all manatee TLs were rounded to 0.1 m. Twelve specimens had both right and left flippers. This formed a postnatal developmental series of 179 flippers from manatees of evenly distributed lengths.

RADIOGRAPHIC TECHNIQUE

Flippers were thawed, cleaned, and standard plain-film radiographs were taken. The majority of radiographs were taken between 1976-1984 with unrecorded radiographic technique at the College of Veterinary Medicine, University of Florida, Gainesville, FL. In 1996, fifteen flippers were radiographed at the College of Veterinary Medicine, Oklahoma State University, Stillwater, OK. Each prepared flipper was placed on top of a film cassette covered with a thin sheet of plastic. Flippers from manatees longer than 1.6 m TL were disarticulated at the elbow joint and radiographed in a lateromedial view using 36x43 cm (14"x18") non-grid, ultra-vision detail screen cassettes and high-detail film. Flippers from manatees shorter than 1.6 m TL were disarticulated at the shoulder joint and radiographed in a mediolateral view using 20x25 cm (8"x10") non-screen film. The film-to-source distance for all flippers was 102-112 cm (40"-44"). Routine perpendicularly oriented X-ray tube exposed film produced images with superimposition of bones. The carpus was thicker than the tips of the digits and therefore the flipper laid at an angle. Thus, to produce an X-ray beam perpendicular to the longitudinal axis of the long-bones and parallel to their cartilage growth plates, the X-ray tube and collimator was angled between 5 and 10 degrees, depending on the size of the flipper. This technique resulted in radiographs with minimal superimposition of bones and a clear penetration of epiphyseal cartilage growth plates. The flippers were radiographed at 52-60 kVp at 400 mA for 0.006-0.5 seconds. Film was processed in an automatic processor.

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EVALUATION TECHNIQUE

All radiographs were examined by naked eye on a standard radiographic illuminator at Oklahoma State University, Stillwater, OK. Each radiograph was examined to identify the separate ossification centers of the carpus and of the epiphyses and diaphyses of the distal radius and ulna, the metacarpals and the phalanges. All radiographs were examined three separate times.

The first appearance data were analyzed and is presented as two different TLs: 1) the "first appearance" of an ossification center was recorded as the TL of the shortest individual in which this ossification center was detected (even though there may have been manatees at the same or greater lengths without this ossification center); 2) a "consistently present" ossification center was recorded as the TL of the shortest individual at which this ossification center was present in all manatees.

These first appearance data were arranged in ascending TL order to produce a sequence of appearance for the manus ossification centers. An ossification center was determined to be radiographically present when defined bony spicules were seen on the film and differentiated from sharp-edged images formed by opaque, sand or grit particles. This evaluation was aided by knowing the topographical location of manus bones in mature manatees. Since the appearance and sequence of appearance of the second and third phalangeal diaphyseal and epiphyseal ossification centers in the fifth digit had an unusual development, these data are presented separately in Chapter 5.

Overall, the 167 Florida manatees in this study can be categorized into four different life stages based on their TL (Bonde *et al.* 1983, O'Shea *et al.* 1985): fetus – ≤ 0.9 m (n=0); dependent calf – 0.9 - 1.5 m (n=32); juvenile – 1.5 - 2.8 m (n=84); sexually mature adult – > 2.8 m (n=51). Males become sexually mature as early as two years of age (2.4 m TL) based on spermatogenesis studies (Hernandez *et al.* 1995). Females become sexually mature at three to four years of age (2.5 - 3.0 m) based on ovarian follicle studies (Marmontel 1995). Moreover, chronological age has been estimated by an examination of bone layering and analysis of growth-layer-groups (GLGs) in the petrous temporal bone of Florida manatees and correlated with known-age specimens (Marmontel 1993). Flippers from 106 of these same age-estimated individuals were radiographed for this study (Appendix B). Marmontel's chronological age data was consulted only after the collection of data from all radiographs was completed. The first appearance of ossification centers in 179 flippers were evaluated radiographically. The TL at first appearance of postnatal ossification centers of all manatee flippers was then correlated with Marmontel's aged specimens to achieve ages in which ossification events occurred.

RESULTS

RADIOGRAPHIC EVALUATION

All carpal and epiphyseal ossification centers were evaluated in 165 of the 179 radiographs taken. Due to overexposure, incorrect positioning, or post-mortem damage to flippers, 14 radiographs were only partially evaluated.

IDENTIFICATION OF SEPARATE POSTNATAL OSSIFICATION CENTERS

The topographic and developmental osteology and nomenclature of the ossification centers of the manus of the Florida manatee is summarized in an illustration (Fig. 3.1). Fifty-two separate ossification centers were identified: 18 diaphyseal, 7 carpal and 27 epiphyseal centers. The ossification centers for the second and third phalanges of the fifth digit were not included in these counts and are described in Chapter 5. The ossification centers identified were: distal radius (cranially) and ulna (caudally), each with a distal epiphysis; seven carpal bones in two rows: the proximal row comprised three bones (cranial to caudal) - the radiale, the intermedium, and the ulnare; and the distal row had four bones (cranial to caudal) - carpals one and two (fused in adults), carpal three, and carpal four; five metacarpal bones, I (cranial) to V (caudal), each with separate proximal and distal epiphyses; digits II-V each had three phalanges - the first and second phalanges each had separate proximal and distal bony epiphyses (with variations in digit V) although separate bony epiphyses were not seen in the third phalanges; digit I had a first phalanx with a proximal epiphysis, and a second phalanx was uncommon (5 out of 179 flippers). The term epiphysis as used in this thesis refers to a bony epiphysis unless otherwise stated. Traditionally, the larger of the two cranial distal carpal bones is called carpal one (Flower 1885). It articulates proximally with the radiale and distally with metacarpals I and II. The smaller bone, which lies proximal to metacarpal II and dorsal to carpal one is carpal two. In this series of radiographs from postnatal manatees the separate carpal and epiphyseal ossification centers are reported below in topographical proximodistal order and in order of their first appearance.

OSSIFICATION CENTERS PRESENT AT BIRTH

In all manatees examined (1.0 - 3.6 m TL) 18 manus long bone diaphyseal ossification centers were consistently present (Fig. 3.2). These were the diaphyses for the radius and ulna, metacarpals I-V, the first phalanges of digits I-V, and the second and third phalanges of digits II-IV. In addition, variation in the number of diaphyseal centers at birth were seen in digits I and V. Occasionally, a second phalanx ossified in digit I (n=5) and the second and third phalanges in digit V were variably present (Chapter 5). An additional 34 ossification centers were detected radiographically in postnatal manatees: the 7 carpal bones and 27 epiphyseal ossification centers.

OSSIFICATION CENTERS APPEARING POSTNATALLY

Distal epiphyses of the radius and ulna:

Ossification was first detected radiographically in the distal epiphysis of the ulna in a 1.9 m TL manatee and in the distal epiphysis of the radius in a 2.0 m manatee (Fig. 3.3, 3.4). These two bony epiphyses were consistently present in manatees at 2.1 m and longer. Normally, a single ossification center developed in the center of the distal epiphyses of both the radius and ulna. In two manatees (2.6 and 2.8 m TL) the distal epiphysis of the ulna had two separate ossification centers and in both cases the cranial center was approximately twice as large as the caudal center.

Carpal bones:

Ossification centers first appeared for the intermedium, ulnare, and carpals three and four at 1.9 m TL (Fig. 3.3, 3.4). These four ossification centers were consistently present in manatees at 2.1 m and longer. The ossification center for carpal bone one first appeared at 2.0 m and was consistently present at 2.2 m. Carpal bone two, the smallest carpal bone lying dorsal to carpal one, first appeared at 2.1 m and was consistently present in manatees at 2.3 m and longer except in one outlying individual (2.5 m). The radiale was the last carpal ossification center to appear at 2.3 m, and was consistently present at 2.8 m TL (Fig. 3.5).

Metacarpals:

The ossification center for the proximal epiphysis of metacarpal V first appeared in a manatee at 1.9 m TL, in metacarpal III at 2.0 m (Fig. 3.3, 3.4), and in metacarpals I, II and IV at 2.1 m. Ossification centers of the proximal epiphyses of metacarpals II, III, IV and V were consistently present in manatees at 2.2 m and longer while that in metacarpal I was variably present until 2.5 m. The distal epiphyseal ossification centers first appeared at 2.0 m TL for metacarpals III, IV and V, and all three were consistently present in manatees at 2.2 m and longer (Fig. 3.5). The ossification center for the second metacarpal distal epiphysis first appeared at 2.1 m and was consistently present at 2.5 m. The distal epiphysis of the atypically-shaped first metacarpal first appeared at 2.4 m and was variably present until 3.0 m TL. The proximal metacarpal epiphyses began appearing in the center of the cartilage epiphysis and continued ossifying to the medial

and lateral edges. In contrast, the distal metacarpal epiphyses often began appearing peripherally, ossifying toward the center. The proximal epiphysis of the second phalanx appeared consistently at 3.3 m TL (Fig. 3.3 & 3.5). The five metacarpal...

Phalanges:

<u>First phalanx:</u>

Proximal epiphysis - The ossification center of the proximal epiphysis of the first phalanx of digit V was first seen in a 2.1 m TL manatee (Fig. 3.3) and was consistently present in manatees at 2.7 m and longer (Fig. 3.5). The proximal epiphysis of the first phalanx for digits III and IV first appeared at 2.1 m and were consistently present in manatees at 2.6 m and 2.5 m and longer, respectively. The proximal epiphysis of digit II first appeared at 2.2 m and was consistently present in manatees 2.9 m and longer. That of digit I first appeared at 2.5 m and was consistently present at 3.1 m TL.

Distal epiphysis - The distal ossified epiphysis of the first phalanx of digit IV first appeared at 2.3 m TL and was consistently present in manatees 2.7 m and longer (Fig. 3.5). The distal epiphysis of digits III and V also first appeared at 2.3 m and were consistently at 2.9 m and 3.0 m, respectively. The ossification center of the distal epiphysis of digit II first appeared at 2.4 m and was consistently present in manatees 3.0 m and longer. In the area where a distal epiphyseal ossification center of the first phalanx of digit I would be expected, a small aggregation of bony spicules was seen in only three specimens (2.9 m, 3.0 m, 3.0 m TL).

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<u>Second phalanx</u>:

Proximal epiphysis - The ossified center for the proximal epiphysis of the second phalanx of digits II, III and IV first appeared in manatees at 2.3 m TL (Fig. 3.3, 3.5). All were consistently present in manatees at 3.0 m and longer. Only five manatees had a second phalangeal diaphysis of digit I, and in none of these radiographs was there a separate proximal or distal epiphyseal ossification center.

Distal epiphysis - The ossification center for the distal epiphysis of the second phalanx for digit IV first appeared at 2.3 m TL and was consistently present in manatees at 3.1 m and longer. The ossified distal epiphysis for digit III first appeared at 2.4 m and was consistently present at 3.3 m and that of digit II first appeared at 2.8 m and was consistently present in manatees 3.4 m and longer. The second phalanx for digit V is described in Chapter 5.

<u>Third phalanx</u>:

The third phalanges of digits II, III and IV are unusual in their half-moon shape. Clearly defined epiphyseal ossification centers were not seen in any third phalanx. However, a small ossification proximal and cranial to the diaphysis of the third phalanx was seen in four manatees (digit III - 2.8 m male, digits II and III - 3.2 m female, and digits II and III - 3.3 m female) and one other individual had an ossification center distal and cranial to the third phalanx (digits II and IV - 3.1 m male). These possible epiphyseal ossification centers ranged from a one millimeter dot to half the length of the third phalanx. The third phalanx for digit V is described in Chapter 5.

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Compilation of the above data on the first appearance was sorted in ascending order to produce a developmental sequence for the ossification centers of the manus (Table 3.1, Fig. 3.3) and ages assigned (Appendix B, Fig. 3.6) as per Marmontel's (1993) petrous temporal bone GLG study.

DISCUSSION

In the Florida manatee, all 18 manus diaphyseal centers were present in calves (1.0 - 1.5 m TL) with exceptions noted in the second and third phalanges of digit V (Chapter 5). All seven carpal and 27 epiphyseal ossification centers in the manus first appeared during the juvenile stage of development (1.5 - 2.8 m TL). In manatees longer than 2.8 m TL the osteological events were restricted to ossification centers becoming consistently present, growth in their size, and fusion of epiphyses with diaphyses (Chapter 4).

SEQUENCE OF DEVELOPMENT

The manatees in this study had a proximodistal sequence of appearance of the epiphyseal ossification centers in the manus (Table 3.1). The proximally located epiphyses were the first to appear followed by the more distal epiphyses; for example, the proximal metacarpal epiphyseal ossification centers first appeared before any phalangeal epiphyses appeared and the epiphyses of the proximal phalanges first appeared before the epiphyses of the distal phalanges appeared. This sequence is similar to that seen in domestic dogs

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(Parcher and Williams 1970), sea lions (Watson *et al.* 1985) and odontocetes (Ogden *et al.* 1981). In ungulates, a similar proximodistal sequence is seen in oxen (Curgy 1965). Thus, the proximodistal sequence of first appearance of ossification centers seen in the manatee manus follows the general mammalian pattern found in terrestrial and marine mammals.

Superimposed on the proximodistal developmental sequence was a caudocranial sequence of first appearance of ossification centers in the carpal bones, in the metacarpals, and in the phalanges. The ossification centers on the ulnar, or caudal, side first appeared sooner than the ossification centers on the radial or cranial side. The relatively larger size and more robust fifth digit, on the ulnar side, is associated with the caudally located muscle mass, the *flexor carpi ulnaris*, which is located caudal to the ulna and inserts on the fifth metacarpal bone (Domning 1978). This earlier development of the fifth digit and larger mature size could be explained by the functional requirements for an expanded muscle attachment site. This developmental precociousness of the caudal skeletal elements might also reflect a more basic tetrapod developmental scheme. For example, an embryonic study of a reptile (Müller and Alberch 1990) has shown that the primary axis of limb development passes through the ulna and continues distally through the fourth digit. The digital arch begins at the fourth carpal bone and radiates medially giving rise to the third and second carpal bones and later, digits. Digit V appears next and finally digit I. In domestic dogs, the epiphyses of metacarpal bones III and IV ossify first followed by the second and finally the fifth (Hare 1961). Further study

with mammalian pre- and postnatal specimens is needed to elucidate these apparent developmental gradients in the manus.

EXCEPTIONS

The carpal and epiphyseal ossification centers first appeared in a manatee at 1.9 m TL, except for one individual (one flipper available), out of 179 flippers. This exception was a young juvenile (MSW96204) at 1.6 m that had seven radiographically visible ossification centers. Possible explanations for this outlier may be that it was from a different population with a different rate of development, extreme individual variation, or possibly an error in recording the field data. The flipper length of this individual was similar to other manatees with approximately the same TL.

The distal epiphysis of the ulna normally has one ossification center but two manatees had two ossification centers. A similar finding has been documented for one dog in a study of 24 dogs (Hare 1961).

In the manatees in this study, possible epiphyses on the third phalanges were seen only seven times in four manatees. When separate epiphyseal ossification centers were present in the third phalanges they were very small in comparison with the well-developed epiphyses in the first and second phalanges. Separate bony epiphyses of the third phalanges are not normally present in domestic ungulates (Getty 1975, Sisson 1975). In contrast, epiphyses on the third phalanges have been reported as normally present and

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well-developed in humans (Greulich and Pyle 1959), four harp seals (Sumner-Smith *et al*. 1972) and a Ross seal (King 1965).

Although there were variations in the total body length at which postnatal ossification centers first appeared in the manatee manus, the sequence of appearance across animals was relatively constant.

The time of initial appearance of ossification centers in different species has been used to establish age-estimation tables, and thus correlate age and/or body size with other life-history data.   Such studies have enabled us to: distinguish differences in the time of development between males and females in humans (Pryor 1925); investigate the homology of skeletal elements in humans (Jones 1942); explain developmental processes that can lead to abnormalities and dysfunction in postnatal individuals as seen in dogs (Watson *et al*. 1986); and identify normal variations in the time of initial ossification in human hands (Pyle and Sontag 1943).

CORRELATION OF BONY DEVELOPMENT WITH CHRONOLOGICAL AGE

This study has been able to combine radiographic analysis of the first appearance of ossification centers in flippers with that of GLG analysis of the petrous temporal bone (Marmontel 1993) in the same manatee.   Correlation of these two techniques is summarized by Figure 3.6 and grouped by TL and life stage categories below.

TL range[1]	Ossification centers present	Life stage[1]	Age[2]
1.0 m	18 - 20* diaphyses	fetus/birth	- 0
1.0 - 1.5 m	no postnatal carpal or epiphyseal centers present	dependent calf	0 - 1
1.5 - 2.8 m	all postnatal carpal and epiphyseal centers first appear	juvenile	1 - 7
2.8 m +	consistently present, growth and fusion of centers	sexually mature adult	3 - 37+

[1] Bonde *et al.* 1983

[2] Marmontel 1993

* the variable presence of the second and third phalangeal diaphyses of digit V is documented in Chapter 5.

Representative bones were selected to illustrate proximodistal and caudocranial sequences in the ossification pattern of the manatee manus. These bones were selected based on their topography, visibility on all radiographs, and suggested possible use in age estimation. Example one: the caudally located ulnar carpal bone was consistently ossified in weaned juveniles (ca. 2.1 m) and corresponded to about two years of age. Therefore, if the ulnare is not ossified the manatee is less than 2.1 m and has not reached two years of age. The proximal epiphysis of the first phalanx of digit IV first appeared at 2.1 m hence the ulnare was always ossified when this epiphyseal ossification center was present, and thus manatees with this ossification should be older than two years. Example two: the cranially located radial carpal bone did not appear until 2.3 m and was never ossified in the manatees less than one year old (the most well represented sample). In fact, it was still unossified in one 2.8 m manatee. The radiale was consistently ossified

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at 2.8 m and corresponded to 3-8 years of age which is at the onset of sexual maturity. The absence of the radiale is therefore a good indicator for aging manatees less than 2.3 m TL and less than one year of age. A small ossification center of the radiale bone would indicate a manatee is approximately in the 2.3 - 2.8 size range and in the juvenile/adult transition. The distal epiphysis of the second phalanx of digit II first appeared at 2.8 m and was consistently ossified in mature adult manatees. Therefore, the radiale is always present when this epiphyseal ossification center is ossified and a manatee would be at least eight years old.

Analyses of the first appearance of these four representative ossification centers (ulnare, proximal epiphysis of the first phalanx of digit IV, radiale, and distal epiphysis of the second phalanx of digit II) revealed that there were no differences between males and females.

The ages at the first appearance of the proximal metacarpal epiphyses are the same as the distal row of carpal bones on which they articulate (Fig. 3.6). Like the carpal bones, the distal metacarpal epiphyses showed a caudal to cranial progression in the ages of initial ossification. More cranial, the distal epiphysis of metacarpal II appeared at the age of one year whereas that of metacarpal I does not appear until ages 2-4 years. The more cranial and the more distal epiphyses had a greater range of TL as well as ages. So, ossification centers such as the distal epiphyses of metacarpal I, the first and second phalanges of

digit II, and the second phalanx of digit III first appeared in late juveniles and sexually mature manatees.

When analyzing age with TL we ask the following question: If the TL is different between two ossification centers but the age is the same, is that TL really different? Within each age (in whole years), based on the number of annual GLGs and without subdividing these counts into smaller subunits, a range of TLs (in tenths of meters) is included. An age, in whole years, is a less discriminatory classification than is a measure with multiple subunits, such as TL, in tenths of meters. This can potentially separate individuals that are very close in skeletal maturity or have the opposite effect of grouping individuals together that should be in different categories. Careful interpretation of the breaking points separating TL and age should be considered because of individual variation.

The reliability of estimating age based on total body length, GLG counts, and manus radiography must be viewed with caution, especially in older/larger manatees. The comparison of total body length and chronological age was verified by comparing TL of GLG-counted (n=833) 1-9 year-old dead manatees with TL of known-age (n=52) 1-9 year-old living, wild manatees (Marmontel *et al.* 1996). These data suggest "that GLG counts in the periotic [petrous temporal bone as used in this thesis] are accurate estimates of age for manatees up to at least 300 cm total length and through the first 10 yr of life" (Marmontel *et al.* 1996). Also, because most of the GLG-aged manatees which have

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flipper radiographs included in this study are in the younger age groups (Appendix B), there is a high confidence level in the data assigned to the 0-4 ages. In the 0-4 ages there were at least 13 individuals per year. For ages older than four years there were less than five (and often one) individual per each year, and thus there is a low confidence level for ages greater than four. These data could be strengthened with GLG counts and flipper radiographs taken from more known-age salvaged animals.

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TABLE 3.1

The Sequence of Radiographic First Appearance of Manus Epiphyseal and Carpal
Ossification Centers Related to Total Body Length (m) in the Florida Manatee

Ossification Center	First Appearance	Consistently Present
Distal ulna	1.9*	2.1
Intermediate carpal	1.9*	2.1
Ulnare carpal	1.9*	2.1
Carpal three	1.9*	2.1
Carpal four	1.9*	2.1
Metacarpal V - prox.	1.9	2.2
Distal radius	2.0*	2.1
Carpal one	2.0	2.2
Metacarpal III - prox.	2.0	2.2
Metacarpal III - dist.	2.0	2.2
Metacarpal IV - dist.	2.0	2.2
Metacarpal V - dist.	2.0*	2.2
Carpal two	2.1	2.3
Metacarpal I - prox.	2.1	2.5
Metacarpal II - prox.	2.1	2.2
Metacarpal IV - prox.	2.1	2.2
Metacarpal II - dist.	2.1	2.5
Digit III - P1 prox.	2.1	2.6
Digit IV - P1 prox.	2.1	2.5
Digit V - P1 prox.	2.1	2.7
Digit II - P1 prox.	2.2	2.9
Radiale	2.3	2.8
Digit III - P1 dist.	2.3	2.9
Digit IV - P1 dist.	2.3	2.7
Digit V - P1 dist.	2.3	3.0
Digit II - P2 prox.	2.3	3.0
Digit III - P2 prox.	2.3	3.0
Digit IV - P2 prox.	2.3	3.0
Digit IV - P2 dist.	2.3	3.1
Metacarpal I - dist.	2.4	3.0
Digit II - P1 dist.	2.4	3.0
Digit III - P2 dist.	2.4	3.3
Digit I - P1 prox.	2.5	3.1
Digit 2 - P2 dist.	2.8	3.4

* MSW96204 (1.6 m) is not included in this data but this ossification center was present.

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Figure 3.1

Outline sketch of all ossification centers that appeared in the manus of the Florida manatee. Dorsal view of left manus. An axial line joins each diaphysis with its proximal and distal epiphyses - for example, metacarpal III displays the proximal epiphysis (p.e.) and the distal epiphysis (d.e.). Radius (R); ulna (U); carpal bones, - radiale (r), intermedium (i), ulnare (u), carpal one (C1), carpal two (C2), carpal three (C3), carpal four (C4); first through fifth metacarpal bones (Mc I-V); first phalanx (P1); second phalanx (P2); third phalanx (P3). The unusual development of the second and third phalanges of digit V is discussed in Chapter 5.

Proximal

Cranial

Caudal

Distal

R U

r i u

C1 C2 C3 C4

Mc I Mc II p.e. Mc III Mc IV Mc V

d.e.

P1 P1 P1 P1 P1

P2 P2 P2 P2 P2

P3 P3 P3 P3
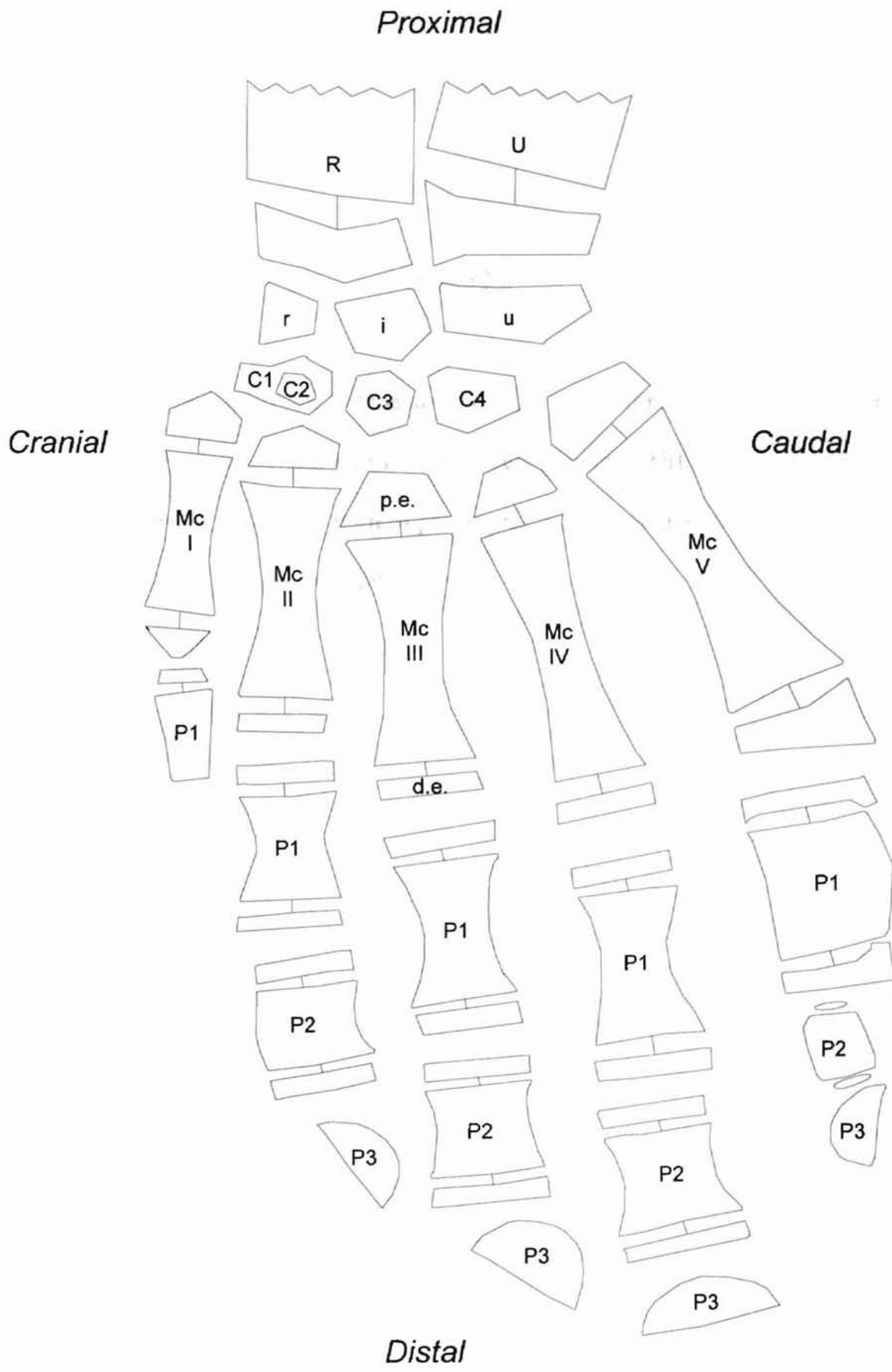
Figure 3.2

Dorsopalmar radiograph of the left manus of a dependent calf male Florida manatee (1.3 m TL) showing the diaphyseal ossification centers present at birth: radius (R) and ulna (U), five metacarpals (I-V) and thirteen phalanges. Two phalanges, rather than the more usual one, were present in digit I of this manatee.
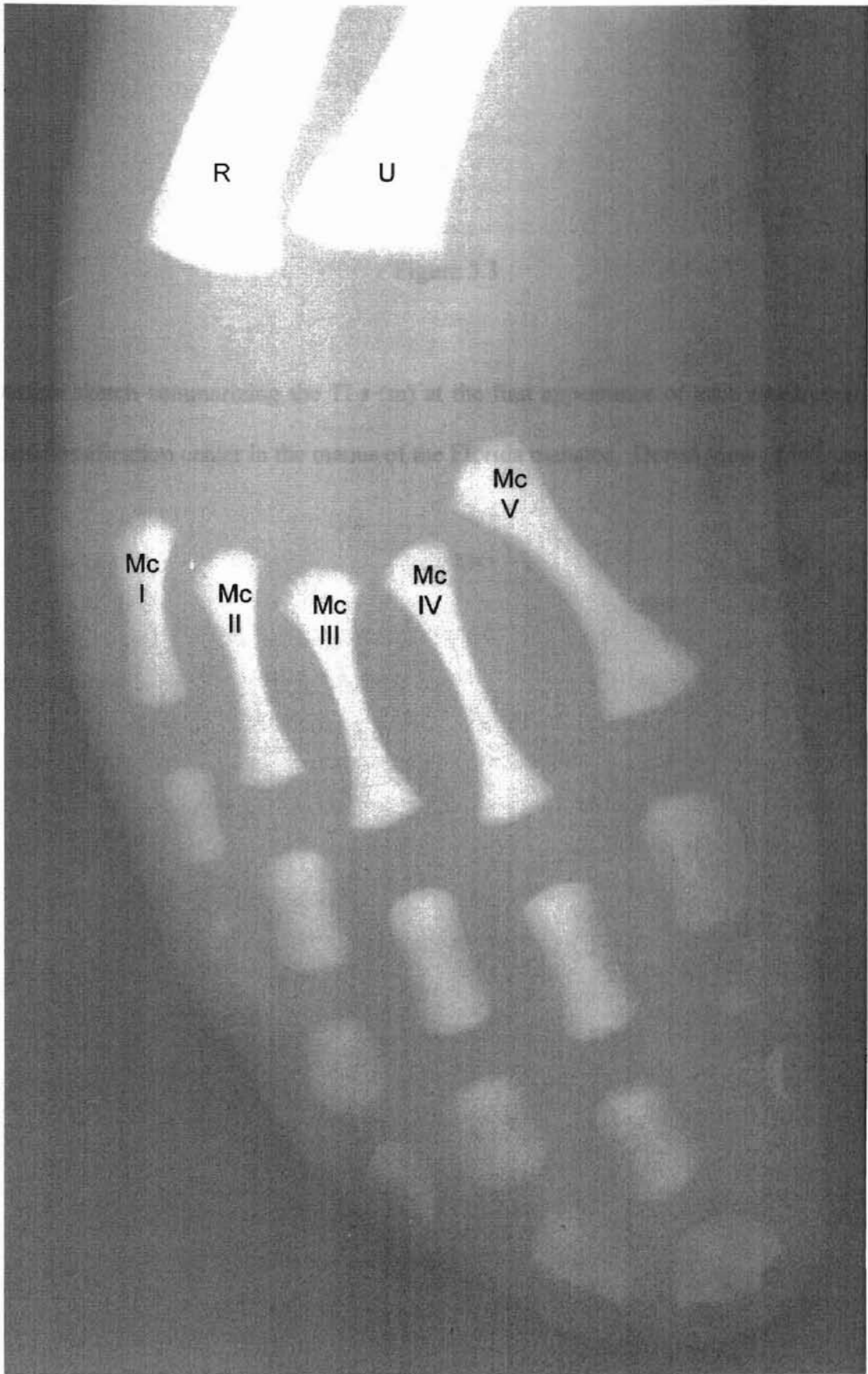
Figure 3.3

Outline sketch summarizing the TLs (m) at the first appearance of each epiphyseal and

carpal ossification center in the manus of the Florida manatee. Dorsal view of left manus.

Figure 3.4

Dorsopalmar radiograph of the left manus of a juvenile male Florida manatee (2.0 m TL) showing the first appearance of postnatal carpal and epiphyseal ossification centers: distal epiphyses (Re, Ue) of the radius (R) and ulna (U); carpal bones - intermedium (i), ulnare (u), carpal one (C1), carpal three (C3), carpal four (C4). The proximal epiphyses of metacarpals III and V, and distal epiphyses of metacarpals III, IV and V were ossified also.
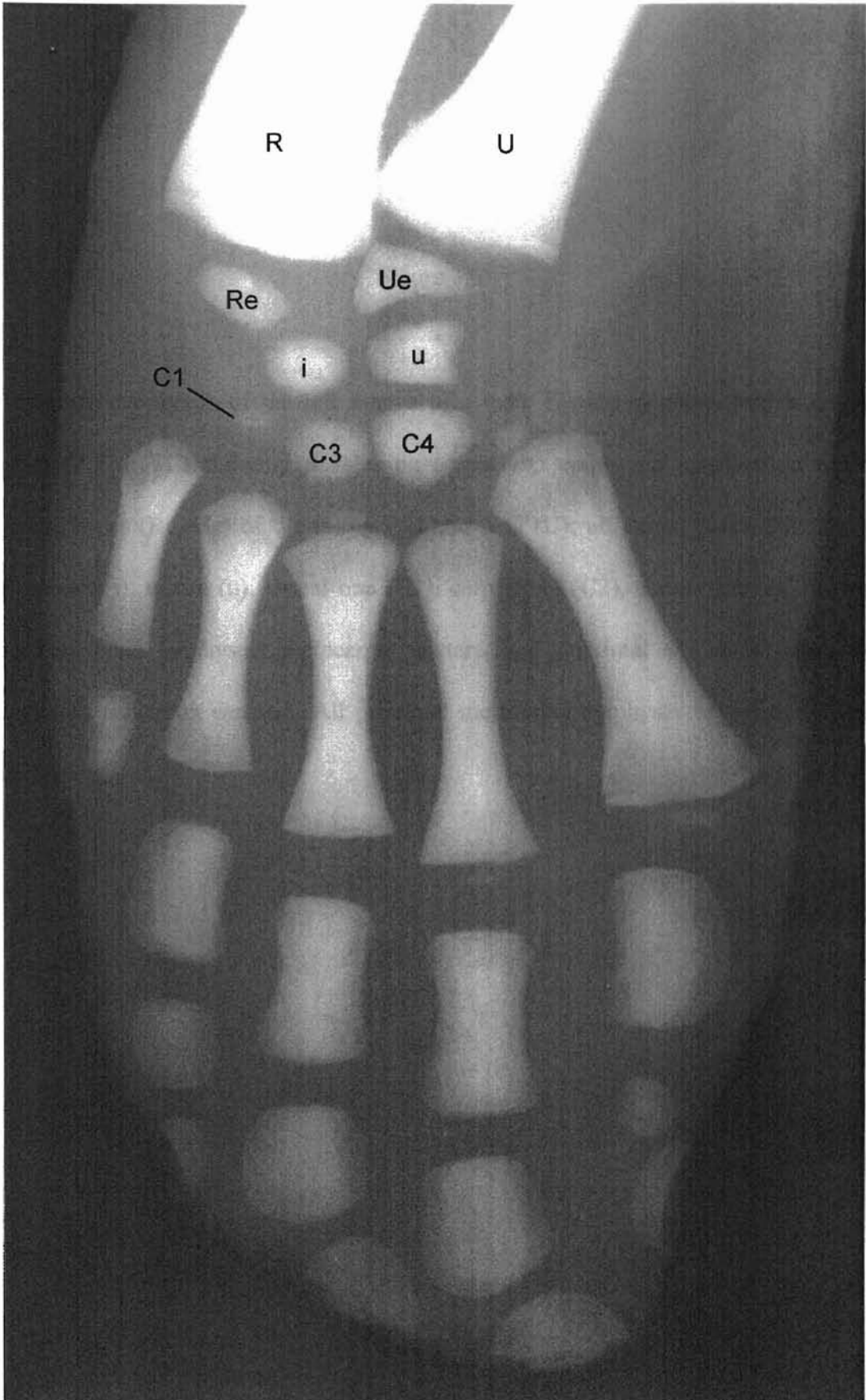
Figure 3.5

Dorsopalmar radiograph of the left manus of a male Florida manatee, just at sexually maturity (2.7 m TL) showing developing carpal and epiphyseal ossification centers: distal epiphyses (Re, Ue) of the radius (R) and ulna (U); all carpal bones - radiale (r), intermedium (i), ulnare (u), carpal one (C1), carpal two (C2), carpal three (C3), carpal four (C4); distal epiphyseal metacarpal centers, and proximal and distal phalangeal epiphyseal ossification centers. All proximal metacarpal epiphyses have fused to their adjacent diaphyses.
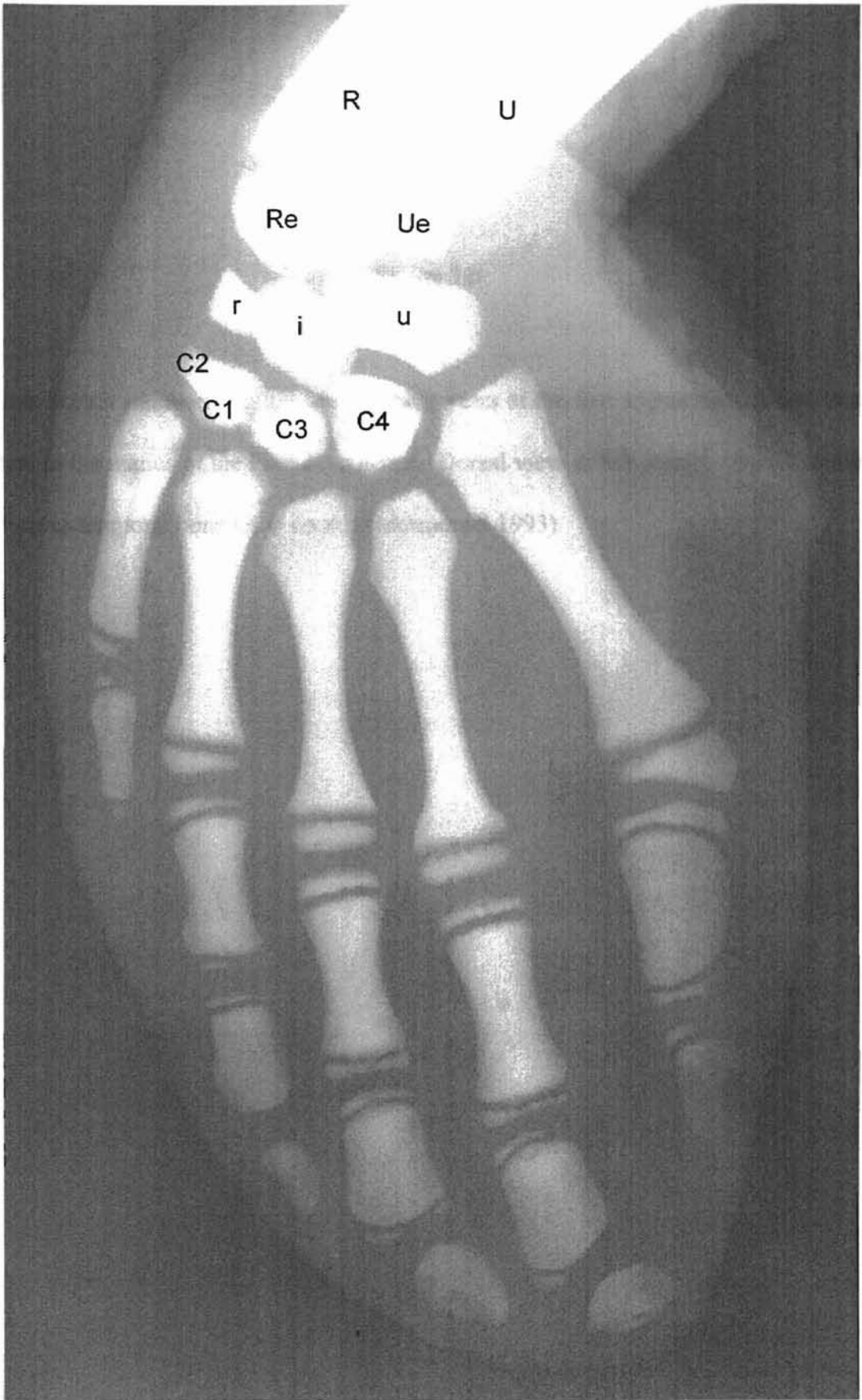
Figure 3.6

Outline sketch summarizing the age in whole years at the first appearance of ossification

centers in the manus of the Florida manatee. Dorsal view of left manus. Age determined

by petrous temporal bone GLG counts (Marmontel 1993).

Fusion and Sequence of Fusion of Epiphyseal and Carpal
Ossification Centers in the Manus of Postnatal Florida Manatees,
Trichechus manatus latirostris

INTRODUCTION

The fusion of an epiphysis to its diaphysis in a long bone is well studied through

histology (Burkitt *et al.* 1993), skeletal preparations (Struthers 1893), and radiography

(Wetherington 1961). Determining the sequence of fusion of epiphyses to diaphyses

reveals a maturation pattern within a species that is relatively constant and can be

compared intra- and interspecifically (Todd and Todd 1938). Epiphyseal fusion has been

examined radiographically in terrestrial and marine mammals including horses (Myers

and Emmerson 1966), domestic dogs Hare 1960), humans (Pryor 1925), harp seals

(Sumner-Smith *et al.* 1972), and common dolphins (Hui 1979). The sequence of

epiphyseal fusion in ungulates and sirenians was investigated by gross morphological

characteristics in a comparative study of museum specimens (Todd and Todd 1938).

They found that the sequence of epiphyseal fusion in the thoracic limb of sirenians was

similar to that seen in ungulates, such as sheep, deer and oxen. A similar sequence was

seen in rodents and humans, thus, manatees and dugongs follow the common pattern of

epiphyseal fusion for mammals.

Bone development is a biological record of the progression of time. The postnatal

longitudinal growth of a mammalian long bone occurs at the cartilage growth plate

between the diaphysis and epiphysis. Here, cartilage cells and matrix are replaced by bone cells and matrix. In addition, endochondral ossification occurs in the cartilage epiphysis. As the typical terrestrial mammal approaches puberty, the epiphyseal bony center enlarges, the cartilage growth plate becomes fully converted to bone, and bony bridges fuse the epiphysis to the diaphysis. The beginning of fusion is defined as a mineralized bridge between the epiphyseal and diaphyseal ossification centers (Haines 1975). The completion of fusion is the complete replacement of the epiphyseal cartilage growth plate by bone and, in terrestrial mammals (Morris 1972), usually coincides with the completion of puberty.

The objectives of this study of the Florida manatee manus were to: 1) determine when each separate epiphyseal ossification center showed "first fusion" to its respective diaphysis as related to total body length (TL); 2) determine when each epiphysis was "consistently fully fused" to its diaphyses as related to TL; 3) determine when each carpal bone reached the adult shape as related to TL; 4) describe the sequence of fusion of the epiphyses as related to TL; and 5) correlate ossification events with chronological age by comparing TL of radiographed manatees with TL of aged manatees (Marmontel 1993).

Examination and analysis of radiographs of 179 flippers of Florida manatees revealed that the first bony bridges were seen spanning the proximal cartilage growth plate of the metacarpal bones at 2.3 m TL. These initial bridges were first seen appearing throughout

the manus until 3.0 m TL. Consistent full fusion was seen in most metacarpals and phalanges at 3.4 m although the distal epiphysis of the fifth metacarpal remained unfused, even in the longest specimen, at 3.6 m TL. The carpal bones reached adult shape by 2.8 m TL, which approximates the attainment of sexual maturity (2.8 m). These specific ranges of TL for the fusion of epiphyses establish the sequential development of the manatee manus bones and form the basis for a possible method to estimate the age of manatees.

MATERIALS AND METHODS

SPECIMENS

Disarticulated flippers were collected and frozen from 167 Florida manatee carcasses, *Trichechus manatus latirostris*, salvaged from the coast and waterways of Florida (1976-1984, 1996). Salvage records from the National Biological Survey, Sirenia Project (Gainesville, FL) provided field number, total body length (TL), sex, side of body of flipper, salvage location and date, and descriptive comments for each individual. The sample consisted of flippers from 92 male and 75 female manatees that ranged in total length from a 1.0 m neonate to a 3.6 m adult (Appendix A). Recorded field measurements of TL were used with a 95% level of confidence and therefore all manatee TLs were rounded to 0.1 m. Twelve specimens had both right and left flippers. This formed a postnatal developmental series of 179 flippers from manatees of evenly distributed lengths.

RADIOGRAPHIC TECHNIQUE

Flippers were thawed, cleaned, and standard plain-film radiographs were taken. The majority of radiographs were taken between 1976-1984 with unrecorded radiographic technique at the College of Veterinary Medicine, University of Florida, Gainesville, FL. In 1996, fifteen flippers were radiographed at the College of Veterinary Medicine, Oklahoma State University, Stillwater, OK. Each prepared flipper was placed on top of a film cassette covered with a thin sheet of plastic. Flippers from manatees longer than 1.6 m TL were disarticulated at the elbow joint and radiographed in a lateromedial view using 36x43 cm (14"x18") non-grid, ultra-vision detail screen cassettes and high-detail film. Flippers from manatees shorter than 1.6 m TL were disarticulated at the shoulder joint and radiographed in a mediolateral view using 20x25 cm (8"x10") non-screen film. The film-to-source distance for all flippers was 102-112 cm (40"-44"). Routine perpendicularly oriented X-ray tube exposed film produced images with superimposition of bones. The carpus was thicker than the tips of the digits and therefore the flipper laid at an angle. Thus, to produce an X-ray beam perpendicular to the longitudinal axis of the long-bones and parallel to their cartilage growth plates, the X-ray tube and collimator was angled between 5 and 10 degrees, depending on the size of the flipper. This technique resulted in radiographs with minimal superimposition of bones and a clear penetration of epiphyseal cartilage growth plates. The flippers were radiographed at 52-60 kVp at 400 mA for 0.006-0.5 seconds. Film was processed in an automatic processor.

The proximal and distal rows of carpal bones were isolated by dissection from four manatees. These specimens were radiographed in a lateromedial and proximodistal view with a soft X-ray technique in a shielded cabinet X-ray unit. Exposures ranged from 60-80 kVp at 3 mA for 2 minutes on non-screen, high-detail sheet film.

EVALUATION TECHNIQUE

All radiographs were examined by naked eye on a standard radiographic illuminator at Oklahoma State University, Stillwater, OK. Each radiograph was examined to identify the beginning and completion of fusion. All radiographs were examined three separate times.

The fusion data were analyzed and is presented as two different TLs: 1) the "first fusion" of an epiphyseal ossification center to its diaphysis was recorded as the TL of the shortest individual in which radiopaque bony bridges were seen spanning the radiolucent epiphyseal cartilage growth plate (even though there may have been manatees at the same or greater lengths without fusion); 2) "consistent full fusion" of an epiphyseal ossification center to its diaphysis was recorded as the shortest TL at which the epiphysis was completely fused to the diaphysis, and the epiphyseal cartilage growth plate fully obliterated by bony bridges in all manatees.

These two fusion events were selected to represent stages of development in the manatee manus because they are recognizable, easily definable, and consistent beginning and end

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points of fusion. These criteria were modified from a 6-stage radiographic classification system created to study the development of odontocete flipper bones (Ogden *et al.* 1981).

The fusion data were arranged in ascending TL order to produce a sequence of epiphyseal fusion for the manus epiphyseal ossification centers. Since the development and fusion of the second and third phalangeal diaphyseal and epiphyseal ossification centers in the fifth digit had an unusual development, these data are presented separately in Chapter 5.

LIFE STAGES

Overall, the 167 Florida manatees in this study can be categorized into four different life stages based on their TL (Bonde *et al.* 1983, O'Shea *et al.* 1985): fetus $-\leq 0.9$ m (n=0); dependent calf – 0.9 - 1.5 m (n=32); juvenile – 1.5 - 2.8 m (n=84); sexually mature adult $->2.8$ m (n=51). Males become sexually mature as early as two years of age (2.4 m TL) based on spermatogenesis studies (Hernandez *et al.* 1995). Females become sexually mature at three to four years of age (2.5 - 3.0 m) based on ovarian follicle studies (Marmontel 1995). Moreover, chronological age has been estimated by an examination of bone layering and analysis of growth-layer-groups (GLGs) in the petrous temporal bone of Florida manatees and correlated with known-age specimens (Marmontel 1993). Flippers from 106 of these same age-estimated individuals were radiographed for this study (Appendix B). Marmontel's chronological age data was consulted only after the collection of all data from all radiographs was completed. The fusion of ossification centers in 179 flippers were evaluated radiographically. The TL at fusion of epiphyses to

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the diaphyses of all manatee flippers was then correlated with Marmontel's aged specimens to achieve ages in which these fusion stages occur.

DISSECTION

The manus of six fresh flippers was dissected to corroborate radiographic findings. The purpose of the dissections was to investigate morphological features that were not apparent in the dorsopalmar radiographs and to verify structures seen in the radiographs. These consisted of: 1) dissecting the proximal row of carpal bones to see if and when the radiale and intermedium fuse; and 2) dissecting the distal row of carpal bones to see when carpal bones one and two fuse to become one bone. Intact proximal and distal rows of carpal bones were dissected from four flippers and then radiographed in a shielded cabinet X-ray unit to look for bony spicules crossing between carpal bones one and two, and between the radiale and intermedium carpal bones.

RESULTS

RADIOGRAPHIC EVALUATION

Fusion stages for all epiphyses and carpal development could be evaluated in 170 out of 179 radiographs. Due to overexposure, incorrect positioning, superimposition of bones, or post-mortem damage to flippers, nine radiographs were only partially evaluated.

FUSION OF EPIPHYSES TO DIAPHYSES

Distal epiphyses of the radius and ulna:

The radial distal epiphysis and diaphysis was usually (164 out of 179 flippers) at the same stage of fusion as the ulnar distal epiphysis and diaphysis. First fusion of the distal epiphyses of both the radius and ulna was first detected in a radiograph of a manatee at 2.9 m TL. Full fusion was consistently seen in manatees at 3.4 m TL and longer.

The distal epiphyses of the radius and ulna also fused together (Fig. 4.1). In addition, the distal third of each diaphysis fused to the other. The radial and ulnar epiphyses and diaphyses fused together at about the same TL, although four manatees had epiphyses that were fused to each other but not to their respective diaphyses. This epiphyseal and diaphyseal fusion could not always be evaluated because the extreme density of the manatee radius and ulna created a radiopaque image which often obliterated the detail of adjacent cartilage growth plates and bones.

Carpal bones:

Carpal bones three and four were the first carpal bones to attain the adult shape in a 2.6 m TL manatee. The radiale, intermedium, ulnare, and carpal bones one and two followed next at 2.8 m. All carpal bones - the radiale, intermedium, ulnare, and carpal bones one through four - were consistently at this adult shape in manatees at 3.1 m and longer (Fig. 4.2, 4.3).

This radiographic study has revealed that the two cranial-most carpal bones in the distal row coalesced. Carpal one, the larger bone, articulated with metacarpals I and II; whereas carpal two, the smaller bone, lay proximal to metacarpal II and immediately dorsal to carpal one (terminology as per Flower 1885). These two carpal bones originated as separate ossification centers but fused early in their postnatal development.

Carpal bones one and two, located distal to the radiale and proximal to the first and second metacarpals, were usually superimposed on one another and the TL at their complete development could not be differentiated on the whole flipper, dorsopalmar radiographs. Two ossification centers were observed radiographically first appearing at different TLs. Two separate carpal bones were found in boiled-out flippers of individuals less than 2.6 m TL. A small, pea-sized carpal two lay cupped on the dorsal surface of the larger carpal one.

The high-detail proximodistal radiographs of the dissected proximal row of carpal bones of four manatees revealed the radiale and intermedium fused in a 2.9 m manatee but not in manatees at 2.8 m, 3.1 m, and 3.2 m TL.

Metacarpals:

The individual proximal and distal epiphyses of the metacarpal bones began fusing centrally near the axis of the metacarpal bone and then continued to ossify peripherally.

First fusion of the proximal epiphyses of all five metacarpal bones occurred in a manatee at 2.3 m TL. These proximal epiphyses were all consistently fully fused at 3.0 m TL.

First fusion of the distal epiphyses of metacarpals III and IV occurred in a manatee at 2.6 m TL. Those of metacarpals I and II occurred at 2.9 m and metacarpal V at 3.1 m TL. In manatees longer than 3.4 m, the distal epiphysis of metacarpals I through IV were consistently fully fused. First fusion of the distal epiphysis of the fifth metacarpal did not occur until 3.1 m. This metacarpal V distal epiphysis was variably fully fused in manatees between 3.1 m and 3.6 m, but in the longest individual, 3.6 m TL, it was not fully fused (Fig 4.3). The distal epiphysis of metacarpal V was the last epiphysis to fully fuse in the manatee manus.

Phalanges:

The proximal and distal epiphyses of the first phalanges of digits I - V (excluding distal epiphyses of digit I) and the proximal and distal epiphyses of the second phalanges of digits II - IV began fusing peripherally, often simultaneously abaxially.

First phalanx:

Proximal epiphysis - First fusion of the proximal epiphyses of the first phalanges for digits III and IV occurred in a 2.4 m TL manatee. At 2.6 m the proximal epiphysis of the first phalanx of digit V showed first fusion, followed by the first phalanx of digit I at

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2.8 m and digit II at 2.9 m. All proximal epiphyses of the first phalanges were consistently fused after 3.4 m TL (Fig. 4.3).

Distal epiphysis - First fusion of the first distal epiphysis occurred in the first phalanx of digit V in a 2.6 m TL manatee followed by digit IV at 2.8 m and digits II and III at 2.9 m. A separate distal epiphyseal ossification center of the first digit was rarely seen (n=3) and not categorized. The distal epiphyses of the first phalanges of digits II - IV were consistently fully fused in manatees 3.4 m TL or longer.

Second phalanx:

Proximal epiphysis - First fusion of the proximal epiphysis of the second phalanx of digit IV occurred in a 2.8 m TL manatee with digits II and III following at 2.9 m TL. Those in digits II - IV were consistently fully fused in manatees 3.4 m TL and longer. A second phalanx in digit I was rare (n=5) and was not evaluated. The unusual development and maturation of the second and third phalanges of digit V is described in Chapter 5.

Distal epiphysis - First fusion of the distal epiphysis of the second phalanges of digits III and IV occurred in a 2.8 m TL manatee and the second digit at 3.0 m TL. Like the proximal epiphyses, the distal ossification centers were consistently fully fused after 3.4 m TL.

DISCUSSION

Radiographic analysis of the Florida manatee manus has revealed two directional sequences of first fusion (excluding the fifth digit): proximodistal and caudocranial (Table 4.1, Fig. 4.2). Similar proximodistal and caudocranial sequences were found in the first appearance of ossification centers (Chapter III). In the fusion of epiphyses, the proximal epiphysis of each metacarpal and phalanx began to fuse before the distal epiphysis of each of these bones. In human hands, however, "the sequence of union is not the sequence of development" (Pryor 1925).

The general proximodistal sequence of fusion seen in the manatee manus is similar to that seen in other marine mammals: for example - in odontocetes, radiographs of the manus from common dolphins, *Delphinus delphis* (Hui 1979), and the distal radius and ulna from Dall's porpoises, *Phocoenoides dalli dalli*, and from the short-finned pilot whales *Globicephala macrorhynchus* (Ogden *et al.* 1981) all demonstrated a proximodistal fusion sequence of epiphyses. In contrast, in the semi-terrestrial harp seals *Pagophilus groenlandicus*, the proximal epiphysis of the distal phalanges fused before other manus epiphyses (Sumner-Smith *et al.* 1972) which is also seen in humans (Greulich and Pyle 1959). And, in mammals generally, the fusion of each ossified epiphysis to its respective diaphysis in the manus occurs at a "relatively constant age" between individuals within a species (Silver 1963). Overall, however, the sequence of fusion in the manatee conforms to the basic mammalian plan of these terrestrial, semi-aquatic and exclusively aquatic mammals.

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The distal epiphysis of the fifth metacarpal bone was the last to fuse (excluding one specimen) and remained "open" for an extended period of time when all other bones in the manus had fused and were skeletally mature (Fig. 4.3). This attribute could be beneficial for estimating the age of older manatees.

The trend of the proximal epiphyses fusing to the diaphyses before the distal epiphyses fuse is demonstrated by the proximal epiphyses of the metacarpals and the first row of phalanges. This fusion follows the same proximodistal sequence as seen in the first appearance of ossification centers. Another possible explanation for this sequence of fusion could be the result of muscle attachment sites. In manatees - *Trichechus manatus* (Murie 1872); *Trichechus inunguis* (Domning 1978) - there are prominent tendinous insertions of muscles on the epiphyseal ends of the bones. For example, *flexor carpi radialis*, four interosseus muscles of the manus, and the superficial and deep digital flexor muscles originate and/or insert on the proximopalmar ends of the metacarpals, distal metacarpals and the proximal ends of the phalanges. Dorsally, the tendinous attachments of muscles *extensor pollicis brevis* and *longus, extensor carpi radialis*, and *digital extensor quinti* insert on the proximal ends of metacarpals I, II, III, and V.

In the manatees in this study, the distal ends of the radius and ulna fused to each other (diaphyses and epiphyses). This parallels the fusion seen in dugongs (Kaiser 1974). Similar radius to ulna fusion is seen in the horse (Myers and Emmerson 1966) and in the

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ox (Emara 1937) but not in terrestrial carnivores (Evans 1993) nor cetaceans (Flower 1885) or seals (Flower 1885).

Individual variation in the rate of skeletal development of terrestrial mammals is affected by many determinants including genetic, hormonal, nutritional, and reproductive. Higher demands for calcium in pregnant and lactating female manatees has been suggested to affect the development of the bones (Marmontel 1996). Perhaps this calcium demand might only affect the later full development of ossification centers and fusion of epiphyses in females. The initial ossification in young, sub-adult females might not be affected enough to make a discernible difference in the time of appearance of ossification centers.

In precocious locomotory terrestrial mammals, such as ungulates, the epiphyses of the thoracic limb phalanges are well ossified at birth and fuse relatively early, whereas in most other terrestrial mammals, this fusion occurs later, but by sexual maturity (Dyce et al. 1996). In contrast, the metacarpal and phalangeal epiphyses of some marine mammals, like cetaceans (Struthers 1863, Flower 1885) and sirenians (Kükenthal 1891), ossify later and remain unfused for an extended period of time. One possible explanation could be that marine mammal limb bone development is retarded since they swim in a buoyant medium and are freed from the skeletal demands to resist gravity that affected their terrestrial ancestors and relatives. This delayed fusion time in manatees allows an extended period of time in which to evaluate bone development.

It has been suggested that a numeric scoring of epiphyseal fusion is more accurate when aging many individuals in a population than individual animals (Harris 1978). For example, numeric scoring of epiphyseal fusion in the red fox *Vulpes vulpes* (Harris 1978) where there was substantial individual variation within the same litter and hence, an average was more accurate for aging. In the manatees in this study, individual variation could account for the broad range of TLs (up to 0.7 m) seen in the first fusion of epiphyses (Table 4.1). Such variations suggest that the sequence of fusion might be a better estimation of age than the bone development of a specific site in an individual and that assigning a number for any ossification event requires caution but does give an estimated range.

Fusion of the radiale, intermedium, and centrale carpal bones is an ordinal characteristic of the Carnivora, such as the dog (Evans 1993) and black bear (Marks and Erickson 1966). The ordinal characteristic in the ungulates, the horse and ox, is the radiale and the intermedium remain separate (Getty 1975, Sisson 1975) which is also typically seen in manatees, although fusion of the radiale and intermedium was reported in one specimen (Quiring and Harlan 1953). In this present study, radiographs of the manus in adults often revealed superimposition of the radiale and intermedium and thus obscured the separateness, or not, of these two bones. Fusion between the radiale and intermedium was found in one (2.9 m) out of four dissected specimens. Adult manatees have a carpus of seven bones. Carpal fusion is exaggerated in dugongs in which a skeletally mature individual has three carpal bones composed of the following coalescences: radiale plus

the intermedium; ulnare plus the accessory carpal bone (Kaiser 1974); and carpal bones

one, two, three and four form one distal carpal bone (Freund 1904).

CORRELATION OF BONY DEVELOPMENT WITH CHRONOLOGICAL AGE

Relative age is the evaluated age of one animal with respect to the state of development

of another (Morris 1972). One of the most useful comparisons of relative age is the

fusion of epiphyses. It was by this process that this study was undertaken and is thus

subject to much individual variation in skeletal development. But, the use of radiographs

to study epiphyses allows a reasonably reliable way of classifying manatees into a series

of ages. And, the delayed development of the manatee's bones prolongs the use of the

epiphyseal fusion method for age estimation.

The ages at which bony bridges started to span the epiphyseal cartilage growth plate

ranged from one to fourteen years old with the majority of manatees lying between the

ages of two and eight years old (Fig. 4.4). The proximal metacarpal epiphyses reached

first fusion at one year of age, which corresponds to the age at weaning. None of the

other ossified epiphyses of the manus showed bony bridges until at least two years of age.

The distal epiphysis of metacarpals III and IV, the proximal epiphysis of the first phalanx

of digits III, IV and V, and the distal epiphysis of the first phalanx in digit V all reached

first fusion around two years of age. The distal epiphyses of metacarpals I and II, most

phalangeal epiphyses, and the distal epiphyses of the radius and ulna reached first fusion

between three to eight years of age. These ages correspond to the range of time of the

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onset of sexual maturity. Finally, the distal epiphysis of the second phalanx of digit II did not show bony bridges until at least eight years of age, which is well into adulthood.

The ages at which epiphyses were completely and consistently fused to their diaphyses happened earliest in the proximal metacarpals in manatees of this study between 6-14 years of age (Fig. 4.5). The carpus consistently had the adult shaped bones between the ages of 8-14 years of age with the radiale at 8-37 years of age. The remaining epiphyses - distal radius and ulna, distal metacarpal, and proximal and distal phalanges - were consistently fused about 25 years of age (Marmontel 1993).

The reliability of estimating age based on total body length, GLG counts, and manus radiography must be viewed with caution, especially in older/larger manatees. The comparison of total body length and chronological age was verified by comparing TL of GLG-counted (n=833) 1-9 year-old dead manatees with TL of known-age (n=52) 1-9 year-old living, wild manatees (Marmontel *et al.* 1996). These data suggest "that GLG counts in the periotic [petrous temporal bone as used in this thesis] are accurate estimates of age for manatees up to at least 300 cm total length and through the first 10 yr of life" (Marmontel *et al.* 1996). Also, because most of the GLG-aged manatees which have flipper radiographs included in this study are in the younger age groups (Appendix B), there is a high confidence level in the data assigned to the 0-4 ages. In the 0-4 ages there were at least 13 individuals per year. For ages older than four years there were less than five (and often one) individual per each year, and thus there is a low confidence level for

ages greater than four. These data could be strengthened with GLG counts and flipper radiographs taken from more known-age salvaged animals.

All of these ages (4+) are based on few individuals and should be used with caution. The only safe claim to make about the age of individuals with consistently fully fused epiphyses is that the manatees are sexually mature. Although some specific total body lengths might change between individuals and populations, the sequence of fusion should be similar to that shown here.

Limitations to the use of radiographic analysis of skeletal development are associated with radiographic technique. The position of the flipper on the X-ray film cassette is critical to get a perpendicular penetration of the epiphyseal cartilage growth plates in individuals longer than approximately 2.2 m TL. The best positioning should allow identification of any bony bridges. Flipper positioning for perpendicular penetration of the epiphyseal cartilage growth plates becomes more difficult with mature adults as the thickness of the flipper increases proximally. Correct radiographic exposure is also vital to produce readable radiographs.

Radiography of salvaged flippers is a routine procedure that can be carried out in a hospital with standard radiographic equipment or with a portable X-ray machine. The feasibility of gathering data in the field will take further work but it has been accomplished with a captive West African manatee, *Trichechus senegalensis*, in the U.S.

(Kaiser 1974) and with 15 captive Amazonian manatees, *Trichechus inunguis*, in an aquarium (de Assis Ribeiro and Best 1984). Since the manatee thoracic limb protrudes from the body wall proximal to the elbow and has flexible joints, the manus should be readily positionable in a live animal for radiography in the field. To implement this technique on wild manatees, however, the capture and radiography are challenges yet to be addressed.

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TABLE 4.1

The Sequence of Fusion of Manus Epiphyseal and Development of Carpal Ossification
Centers Related to Total Body Length (m) in the Florida Manatee

Ossification Center	First Fusion/ Development (m)	Consistent Full Fusion/ Development (m)	Age* at Consistent Fusion
Metacarpal I - prox.	2.3	3.0	6-14
Metacarpal II - prox.	2.3	3.0	6-14
Metacarpal III - prox.	2.3	3.0	6-14
Metacarpal IV - prox.	2.3	3.0	6-14
Metacarpal V - prox.	2.3	3.0	6-14
Digit three - P1 prox.	2.4	3.4	25+
Digit four - P1 prox.	2.4	3.4	25+
Carpal three	2.6	3.1	8-14
Carpal four	2.6	3.1	8-14
Metacarpal III - dist.	2.6	3.4	25+
Metacarpal IV - dist.	2.6	3.4	25+
Digit five - P1 prox.	2.6	3.4	25+
Digit five - P1 dist.	2.6	3.4	25+
Radiale	2.8	3.1	8-37
Intermediate carpal	2.8	3.1	8-14
Ulnare carpal	2.8	3.1	8-14
Carpal one + two	2.8	3.1	8-14
Digit three - P2 dist.	2.8	3.4	25+
Digit four - P1 dist.	2.8	3.4	25+
Digit four - P2 prox.	2.8	3.4	25+
Digit four - P2 dist.	2.8	3.4	25+
Distal radius	2.9	3.4	25+
Distal ulna	2.9	3.4	25+
Metacarpal I - dist.	2.9	3.4	25+
Metacarpal II - dist.	2.9	3.4	25+
Digit one - P1 prox.	2.9	3.4	25+
Digit two - P1 prox.	2.9	3.4	25+
Digit two - P1 dist.	2.9	3.4	25+
Digit three - P1 dist.	2.9	3.4	25+
Digit two - P2 prox.	2.9	3.4	25+
Digit three - P2 prox.	2.9	3.4	25+
Digit two - P2 dist.	3.0	3.4	25+
Metacarpal V - dist.	3.1	3.6+	25+

* Age correlated by petrous temporal bone GLG counts (Marmontel 1993).

Figure 4.1

Dorsopalmar radiograph of the left manus of an adult female Florida manatee (2.9 m TL) representing an advanced degree of skeletal maturation. All epiphyseal ossification centers are present and well developed. The distal epiphysis (Re) of the radius (R) has fused to the distal epiphysis (Ue) of the ulna (U). Carpal bones have reached adult shape - radiale (r), intermedium (i), ulnare (u), carpal one (C1), carpal two (C2), carpal three (C3), carpal four (C4). All proximal metacarpal epiphyses have fused to their adjacent diaphyses. The proximal and distal epiphyses of the phalanges are in various degrees of fusion to their diaphyses. An osteolytic lesion is present on the second phalanx of digit IV.
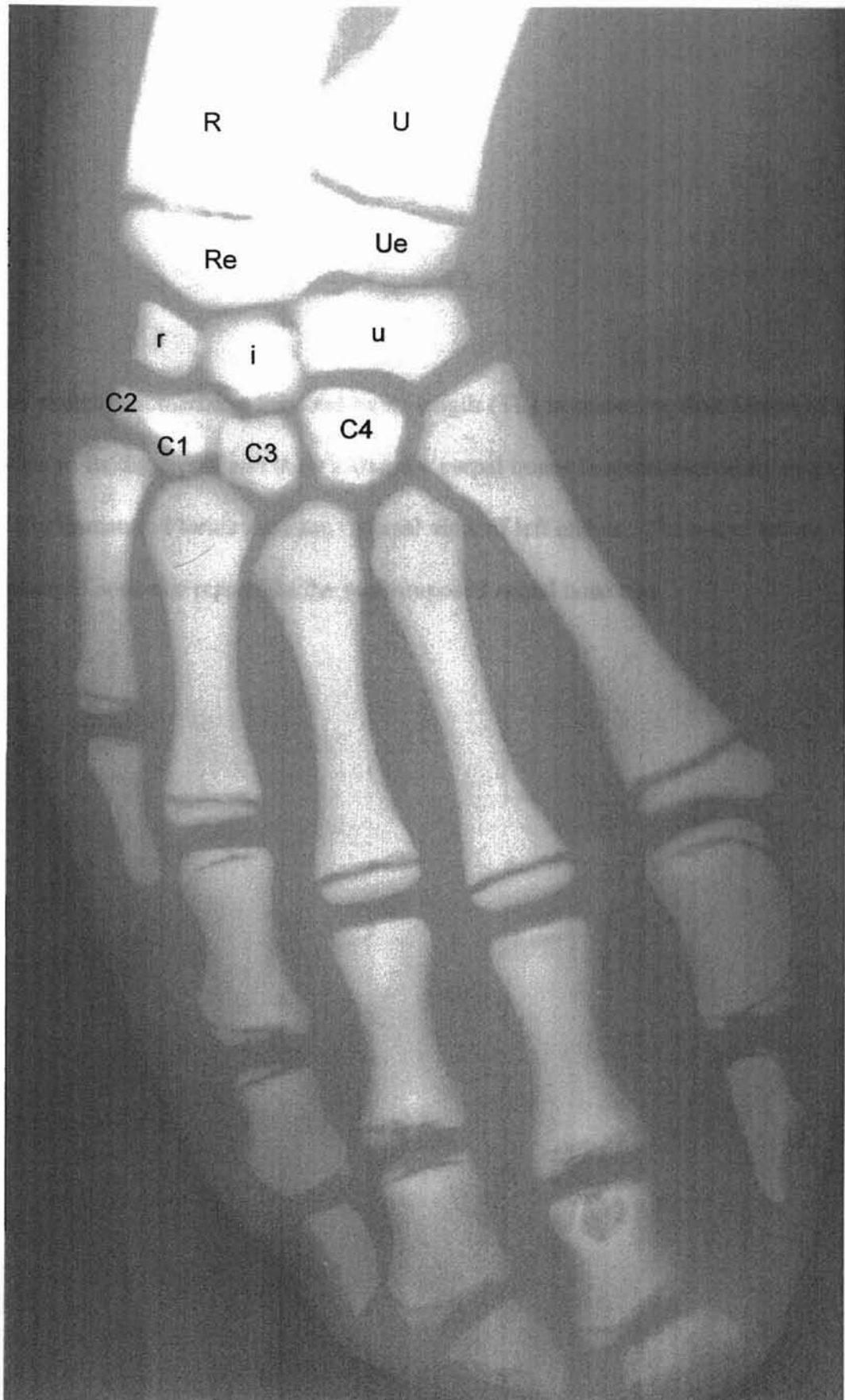
Figure 4.2

Outline sketch summarizing the total body length (TL) in meters at first fusion of each

epiphysis to its diaphysis, and the TL that the carpal bones first attained adult shape in a

skeletally immature Florida manatee. Dorsal view of left manus. The round hatched line

within carpal bone one represents the superimposed carpal bone two.

2.9

2.9

2.8   2.8   2.8

2.8   2.6   2.6

2.3   2.3

2.3   2.3   2.3

2.9   3.1

2.9   2.9

2.9   2.6   2.6

2.6

2.9   2.4

2.4   2.6

2.9   2.9

3.0   2.9

2.8

2.9

2.8

2.8

89
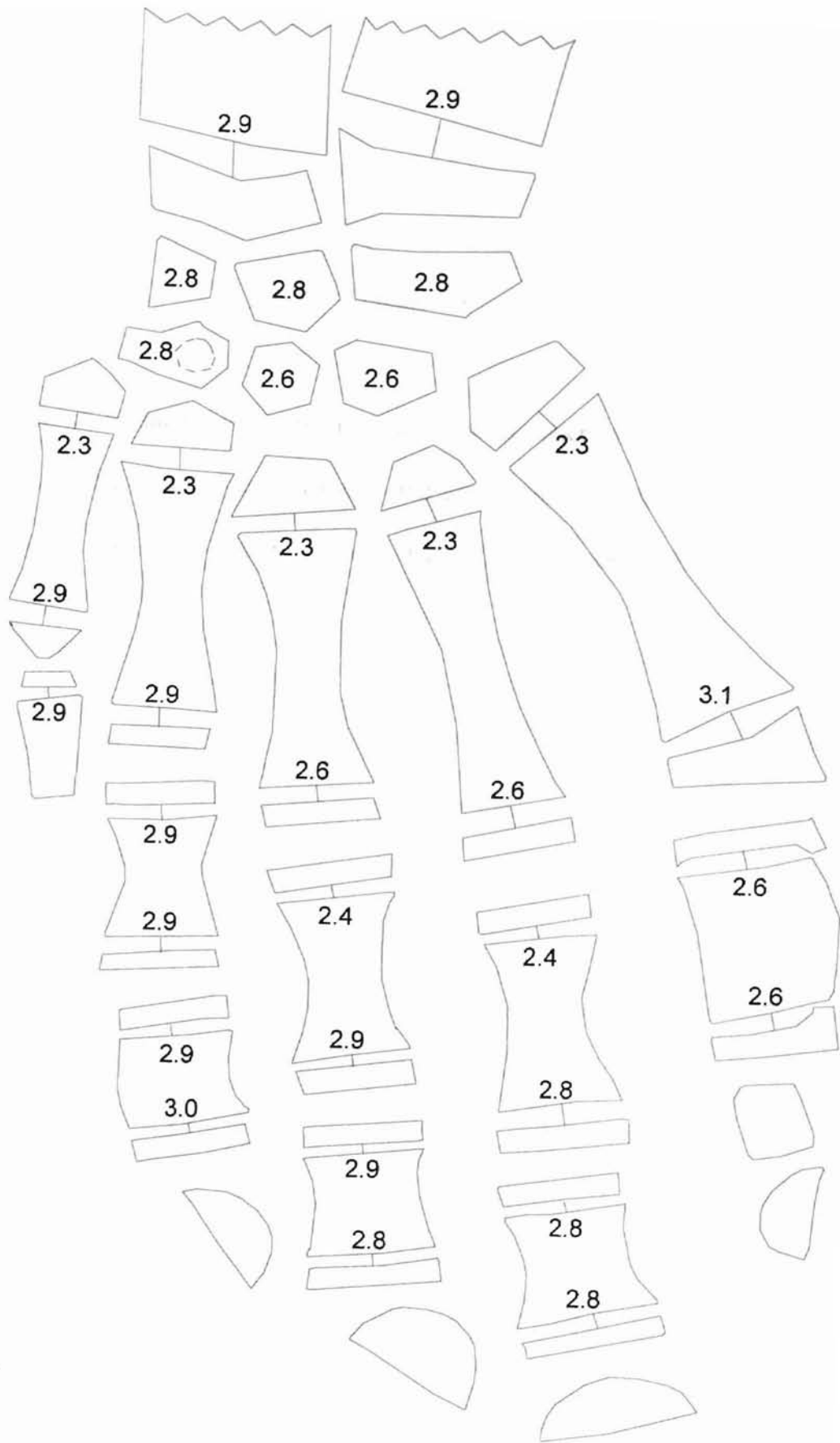
Figure 4.3

Outline sketch summarizing the total body length in meters when each epiphysis was consistently fully fused to the diaphysis, and carpal bones reached adult shape, in all skeletally mature Florida manatees longer than this measurement. Dorsal view of left manus.

3.4   3.4

3.1   3.1   3.1

3.1   3.1   3.1

3.0   3.0

3.0   3.0   3.0

3.4   3.6+

3.4   3.4   3.4   3.4

3.4   3.4   3.4

3.4   3.4   3.4   3.4

3.4   3.4   3.4

3.4   3.4   3.4

3.4   3.4

3.4
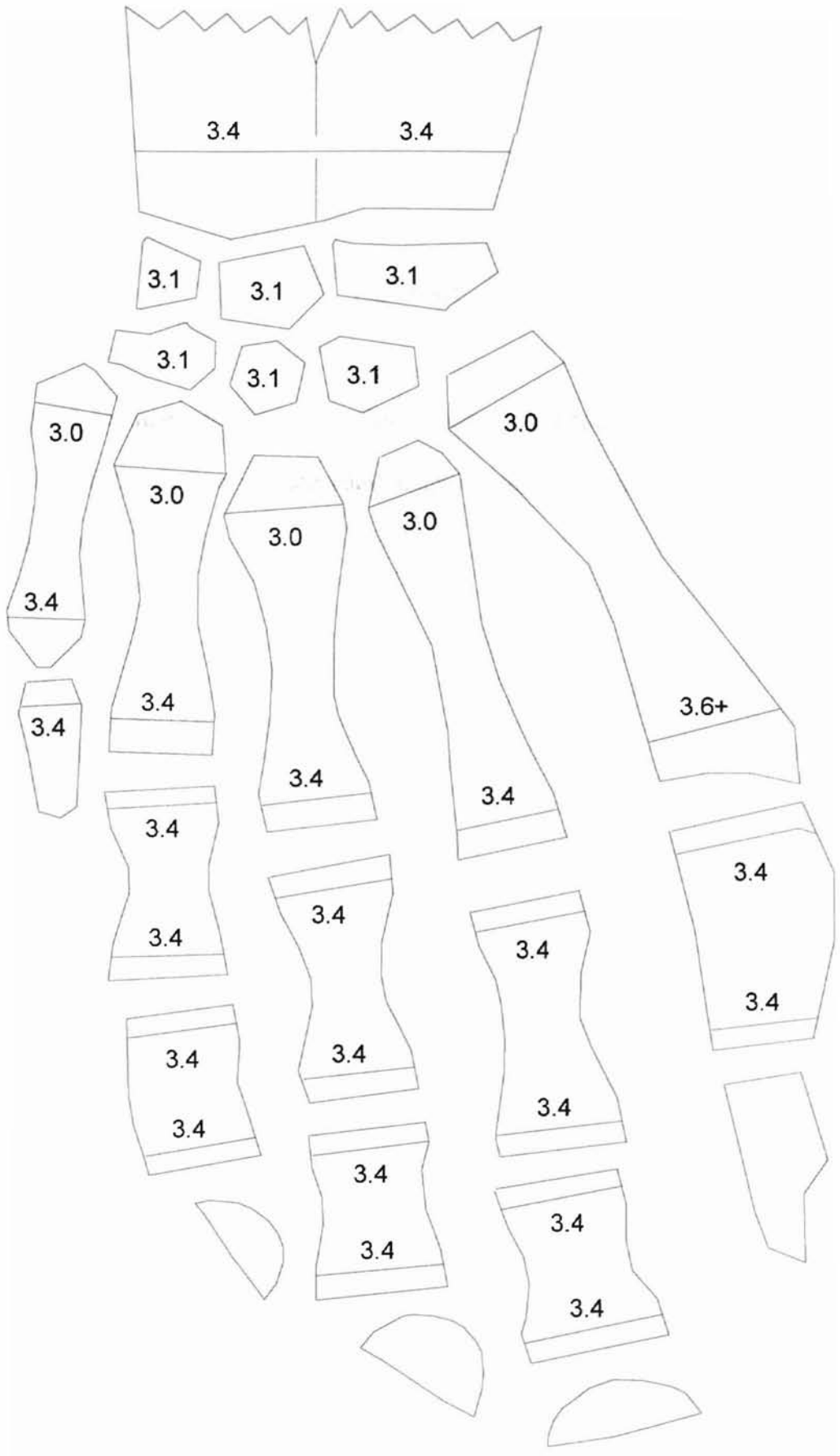
Figure 4.4

Outline sketch summarizing the ages at first fusion of each epiphysis to its diaphysis and when the carpal bones first reached adult shape of skeletally mature Florida manatees. Dorsal view of left manus.
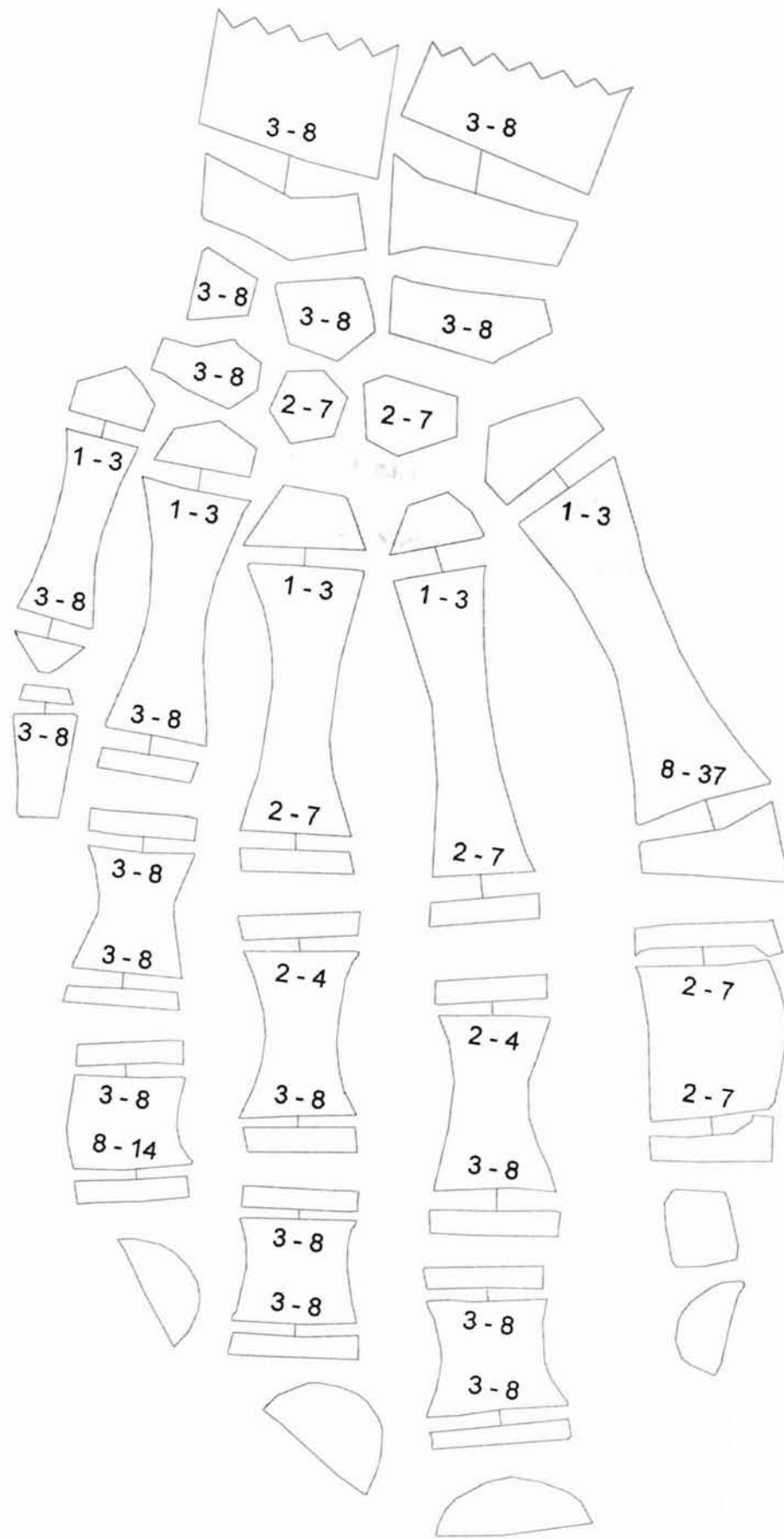
Figure 4.5

Outline sketch summarizing the ages when each epiphysis was consistently fully fused to its diaphysis and carpal bones reached adult shape of skeletally mature Florida manatees. Dorsal view of left manus.

CHAPTER V
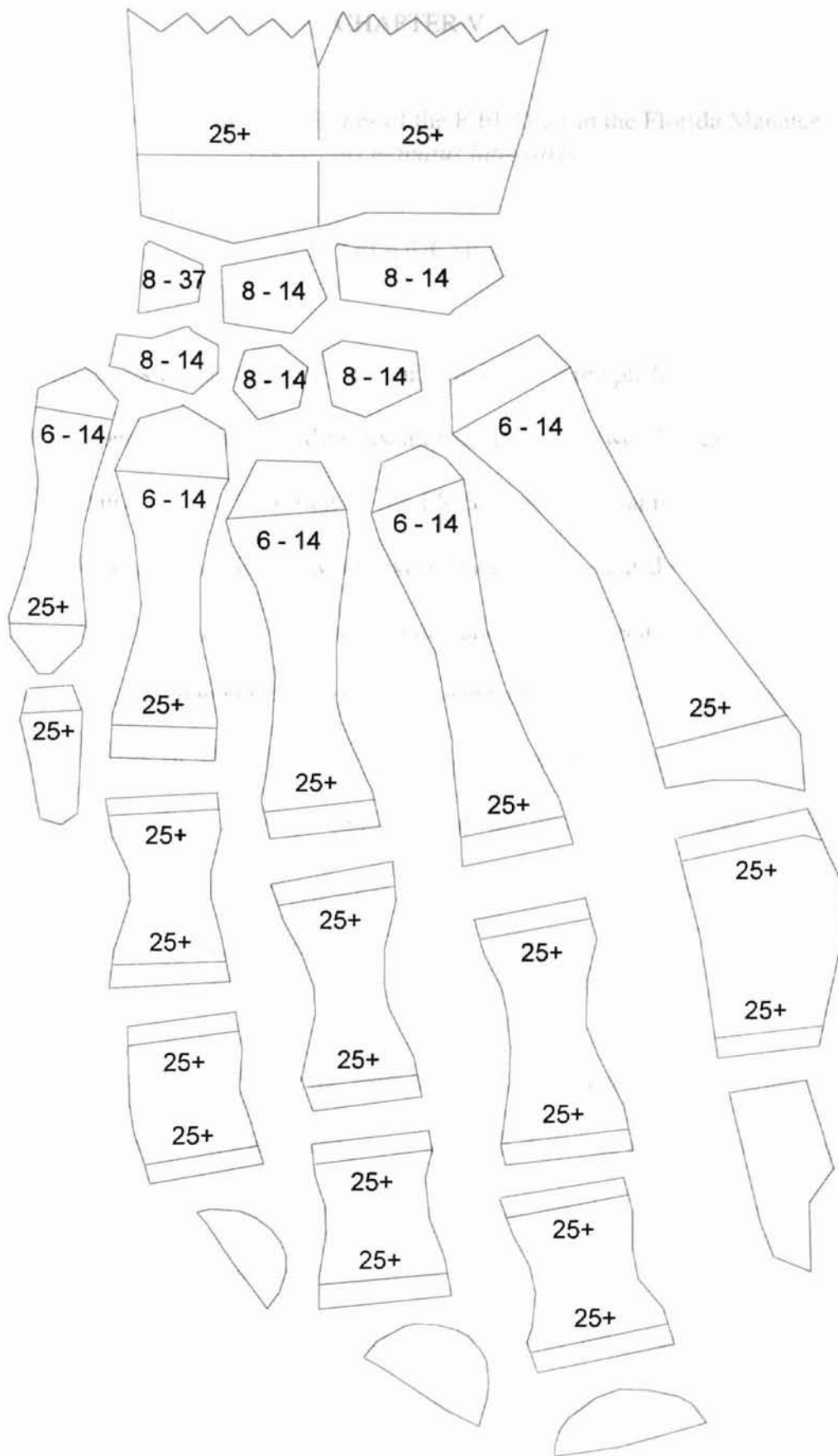
Unusual Development of the Bones of the Fifth Digit in the Florida Manatee,
Trichechus manatus latirostris

INTRODUCTION

Terrestrial mammals characteristically are endowed with three phalanges in each digit of the manus and pes, with the first digit usually restricted to two (Vaughan 1986). The number of phalanges in the fifth digit of the Florida manatee manus, however, has been variously reported as one (Home 1821), two (Cuvier 1809), and three (Sukhanov *et al.* 1986) although these reports are based on only one or two specimens. Coalescence of phalanges is rare in mammals whereas cetaceans characteristically exceed the basic mammalian number of phalanges; for example, the pilot whale has up to fourteen phalanges per digit (Flower 1885). Manatees, on the other hand, conform to the basic mammalian plan and are usually reported with a phalangeal formula of I-2, II-3, III-3, IV-3, V-3 (Vrolik 1852, Murie 1872).

During this radiographic study on the osteological development of the Florida manatee manus it was apparent that the phalanges of the fifth digit had an unusual development and ossification pattern. On this basis, I reevaluated the number of phalanges of the fifth digit in a developmental series of radiographs of 179 flippers. The objectives of this study of the Florida manatee manus were to: 1) identify the presence and location of each separate diaphyseal and epiphyseal ossification center in the fifth digit; 2) determine

when each phalangeal ossification center first appeared as related to total body length (TL); and 3) determine the sequence of fusion of the epiphyses to the diaphyses.

This study revealed that individual manatees had either one, two or three bony phalanges in the fifth digit with varying numbers of ossified epiphyses. The results of this cross-sectional study suggest that over time the second and third phalanges of the fifth digit coalesce to form a "composite terminal phalanx".

MATERIALS AND METHODS

SPECIMENS

Disarticulated flippers were collected and frozen from 167 Florida manatee carcasses, *Trichechus manatus latirostris*, salvaged from the coast and waterways of Florida (1976-1984, 1996). Salvage records from the National Biological Survey, Sirenia Project (Gainesville, FL) provided field number, total body length (TL), sex, side of body of flipper, salvage location and date, and descriptive comments for each individual. The sample consisted of flippers from 92 male and 75 female manatees that ranged in total length from a 1.0 m neonate to a 3.6 m adult (Appendix A). Recorded field measurements of TL were used with a 95% level of confidence and therefore all manatee TLs were rounded to 0.1 m. Twelve specimens had both right and left flippers. This formed a postnatal developmental series of 179 flippers from manatees of evenly distributed lengths.

RADIOGRAPHIC TECHNIQUE

Flippers were thawed, cleaned, and standard plain-film radiographs were taken. The majority of radiographs were taken between 1976-1984 with unrecorded radiographic technique at the College of Veterinary Medicine, University of Florida, Gainesville, FL. In 1996, fifteen flippers were radiographed at the College of Veterinary Medicine, Oklahoma State University, Stillwater, OK. Each prepared flipper was placed on top of a film cassette covered with a thin sheet of plastic. Flippers from manatees longer than 1.6 m TL were disarticulated at the elbow joint and radiographed in a lateromedial view using 36x43 cm (14"x18") non-grid, ultra-vision detail screen cassettes and high-detail film. Flippers from manatees shorter than 1.6 m TL were disarticulated at the shoulder joint and radiographed in a mediolateral view using 20x25 cm (8"x10") non-screen film. The film-to-source distance for all flippers was 102-112 cm (40"-44"). Routine perpendicularly oriented X-ray tube exposed film produced images with superimposition of bones. The carpus was thicker than the tips of the digits and therefore the flipper laid at an angle. Thus, to produce an X-ray beam perpendicular to the longitudinal axis of the long-bones and parallel to their cartilage growth plates, the X-ray tube and collimator was angled between 5 and 10 degrees, depending on the size of the flipper. This technique resulted in radiographs with minimal superimposition of bones and a clear penetration of epiphyseal cartilage growth plates. The flippers were radiographed at 52-60 kVp at 400 mA for 0.006-0.5 seconds. Film was processed in an automatic processor.

The fifth digit was isolated by dissection from three manatees. These specimens were radiographed in a dorsopalmar view with a soft X-ray technique in a shielded cabinet X-ray unit to produce high-detail films and to look for bony spicules crossing between epiphyses and diaphyses. Exposures ranged from 60-80 kVp at 3 mA for 2 minutes on non-screen, high-detail sheet film.

EVALUATION TECHNIQUE

All radiographs were examined by naked eye on a standard radiographic illuminator at Oklahoma State University, Stillwater, OK. Each radiograph was examined to determine presence and location of the separate diaphyseal and epiphyseal ossification centers of the first, second, and third phalanges in the fifth digit. All radiographs were examined three separate times.

An ossification center was determined to be radiographically present when bony spicules were seen on the film and could be differentiated from sharp-edged images formed by opaque, sand or grit particles. This evaluation was aided by knowing the topographical location and shape of the phalangeal bones in mature manatees. Radiographic fusion was defined as bony spicules bridging the gap between an epiphyseal ossification and its bony diaphysis. The data were then analyzed to determine the total body length (TL) at which each ossification center first appeared, and subsequently fused with an adjacent ossification center.

DEVELOPMENTAL PATTERNS

The development of ossification centers of the second and third phalanges of the fifth digit was classified according to the following patterns as seen on radiographs:

1 - Neither the ossified diaphysis of the second nor the third phalanx was seen.

2 - Only the ossified diaphysis of the second phalanx was seen.

3 - Only the ossified diaphysis of the third phalanx was seen.

4 - The ossified diaphyses of both the second and third phalanges were present and were separate bones.

5 - Epiphyseal ossification centers varied in presence and stages of fusion.

6 - The "composite terminal phalanx" was present.

Each flipper radiograph was placed in one of five groups (A-E), each group spanning a 0.5 m TL increment, which approximately correlated with life history stages: Group A = 1.0 - 1.5 m; Group B = 1.6 - 2.0 m; Group C = 2.1 - 2.5 m; Group D = 2.6 - 3.0 m; Group E = 3.1 - 3.6 m. All flippers were assigned to one of the developmental patterns based on the number of diaphyseal and epiphyseal centers present in the second and third phalanges (Table 5.1).

LIFE STAGES

Overall, the 167 Florida manatees in this study can be categorized into four different life stages based on their TL (Bonde *et al.* 1983, O'Shea *et al.* 1985): fetus − ≤ 0.9 m (n=0);

dependent calf – 0.9 - 1.5 m (n=32); juvenile – 1.5 - 2.8 m (n=84); sexually mature adult – > 2.8 (n=51). Males become sexually mature as early as two years of age (2.4 m TL) based on spermatogenesis studies (Hernandez *et al.* 1995). Females become sexually mature at three to four years of age (2.5 - 3.0 m) based on ovarian follicle studies (Marmontel 1995).

RESULTS

RADIOGRAPHIC EVALUATION

All diaphyseal and epiphyseal ossification centers could be evaluated in 174 of the 179 radiographs. Due to overexposure, incorrect positioning, or post-mortem damage to flippers in the fifth digit region, 5 radiographs were not evaluated.

DESCRIPTION OF THE FIFTH DIGIT

The fifth digit in the Florida manatee was always seen with an ossified first phalanx, and which, in older manatees, had a proximal and distal ossified epiphysis. The first phalanx also had a caudally rounded, rectangular diaphysis and was the largest of the three phalanges. The second and third phalanges were seen in six different diaphyseal and epiphyseal ossification patterns (Table 5.1, Fig. 5.1). First, neither the ossified diaphysis of the second nor the third phalanx was present. Second, only a small, round second phalangeal diaphysis was present. Third, only a crescent-shaped third phalangeal diaphysis was present. Fourth, both the ossified diaphyses of the second and third

phalanges were present and were separate bones. Fifth, epiphyseal ossification centers, varying in presence and stages of fusion, were present. Sixth, a single bone herein termed the "composite terminal phalanx", resembling the second phalanx proximally and the third phalanx distally, was present (Fig. 5.1).

FIRST APPEARANCE OF PHALANGEAL DIAPHYSES

First phalanx:

The diaphyseal ossification center for the first phalanx of the fifth digit in the Florida manatee was detected in all radiographs (1.0 - 3.6 m TL), i.e., it was ossified at birth.

Second phalanx:

The separate diaphyseal ossification center for the second phalanx was first detected in a 1.3 m TL female and in a 1.0 m male manatee (smallest manatee in this study). In females, this diaphysis was consistently present in manatees at 2.4 m and longer. The diaphysis for the second phalanx in males was consistently present in male manatees at 1.4 m and longer.

Third phalanx:

The separate diaphyseal ossification center for the third phalanx was detected in a 1.0 m female and in a 1.2 m male manatee. This ossification center was consistently present in females at 1.3 m, and in males at 1.7 m. These second and third phalangeal diaphyses of the fifth digit were the last of all the phalanges to ossify in the neonate manatee.

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"Composite terminal phalanx":

The "composite terminal phalanx" first appeared in a 1.0 m female and in a 1.3 m male. This single bone was present, at varying TLs throughout the series of flippers, including the longest manatee at 3.6 m, although, its frequency of occurrence increased with TL (Table 5.1).

FIRST APPEARANCE OF EPIPHYSES

The proximal epiphyseal ossification of the first phalanx was first detected in 2.1 m TL manatees and the distal epiphysis at 2.3 m. An ossified proximal epiphysis of the second phalanx was first detected at 2.3 m. An ossified distal epiphysis of the second phalanx, when the second and third phalanges remained separate, was first detected at 2.4 m. Epiphyseal ossification centers were not observed for the third phalanx.

FUSION OF EPIPHYSES

Fusion of the proximal epiphysis of the first phalanx to its diaphysis was first detected in manatees at 2.6 m TL and the distal epiphysis in manatees at 2.8 m. The presence of separate epiphyses of the second phalanx and their fusion was extremely variable throughout all TL groups.

Of the twelve paired flippers radiographed, two female (1.3 m and 2.3 m) and two male (1.4 m and 2.2 m) manatees displayed side of body differences in the number of diaphyses and/or epiphyses present (Table 5.1).

DISCUSSION

The great variability in the number of separate diaphyses and epiphyses for the second and third phalanges in the fifth digit of the Florida manatee is unusual when compared to the basic mammalian plan. Moreover, the main digits II - IV did not show this variability. Individual manatees of similar total body lengths (TL) had six possible developmental patterns of ossification of the second and third phalanges of the fifth digit. Manatees had either one, two or three phalanges in digit V. This finding could explain some of the varying numbers of phalanges in the fifth digit as previously reported (Flower 1885, Kaiser 1974): two (Home 1821, Vrolik 1852, Bahrdt 1933) and three phalanges (Vrolik 1852, Owen 1866, Murie 1872). Also, the reported variations might depend on the age of the manatee under observation, since younger manatees usually had three phalanges and there was an increasing tendency for older manatees to have only two phalanges. The method of preparation of the skeleton could also affect the number of phalanges recorded because small bones are easily lost. This variability in number of phalanges may signify low functional importance since vestigial or rudimentary organs show varied degrees of development within a species (Darwin 1859). And, although a

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bony structure was always present, the structural details of the bones might not be vital to the desired function of the flipper.

This study revealed only one bone distal to the first phalanx in the fifth digit of many adult Florida manatees. The shape of this single bone, the "composite terminal phalanx", is closely similar to the outline of the unfused second and third phalanges in other individuals. The radiographic gap between the first phalanx and this "composite terminal phalanx" is comparable to the other interphalangeal gaps in digits II - IV. Therefore, in this cross-sectional study based on examination of radiographs, bones and dissection, these data suggest that this "composite terminal phalanx" is the result of fusion between the second and third phalanges, which is in accordance with the traditional hypothesis on loss of skeletal elements (Müller and Alberch 1990).

This coalescence of diaphyseal phalanges denotes a deviation from the basic mammalian plan. In dugongs there is fusion between phalanges in some museum specimens and is claimed due to the relatively immobile state of the phalanges in the flipper (Leboucq 1889). The second and third phalanges of the fifth digit were the only phalanges to fuse in the Florida manatee manus.

The proximodistal sequence of fusion of epiphyses seen in other digits of the manatee manus (Chapter 4) was not seen in the second and third phalanges of the fifth digit. The extreme variability in the ossified presence of epiphyses, much less their fusion to the

diaphyses, shows the unusual pattern of ossification and is therefore not surprising that the number of phalanges in the fifth digit has been variably reported in the literature.

SUMMARY

Extreme variation was seen in the ossification patterns of the second and third phalanges with the number of diaphyseal and epiphyseal ossification centers varying between zero and four. The diaphyses of the second and third phalanges were sometimes not present in young manatees and, consequently, were the last diaphyses to ossify in the manatee manus. Nonetheless, a single bone, the "composite terminal phalanx", was present distal to the first phalanx in all total body length (TL) classes from 1.0 m to 3.6 m TL. Therefore, in this cross-sectional study based on radiographs, bones and dissection, the data suggests that this "composite terminal phalanx" was the result of fusion between the second and third phalanges of the fifth digit. These second and third phalanges of digit five were the only phalanges to fuse in the Florida manatee manus and might account for the reported variation in phalangeal counts for the fifth digit of the manatee manus.

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TABLE 5.1

Developmental Patterns of Ossification Centers of the Second and Third Phalanges in the Fifth Digit of the Florida Manatee

		1	2	3	4	5	6
		No diaphyses present	P2		P2 / P3	Epiphyses present	ctp
	TL			P3			
	1.0		M	FF			F
	1.1	*MMM					
Group A	1.2	FFM	MMMMM		M		
	1.3	FFMMM	F	FM	MMMMM		FFM
	1.4				MM		FFF
	1.5		M		F		M
	1.6						
	1.7				MMM		
Group B	1.8						
	1.9				MMM		
	2.0			FF	FMMM		MMM
	2.1				FFMMMM		FFMM
	2.2				FFM	MM	FF
Group C	2.3			F	FF	F	FMMM
	2.4				FFFFFMM	FFMM	
	2.5				FFFFMM	M	FMMM
	2.6				FM	MMM	FMM
	2.7				FM	FMMMMMM	FFFMM
Group D	2.8				F	FFFFMM	M
	2.9				M	FFFFMMMMMM	MM
	3.0				F	FFFFMMM	MM
	3.1					FFFFM	FFM
	3.2					F	FFMM
Group E	3.3					F	
	3.4						FM
	3.5						FF
	3.6						F

*Paired flippers indicated by underlining.   M = male, F = female.

Figure 5.1

Dorsopalmar radiographs of the left fifth digit from seven representative Florida manatees (1.2 - 3.1 m TL) showing the variation of diaphyseal and epiphyseal ossification centers for the second and third phalanges. The first five radiographs are from manatees of similar TL but reveal five different developmental patterns. Distal end of metacarpal V (Mc V), first phalanx (P1), second phalanx (P2), third phalanx (P3), composite terminal phalanx (ctp).

CHAPTER VI
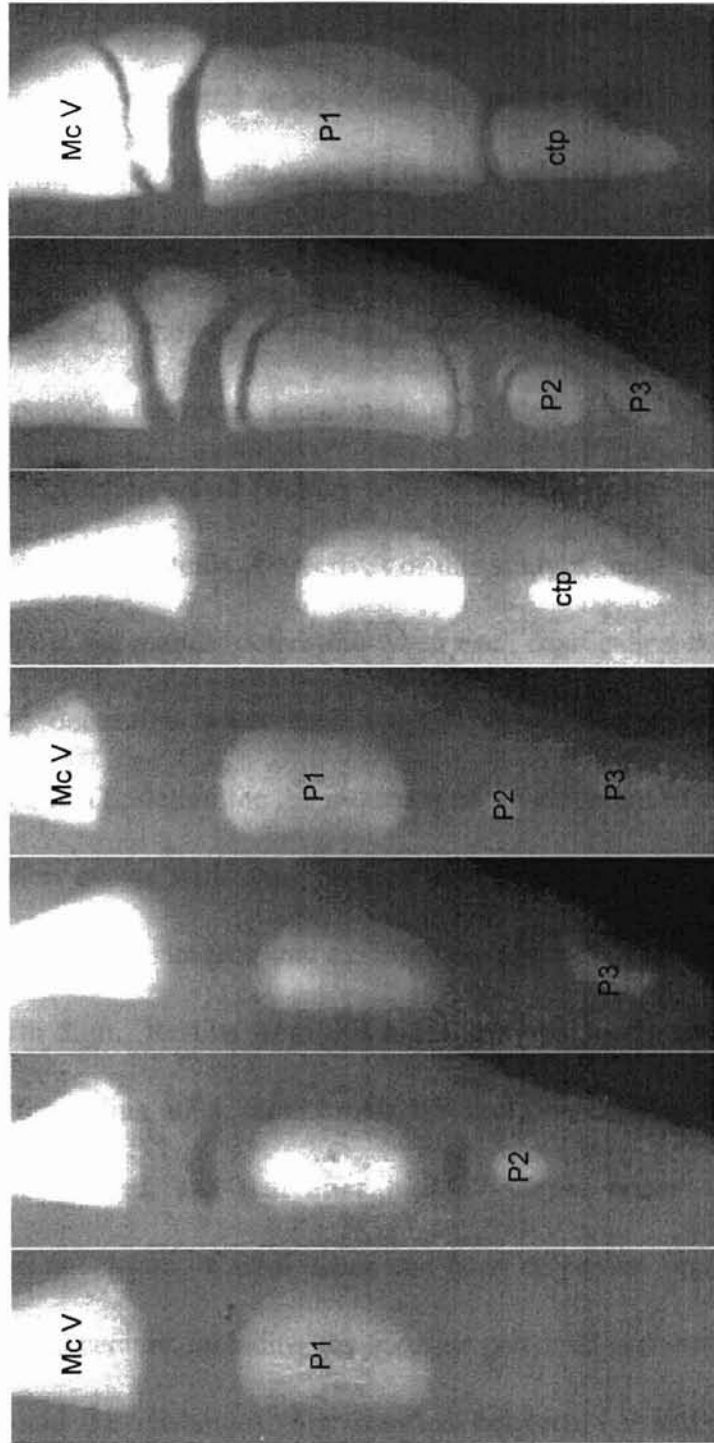
Summary of Thesis

Radiographs from a developmental series of 179 flippers salvaged from 167 dead Florida manatees, *Trichechus manatus latirostris*, (1.0 - 3.6 m total body length (TL)) were examined for the first appearance and fusion of 34 carpal and epiphyseal ossification centers in the manus. Chronological age has been estimated by counts of growth-layer-groups in the petrous temporal bone and correlated with known-age manatees (Marmontel 1993). Radiographed flippers from 106 of these age-estimated individuals were included in the current study. Objectives of this study were to: identify the separate ossification centers in the manus; determine when each ossification center first appeared as related to TL; determine when each epiphyseal ossification center fused to its diaphysis as related to TL; determine the sequence of development of ossification centers; correlate ossification events with chronological age by comparing TL of radiographed manatees with TL of aged manatees; and examine the unusual developmental ossification patterns of the fifth digit. Results from this cross-sectional study revealed that at birth, the diaphyses of the radius, ulna, metacarpals I-V and phalanges (I-1, II-3, III-3, IV-3, V-1-3) were well ossified, and that after birth, 7 carpal bones and 27 epiphyseal ossification centers developed. Carpal bones and bony epiphyses first appeared at 1.9 m TL. Other epiphyseal centers, including the separate proximal and distal epiphyses of the five metacarpals and the phalanges, first ossified between 1.9 and 2.8 m TL (sexual maturity). These postnatal centers first appeared in proximodistal and caudocranial

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sequences. First fusion, bony bridges spanning the epiphyseal cartilage growth plate, was first seen in metacarpal bones at 2.3 m TL and continued to appear throughout the manus until 3.0 m TL. Full fusion of epiphyses to diaphyses continued through 3.6 m TL. The number of diaphyseal and epiphyseal ossifications in the second and third phalanges of the fifth digit varied from zero to four. Moreover, the data suggests that these second and third phalanges coalesce into a single bone, the composite terminal phalanx. Correlation of these radiographic data with known-age specimens may be useful for the estimation of age of salvaged and living Florida manatees.

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APPENDICES

Collection Data for 167 Florida Manatees, *Trichechus manatus latirostris*,
with 179 Flippers Radiographed Included in This Study,
Arranged in Ascending Total Body Length (TL).

Field #	Sex	TL (m)	Salvage Date	Florida County	Flipper Side of Body*
MEC9645	M	0.96	7/1/96	Brevard	L
M 191	F	1.03	4/3/80	Volusia	u
M 272	F	1.04	3/4/82	Citrus	R&L
M 190	F	1.10	4/3/80	Volusia	u
M 213	M	1.17	12/3/80	Brevard	R&L
M 178	M	1.19	12/28/79	Brevard	R
M 341	M	1.20	7/7/83	Clay	R&L
MSW96205	M	1.22	7/6/96	Charlotte	R
M 155F	M	1.23	6/5/79	Brevard	R&L
M 207	F	1.23	10/2/80	Flagler	R
M 148	F	1.24	5/4/79	Hillsborough	u
MEC9646	M	1.24	1/7/96	Brevard	L
M 243	M	1.26	5/28/81	Hillsborough	u
M 185	F	1.30	2/21/80	Citrus	u
M 271	M	1.30	2/28/82	Citrus	R
M 132	M	1.31	12/23/78	Hillsborough	R
M 214	F	1.32	12/7/80	Levy	R&L
M 135	M	1.33	2/8/79	Citrus	R
M 160	M	1.33	7/2/79	Manatee	R
M 80-3	M	1.34	1/18/80	Broward	R
M 141	F	1.34	3/16/79	Citrus	R
M 167	F	1.35	8/20/79	Duval	u
M 231	M	1.36	3/30/81	Volusia	u
M 361	M	1.36	1/13/84	Citrus	R
M 80-26	M	1.375	11/25/80	Lee	R&L
M 177	M	1.39	12/13/79	Brevard	u
M 193	F	1.39	5/29/80	Dixie	u
M 173	F	1.41	9/30/79	Volusia	R
M 186	F	1.41	2/21/80	Broward	u
M 76-26	M	1.42	11/22/76	Dade	u
M 172	F	1.42	9/10/79	Brevard	R
M 215	M	1.47	12/16/80	Duval	u
M 161	F	1.52	7/3/79	Putnam	R
M 188	M	1.52	3/17/80	Hillsborough	u
MSW96204	M	1.58	6/29/96	Lee	R
M 79-1	M	1.72	1/10/79	Collier	u
M 144	M	1.75	4/17/79	Putnam	u

M 227	M	1.76	3/5/81	Duval	L
M 232	M	1.90	4/2/81	Brevard	*u*
M 244	M	1.90	6/2/81	Brevard	*u*
M 171	M	1.92	9/10/79	Duval	*u*
M 145	M	1.96	4/19/79	Putnam	*u*
M 350	M	1.99	12/7/83	Duval	R
M 78-19	M	2.00	3/4/78	Lee	*u*
M 80-4	F	2.00	1/24/80	Okeechobee	R
M 79-2	M	2.02	1/12/79	Martin	R&L
M 170	M	2.04	9/5/79	Brevard	R
M 79-26	M	2.05	12/22/79	Martin	R
M 180	M	2.07	1/16/80	Dixie	*u*
M 273	F	2.08	3/4/82	Lee	R&L
M 311	F	2.10	8/28/82	Miami Seaquarium	*u*
MSW-6	M	2.11	1/13/84	Lee	R
M 364	M	2.12	1/29/84	Duval	R
M 406	F	2.16	8/25/84	Volusia	R
M 78-22	M	2.17	3/8/78	Martin	*u*
M 154	M	2.175	5/29/79	Brevard	*u*
M 211	F	2.18	10/30/80	Volusia	*u*
MSW-10	F	2.18	1/21/84	Charlotte	R
M 314	M	2.19	10/29/82	Citrus	R&L
MSW-8	M	2.21	1/18/84	Collier	R
M 362	F	2.22	1/19/84	Brevard	R
M 224	F	2.23	2/4/81	Brevard	*u*
M 79-6	F	2.24	2/17/79	Collier	*u*
M 79-9	M	2.25	2/20/79	Charlotte	*u*
M 137	M	2.28	2/13/79	Brevard	*u*
MSW-7	F	2.28	1/14/84	Collier	R
M 78-44	M	2.30	12/31/78	Dade	*u*
M 179	F	2.30	1/4/80	Harrison	R&L
M 398	F	2.30	7/27/84	Volusia	R
M 366	F	2.31	1/29/84	Duval	R
M 80-7	F	2.32	2/13/80	Martin	R
M 79-24	M	2.35	11/15/79	Dade	*u*
M 308	M	2.37	8/28/82	Putnam	R
M 219	M	2.41	1/23/81	Duval	*u*
MSW-11	F	2.41	1/23/84	Charlotte	R
M 182	F	2.42	1/28/80	Brevard	*u*
M 373	F	2.43	2/20/84	Duval	R
M 158	F	2.44	6/21/79	Manatee	*u*
M 220	M	2.44	1/23/81	Brevard	*u*
M 78-30	F	2.45	6/19/78	Dade	*u*
M 147	F	2.45	5/1/79	Brevard	*u*

M 363	M	2.47	1/19/84	Duval	R
MSW-2	F	2.47	11/19/83	Collier	R
M 192	M	2.48	5/29/80	Brevard	u
M 354	F	2.50	11/13/83	Lee	L
M 357	M	2.50	1/7/84	Clay	R
M 358	F	2.50	1/8/84	Duval	R
M 217	M	2.52	1/17/81	St. Johns	u
MSW-3	F	2.54	12/1/83	Lee	R
M 78-42	M	2.55	11/13/78	Dade	u
M 216	F	2.55	1/12/81	Manatee	R
M 153	M	2.56	5/26/79	Brevard	u
MEC9642	M	2.56	6/22/96	Brevard	R
M 78-2	M	2.57	1/23/78	St. Lucie	u
M 226	F	2.59	3/4/81	Duval	u
M 79-10	F	2.60	3/29/79	Dade	L
M 79-23	M	2.63	10/31/79	Dade	u
M 131	M	2.63	12/12/78	Lee	u
M 149	F	2.63	5/11/79	Putman	u
M 218	M	2.63	1/22/81	Duval	R
M 239	M	2.63	5/16/81	Lee	u
MSW96200	M	2.64	6/16/96	Lee	L
M 222	F	2.65	1/30/81	Nassau	u
M 348	M	2.67	9/30/83	Nassau	R
MSW-15	M	2.69	2/3/84	Lee	R
M 139	F	2.70	3/5/79	Brevard	u
M 223	M	2.71	2/3/81	Duval	u
M 79-4	M	2.72	2/12/79	Martin	u
M 212	F	2.72	11/3/80	Levy	u
M 352	F	2.72	12/12/83	Brevard	R
M 134	M	2.73	1/25/79	Brevard	R
M 174	M	2.73	10/4/79	Brevard	u
M 183	F	2.74	2/14/80	Brevard	u
M 194	F	2.74	6/15/80	Brevard	u
M 369	M	2.74	2/6/84	Duval	R
MNW9614	M	2.74	6/26/96	Hillsborough	L
M 365	M	2.76	1/29/84	Duval	R
M 166	M	2.77	8/5/79	Volusia	u
M 197	M	2.77	7/9/80	Brevard	u
M 168	F	2.80	8/20/79	Brevard	u
MSW-14	M	2.82	1/30/84	Lee	R
SWFTM9611	M	2.82	6/16/96	Brevard	L
M 200	F	2.83	8/20/80	Brevard	u
M 138	F	2.87	2/14/79	Duval	u
M 184	F	2.87	2/16/80	Brevard	u
M 387	M	2.87	5/5/84	Volusia	R

M 150	F	2.89	5/13/79	Martin	*u*
M 230	F	2.90	3/23/81	Brevard	*u*
M 78-15	M	2.91	2/27/78	Dade	*u*
M 80-6	M	2.91	2/5/80	Dade	R
M 136	M	2.92	2/8/79	Duval	R
M 143	M	2.92	4/17/79	Glynn	*u*
M 353	M	2.92	12/27/83	Brevard	R&L
MSW-4	M	2.92	1/8/84	Lee	R
MSW96206	M	2.93	7/7/96	Charlotte	L
M 199	F	2.94	8/20/80	Brevard	*u*
M 152	F	2.96	5/26/79	Brevard	*u*
M 208	M	2.97	10/12/80	Brevard	*u*
M 79-25	F	2.98	11/29/79	Glades(?)	*u*
M 307	F	3.00	8/17/82	Glynn	R
M 159	F	3.01	6/30/79	Brevard	R
M 371	F	3.02	2/9/84	Nassau	R
M 356	F	3.03	1/7/84	Nassau	R
MSE9621	M	3.03	7/5/96	Martin	L
M 351	M	3.06	12/9/83	Duval	R
M 140	M	3.07	3/16/79	Martin	*u*
M 242	M	3.07	5/26/81	Brevard	*u*
M 229	M	3.09	3/16/81	Duval	*u*
MEC9640	F	3.09	6/16/96	Brevard	R
M 374	F	3.13	2/22/84	Indian River	R
MEC9644	F	3.13	6/23/96	Brevard	R
M 142	M	3.14	4/10/79	Brevard	*u*
M 79-3	F	3.15	2/4/79	Lee	*u*
M 196	F	3.16	7/3/80	Levy	*u*
MSW96201	F	3.17	6/23/96	Collier	R
M 235	M	3.18	5/6/81	Putman	*u*
M 234	F	3.19	4/30/81	Brevard	*u*
M 146	M	3.20	4/25/79	Brevard	*u*
M 198	F	3.22	8/13/80	Putnam	*u*
M 225	F	3.25	2/12/81	Duval	*u*
M 247	M	3.25	7/16/81	Volusia	L
MNE9613	F	3.29	6/19/96	Clay	R
M 77-28	F	3.30	9/21/77	Dade	L
MSW-5	M	3.44	1/10/84	Lee	R
M 236	F	3.46	5/7/81	Indian River	*u*
M 155	F	3.55	6/5/79	Brevard	R&L
MSW96202	F	3.60	6/26/96	Glades	R

* L = left, R = right, *u* = unknown, M = male, F = female

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APPENDIX B

Estimated Ages from 106 Manatees with Growth-Layer-Group Counts
of the Petrous Temporal Bone by Marmontel (1993) Which Have Been
Included in This Study and Related to Total Body Length (m).

Age	# of Aged Manatees	Range	Mean	Median
0 - 1	26	1.0 - 2.1	1.5	1.4
1 - 2	15	1.2 - 2.4	1.9	2.1
2 - 3	13	2.3 - 2.7	2.5	2.4
3 - 4	15	2.3 - 2.9	2.6	2.6
4 - 5	4	2.3 - 2.8	2.6	2.6
5 - 6	3	2.7 - 2.9	2.8	2.9
6 - 7	5	2.6 - 3.0	2.7	2.6
7 - 8	5	2.5 - 2.9	2.7	2.7
8 - 9	5	2.8 - 3.2	2.9	2.9
9 - 10	2	3.0 - 3.2	3.1	3.1
10 - 11	2	3.0	3.0	3.0
11 - 12	2	2.9 - 3.2	3.0	3.0
12 - 13	1	3.1	3.1	3.1
13 - 14*	1	3.2	3.2	3.2
14 - 15	1	3.0	3.0	3.0
17 - 18	1	3.1	3.1	3.1
24 - 25	1	3.3	3.3	3.3
25 - 26	1	3.2	3.2	3.2
25 - 26*	1	3.6	3.6	3.6
28 - 29*	1	3.3	3.3	3.3
37 - 38*	1	3.1	3.1	3.1

* Because of bone resorption, not all GLGs could be counted therefore the total GLG
count was estimated (Marmontel 1993).

VITA

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Master of Science

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