VARIATION IN THE NUMBER OF CARPAL BONES IN BOTTLENOSE DOLPHINS (TURSIOPS TRUNCATUS) FROM THE GULF OF MEXICO

By

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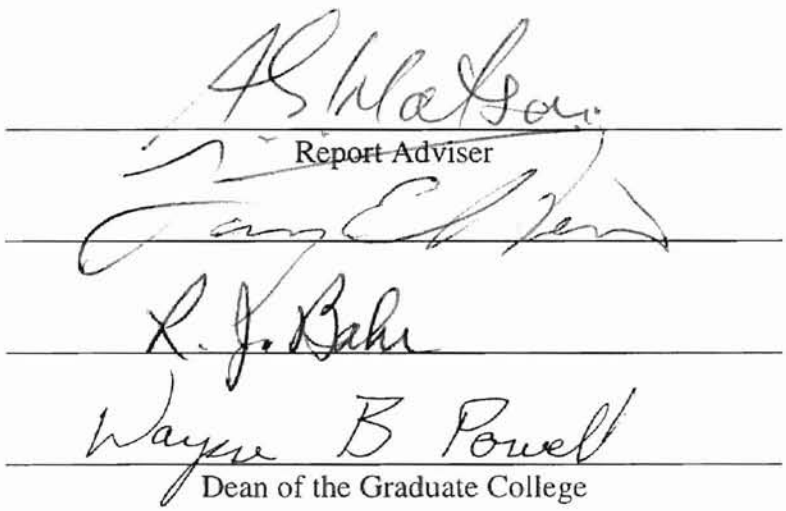

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NOMENCLATURE

a	accessory
c1	carpal one
c2	carpal two
c3	carpal three
c4+5	carpal four-plus-five
i	intermedium
r	radiale
r'	small radiale
r"	second radiale
u	ulnare
I	metacarpal one
II	metacarpal two
III	metacarpal three
IV	metacarpal four
V	metacarpal five

Chapter 1

INTRODUCTION AND OBJECTIVES

Introduction

The purpose of this study was to describe and quantify the variation in the number and topography of carpal bones in mature bottlenose dolphins (Tursiops truncatus) from the Gulf of Mexico.

The bottlenose dolphin is a globally distributed (Leatherwood et al. 1983) polymorphic species (Hersh and Duffield 1990, Ross 1990, Mead and Potter 1995), in which several genetically distinct regional populations are recognized (Dowling and Brown 1993). These dolphins are about a meter in total body length at birth (Leatherwood et al. 1983), reach sexual maturity at 2.2 - 2.4 m (Evans 1987), and, as adults, may exceed 3 m in maximum body length (Leatherwood et al. 1983, Evans 1987). The coastal habitat and wide distribution make the bottlenose dolphin one of the most studied cetaceans.

The number of bones in the carpus of the bottlenose dolphin is listed as four (Schwab 1988), five (Rommel 1990), six and seven (Schwab 1988), although these recent reports are based on few individuals. The few studies which examined the homologies of these carpal bones do not always agree on

the identity of the individual bones (Fitzgerald 1970, Rommel 1990, Watson et al. 1994) and have used names (Fitzgerald 1970) that may be inconsistent with their topographical relationships. A study based on a large number of dolphins, including individuals of both sexes and a variety of ages, should help clarify the variation in, and homology of, the bones of the carpus in the bottlenose dolphin.

Bottlenose dolphins strand frequently along the coast of the Gulf of Mexico, a condition which provides an abundance of specimens for anatomical examination. This study used radiography to examine both thoracic limbs salvaged from 27 mature bottlenose dolphins from the Gulf of Mexico. These limbs were compared for variations in the number and topography of carpal bones. Throughout this report, anatomical terminology, unless otherwise noted, follows Nomina Anatomica Veterinaria (Frewein et al. 1994), in which carpal bone nomenclature is that derived from Gegenbaur (1864).

Objectives

The specific objectives of this research were:

1. To review the literature on the odontocete carpus (chapter 2).
2. To describe the topography and frequency of occurrence of individual bones in the carpus of Gulf of Mexico bottlenose dolphins (Tursiops truncatus), 2.0 m and longer in total body length, stranded on the Texas coast (chapter 3).

3. To describe the morphological equivalence and examine homologies of these carpal skeletal elements (incorporated into chapter 3).
4. To use statistical analysis of data to determine if side of body or sex is a source of variation in the number of carpal bones (incorporated into chapter 3).

Chapter 2

LITERATURE REVIEW OF THE ODONTOCETE CARPUS

Introduction

In cetaceans the thoracic limbs have rotated so that the medial and lateral edges of the limb in terrestrial mammals have become the cranial and caudal edges of the flipper. In addition, the limb has become compressed mediolaterally and expanded craniocaudally to form a streamlined control surface (Felts 1966) used for steering. Through these modifications the bones of the antebrachium and manus have, likewise, become both compressed and expanded. Furthermore the range of joint motion in the flipper is much less than in the thoracic limbs of terrestrial mammals: the synovial shoulder joint retains mobility, whereas all distal joints have flat articular surfaces that severely limit their range of motion. In particular, all articular and non-articular surfaces of the bones of the carpus lie at right angles to each other.

The short bones of the thoracic limb forming the carpus are located distal to the radius and ulna and proximal to the metacarpals (Dyce et al. 1996). In most typical terrestrial mammals the carpus is extended and flexed

as the animal uses the limb to provide propulsion. In cetaceans, however, these joints are more rigid and serve as part of the flattened flipper.

The generalized primitive plan for the tetrapod carpus contains three rows of bones (Goodrich 1930). In this plan the proximal row consists of three bones, in medial to lateral order: the radiale, intermedium, and ulnare; and in the Reptilia and Mammalia, the accessory carpal bone palmarolaterally (Goodrich 1930). The middle row consists of two or three centralia, and the distal row contains carpals one to five, one immediately proximal to each of the metacarpal bones. Most living tetrapods have fewer bones within the carpus than in this generalized plan. The reduction in the number of bones is thought to be due to fusion among, or lack of development of, these elements within the carpus.

The Mammalia most likely evolved from cynodonts, a group of mammal-like reptiles belonging to the order Therapsida (Vaughan 1986). The carpus of Thrinaxodon, contained eleven bones: a proximal row composed of a radiale, intermedium, ulnare, and an accessory carpal bone; a middle row containing two centralia located distal to the radiale and intermedium; and a distal row with five carpal bones (Jenkins 1971).

Although there are skeletal elements in terrestrial mammals (Cerny and Brandstatter 1990) and in cetaceans (Klima 1990) that develop directly from a mesenchymal condensation into bone during ontogeny, typically many elements have an intermediate cartilaginous stage. The carpal bones in

terrestrial mammals normally pass through this three step sequence (O'Rahilly 1957). At any one of these stages, a carpal element may halt in development, be resorbed, or coalesce with adjacent elements and lose its identity. Thus, at each stage of ontogeny, a skeletal element may be represented in any one of these three histological states. In this report, the more general term "skeletal element" will be used when the composition is not specified; i.e., the element is either bone, cartilage, or mesenchyme. The term "ossification center" refers to a developmental center in which precursor tissue is being replaced by bone tissue. The term "bone" refers to an organ as an individual unit of the mature skeleton. For example, the radius bone in the domestic dog is a single skeletal element which develops from three ossification centers in which bone tissue replaces the cartilage model (Evans 1993). The three ossification centers of the radius are the diaphysis and two epiphyses. In contrast, the radial carpal bone of the dog also develops from three ossification centers which represent three separate skeletal elements, the radiale, intermedium, and a centrale, which fuse during development (Evans 1993). The terms "skeletal element," "bone" and "ossification center" will be used throughout this report in the manner described above.

This report will follow the carpal terminology of Nomina Anatomica Veterinaria (Frewein et al. 1994), which uses the terms first developed by Gegenbaur (1864). The human derived term "scaphoid" (or "navicular") will be referred to as the radiale, the "lunate" (or "semilunar") as the

intermedium, the "cuneiform" (or "triangular") as the ulnare, and the "pisiform" as the accessory carpal bone. In the distal row, the "trapezium," "trapezoid," and "capitate," will be referred to as carpal bones one, two, and three. The term "unciform" has been used to refer to both carpal bone four and to fusions of carpal bones four and five. Because of this ambiguity, the term "unciform" will be used when it is not clear which usage the author intended.

Comparison of the carpus among odontocete families

The carpus of cetaceans is of particular interest because of the alteration it has undergone during evolution. This joint has evolved into a semi-rigid plate of bones and cartilage (Felts 1966). At the same time, some species appear to have undergone a reduction in the number of carpal elements (Eales 1954). Whether this reduction is due to loss, or fusion, of carpal elements is often unclear. The number of bones found in the carpus can differ among individuals of the same species, and even between the limbs of an individual (Eales 1954, Pilleri and Gihr 1976a, Schwab 1988, Marshall et al. 1992). Proximodistal shortening of the limb can obscure the distinctiveness of separate proximal and distal rows in the carpus (Flower 1885, Leboucq 1889). These factors make it difficult to infer homologies of odontocete carpal bones.

The majority of cetacean species, approximately 88% (Leatherwood et al. 1983), belong to the suborder Odontoceti. These animals are all pentadactylous, but vary greatly in size, diet, and lifestyle. The bulk of the research on cetacean carpal composition has been on odontocetes, perhaps because the global availability and small size of some species make it easy to obtain and handle specimens. This literature review will examine descriptions of nine species of odontocetes from five families: Physeteridae, Monodontidae, Ziphiidae, Platanistidae, and Delphinidae (measurements in meters, m, are of total body length, TL, unless listed otherwise). The carpal bones of the bottlenose dolphin have been described by several authors, but there is no consensus on the number or names of these bones. Accordingly, this species is a prime candidate for a study which incorporates a large number of specimens, thereby reducing sampling bias.

Physeteridae - Examination of the cleaned bones of the skeleton of an adolescent male sperm whale (Physeter macrocephalus, 18.29 m) produced one of the first detailed descriptions of the complete skeleton in this species (Flower 1868). The ossification process of the carpus in the sperm whale appears to be unlike that of other odontocetes: "in many cases, a kind of epiphysial ossification has taken place in this cartilage, so that the bones were surrounded by a more or less complete case of thin osseous matter, which appears to unite with them" (Flower 1868).

Five similarly shaped bones were found in the carpus of this sperm whale (Fig. 1), but the proximal and distal rows were not as distinct as in other cetaceans, being proximodistally compressed so "as to appear almost to form a single row" (Flower 1868). The proximal row of the carpus contained three bones which were closely related to the distal ends of the radius and ulna, being slightly proximal to the other bones of the carpus. The most cranial carpal bone, located immediately distal to the cranial half of the radius, was identified as the radiale or a fused carpal one and radiale. The center-most bone, located between the distal ends of the radius and ulna, was identified as the intermedium. The next more caudal bone of the proximal row was directly distal to the caudal half of the ulna and was identified as the ulnare. A sixth bone, the accessory carpal bone, is located caudal to the ulnare in sperm whales (Flower 1868).

The two remaining bones in the carpus were located more distally. The more cranial of these bones articulated with metacarpal two, the radius, and a portion of the radiale and intermedium. This bone was identified as carpal two or a fused carpals two and three. The more caudal of the two distal bones was identified as an unciform. It articulated with the ulna, metacarpal three, and metacarpal four, and was located between the intermedium and ulnare,

Monodontidae - Eales's (1954) study of the manus of the narwhal (Monodon monoceros) incorporated fetuses, thus revealing information about

the ontogenetic stages of carpal development and the fusions that can occur within the carpus prior to ossification. These data were from two fetal specimens 0.137 m and 0.15 m long (presumably TL). The fetuses were compared with radiographs from a sub-adult narwhal (length and sex unknown, but prior to having undergone distal epiphyseal fusion of the radius and ulna).

Dissection of the carpi of the fetal narwhals revealed a proximal row of three cartilaginous elements: the radiale, intermedium, and ulnare (Fig. 2-a). A small, distinct centrale is located distally between the radiale and intermedium, confirming the earlier observation of Leboucq (1889). The intermedium articulates distally with carpal three, carpal four, and the centrale. The ulnare is the smallest of the three elements in the proximal row and articulates proximally with the ulna and distally with carpal four, carpal five and metacarpal five. The distal row in the fetus is composed of four cartilaginous elements identified as carpals two through five. Carpal one was not observed, but since metacarpal one extends proximally, it was hypothesized that this proximal elongation resulted from carpal one earlier fusing with metacarpal one. Carpal two articulates with the proximal end of metacarpal two, metacarpal one, the centrale, and carpal three. Carpal three articulates proximally with the centrale and intermedium, distally with metacarpals two and three, and caudally with carpal four. Carpal four lies proximal to metacarpals three and four, articulating with both, and

proximally with the intermedium and ulnare, and caudally with carpal five. A small carpal five articulates with the ulnare, carpal four, the cranial portion of the proximal border of metacarpal five, and the caudal portion of the proximal border of metacarpal four. In addition to these carpal elements, Leboucq (1889) described a small accessory carpal element fused to the distal end of the ulna in his dissections of four fetal narwhal.

Radiographic examination of a sub-adult narwhal reveals that, in place of the cartilaginous carpals four and five in the fetus, there is a single bone that does not approach metacarpal five (Fig. 2-b). This bone was identified as a fusion of carpals four and five. In this individual, the carpal bones "occupy islands in fibrous connective tissue and hence do not make contact with one another" (Eales 1954).

Yablakov's (1974) summary of his research (methodology not stated) into the anatomy of the beluga (Delphinapteras leucas) states that the carpus is highly variable. The most common carpal pattern (Fig. 3) for his specimens (presumably adults) was a proximal row containing the radiale, intermedium, ulnare, and the accessory carpal bone, with the distal row containing carpal bones one through four. Centralia occurred between the proximal and distal row in some specimens. In addition, carpals one, two, three, and the accessory carpal bone were absent in some individuals.

Ziphiidae - Dissection of the carpi of a 6.25 m male bottlenose whale (Hyperoodon ampullatus) revealed a unique carpal composition (Turner

1909). This immature whale had unfused distal epiphyses of the radius and ulna and most of the carpal elements "consisted of osseous nodules in masses of cartilage" (Turner 1909).

The proximal row of the carpus in this whale consisted of a partially ossified radiale, intermedium, and ulnare, and a cartilaginous accessory carpal element which articulated with the ulna, ulnare, and metacarpal five. The distal row of the carpus was unusual in that it contained five carpal elements (Fig. 4), one directly proximal to each metacarpal bone. Carpals two and five were cartilaginous, while carpals one, three, and four had small ossification centers in their cartilaginous models.

Platanistidae - A radiographic study of eleven pairs of flippers from two species of Asian river dolphin (Platanista spp.) demonstrated interesting differences in the carpi of these two closely related species (Pilleri and Gihr 1976a). Five sub-adult Indus susu (P. minor, three males and two females, 1.17 - 1.26 m) and six adult Ganges susu (P. gangetica, two males and four females, 1.80 - 2.40 m) were examined using radiography.

No variation was found in the six carpal elements observed in all Indus susu specimens. The proximal row consisted of three bones, and the distal row of three bones (Fig. 5-a). The most cranial bone of the proximal row articulates proximally with the cranial edge of the distal surface of the radius, and distally with metacarpal one. Pilleri and Gihr described this as a fusion of carpal one and the radiale. This bone occupies only part of the area

distal to the radius and cranial to the intermedium, however, which seems to indicate that it represents only a portion of the radiale, and possibly carpal one as well. Another bone, identified by Pilleri and Gihr as a centrale, lies caudal to the radiale-carpal one composite and occupies the remainder of the carpal space filled by the radiale in other odontocetes. This bone articulates proximally with the radius and distally with carpal two. The topographical relationship of this bone to the radius suggests that it is a portion of the radiale that develops as a separate entity, rather than a centrale, which by definition, is usually found between the proximal and distal carpal rows and does not articulate with the radius (Goodrich 1930). The intermedium is the most caudal of the bones in the proximal row and lies between the distal ends of the radius and ulna, articulating proximally with both of these, and distally with carpal two and carpal three.

The most cranial of the bones in the distal row is carpal two, articulating proximally with both the intermedium and the centrale, cranially with the caudal surface of metacarpal one, distally with metacarpal two, and caudally with carpal three. Carpal three articulates proximally with the intermedium, cranially with carpal two, distally with metacarpal three, and caudally with carpal four-plus-five. Caudal to, and articulating with the caudal margin of carpal three is the caudal-most bone of the distal row - carpal four-plus-five. This bone also articulates with the intermedium, the distal ulna, metacarpal four, and the cranial portion of metacarpal five.

There was no independent ossification center for the ulnare in these sub-adult Indus susu. Pilleri and Gihr (1976a) explained this absence as a failure of the ulnare to develop, or an incorporation of it into the distocaudal surface of the ulna, which in turn articulated with the caudal portion of the proximal border of metacarpal five. No evidence was offered for either of these explanations.

The six adult Ganges susu had a similar pattern of six carpal bones as described for Indus susu, although there was a tendency for fusion of the two bones that occupy the region of the radiale (Fig. 5-b). This fusion was seen in at least one flipper in each animal and bilaterally in one male and one female.

Delphinidae - Three species in this family were examined because it contains Tursiops truncatus, the species that was studied in this report. The striped dolphin (Stenella coeruleoalba) is a gregarious, pelagic dolphin which reaches 2.7 m (Leatherwood et al. 1983). Radiographs of the right flippers from 54 male and 44 female dolphins were examined (Calzada and Aguilar 1996). These specimens ranged from fetuses up to animals estimated to be 34 years old. The most frequent pattern (80%, n = 78) for the carpus (Fig. 6) contained two rows: a proximal row with three carpal bones, identified as the radiale, intermedium, and ulnare, and a distal row with two bones which were identified as carpal three and a bone resulting from the fusion of carpals four and five (Calzada and Aguilar 1996). Variations due to additional bones

were observed in 20 percent of flippers. These included the accessory carpal bone (at an unknown frequency); a centrale between the intermedium and the radiale in two flippers, and a single bone, tentatively identified as carpal one or carpal two, that is located between the radiale and the metacarpal one (frequency unknown) (Calzada and Aguilar 1996).

The false killer whale (Pseudorca crassidens) is a large oceanic delphinid that can reach 6.1 m (Leatherwood et al. 1983). A radiographic study of 95 pairs of flippers and 23 unpaired flippers from false killer whales examined skeletal variation within the manus (Gihr et al. 1982). Five carpal bones - radiale, intermedium, ulnare, carpal three, and the fusion of carpals four and five - appeared in similar topographies to those in the striped dolphin above (Fig. 7). Gihr et al.'s identification of the more caudal of the two distal bones as fused carpal four-plus-five, concurs with that of Calzada and Aguilar (1996), but Gihr et al. consider the more cranial of these bones to be a fusion of carpal two and carpal three, whereas Calzada and Aguilar consider it to be carpal three only. Another bone, identified by Gihr et al. as a centrale, frequently appears between the radiale and intermedium and articulates proximally with the radius. This additional bone, immediately caudal to the radiale, occupies a portion of the area filled by the radiale in other odontocetes. Forty-four different variations were observed within the carpus of these specimens, differing in the absence, presence, and degree of fusion of this so-called centrale with the other carpal bones, as well as fusions

among the other basic five carpal bones. In addition, two bones were present at the cranial margin of the flipper distal to the radiale in the region of the first digit: Gihr et al. (1982) identified these as carpal one and metacarpal one, a poorly supported conclusion since the more proximal of this pair has been topographically identified as a shortened metacarpal one (Flower 1868).

The flippers of 11 bottlenose dolphins (Tursiops truncatus) from the east coast of the USA were cleaned and dried or radiographically examined as part of a study on the osteology of this species (Rommel 1990). Eight dolphins had five carpal bones, one had four, and two had six, but differed in the arrangement of these bones. The most common arrangement of carpal bones was two rows (Fig. 8). The proximal row contained three bones - the radiale, intermedium, and ulnare. The radiale was the most cranial of these three and lay distal to the radius. The distal surface of the radiale articulated with metacarpal one. The intermedium lies between the radiale and the ulnare, and its proximal surface articulated with the distal ends of both the radius and ulna. The ulnare was distal to the ulna and was the most caudal of the carpal bones of the proximal row. The distal surface of the ulnare articulated with metacarpals four and five. "Secondary ossifications" were observed for the radiale and ulnare (one specimen each).

The distal row consisted of two bones. The more cranial bone was identified as carpal three. The proximal surface of this bone articulated with the radiale and the intermedium, while the distal surface articulated with

metacarpals two and three. The more caudal bone was identified as a fusion of carpals four and five. The proximal surface of this bone articulated with the intermedium and ulnare. Its distal surface articulated with the caudoproximal surface of metacarpal three, and its caudal surface with the cranioproximal surface of metacarpal four.

A radiographic-dissection study of 163 bottlenose dolphin flippers from the Gulf of Mexico revealed that the accessory carpal element was present at the distocaudal corner of the ulna, and that it may ossify in dolphins longer than 2.3 m (Stepaniuk and Watson 1995) (Fig. 9).

A bilaterally symmetrical six-bone carpus has been described for a unilaterally polydactylous bottlenose dolphin (male, 2.20 m) from the Gulf of Mexico (Watson et al. 1994). Radiographs and dissection of the flippers in this dolphin revealed a carpus with a proximal row that consisted of four bones. The radiale, intermedium, and ulnare occupied similar positions and maintained the same proximal articulations as has been described for the bottlenose dolphins of the western Atlantic (Rommel 1990). In addition there was another bone in the proximal row located cranial to the radiale and caudal to the cranial margin of the radius. The two bones of the distal row articulated in the same manner as has been reported previously (Rommel 1990) but the more caudal of these bones was identified as carpal four.

Another radiographic-dissection study of Gulf of Mexico bottlenose dolphins (Holmes 1993) examined seven flippers from four animals (1.71 -

2.26 m). The author found that: "In general, there are five carpal bones and one cartilage (pisiformis) [accessory carpal]" (Holmes 1993). The proximal row consisted of a radiale, intermedium, and ulnare. The accessory carpal, was located caudal to the distal end of the ulna and contained a small nodule of bone in a larger cartilage model. It was also noted that "the distal row consists of two bones which were designated as C1 and C2," which appeared to correspond to Rommel's (1990) carpal three and carpal four-plus-five, respectively.

Another radiographic study which included examination of the left thoracic limbs from 11 bottlenose dolphins (Fitzgerald 1970) revealed a basic five-bone carpal pattern like that reported above (Rommel 1990). The proximal row contained a radiale, intermedium, and ulnare, while the distal row contained two bones identified as carpal two and an unciform. Although a pattern of five bones in two rows was observed, there were several variations due to differences among individuals in the number and location of additional bones. A centrale-like sixth bone, located between the intermedium and ulnare, was found in three dolphins, although it was referred to as a secondary ossification center of the intermedium (Fitzgerald 1970). One other specimen appears to have an additional ossification center fused to the caudal border of the ulnare, which probably represents the accessory carpal bone. Four specimens had an additional bone cranial to the radiale, which was identified as the radial sesamoid bone.

The validity of this latter interpretation cannot be verified since no evidence was offered; i.e., the radiographs were not reproduced and tendinous attachments were not described. In terrestrial mammals, e.g., the dog, a sesamoid bone is embedded in the tendon of the abductor pollicis longus and lies medial to the radiale near the proximal end of metacarpal one (Evans 1993). In the bottlenose dolphin, Leboucq (1889) noted the presence of an additional bone cranial to the radiale and interpreted it as a proximal carpal belonging to an additional digital ray (prepollex), while Rommel (1990) refers to it as a "secondary ossification" for the radiale.

The skeletal components of the left and right manus of four mature bottlenose dolphins (2.05 - 2.77 m) from the Gulf of Mexico were examined using radiographs and dissection (Schwab 1988). The number of carpal bones varied from four to five. The author interpreted carpal variation as the result of fusion. The specimens with five carpal bones had proximal rows containing a radiale, intermedium, and ulnare. The bones of the distal row were "designated as c1 and c2" and their homology was not determined, but they correspond to Rommel's (1990) carpal three and carpal four-plus-five, respectively. Schwab claimed that two dolphins had six bones in each carpus; however, the so-called sixth carpal bone was the cranial lobe of a dumbbell-shaped radiale. Although Schwab stated that there was no asymmetry between the carpi of individuals, his Table 12 (p. 123) shows that one dolphin

had flippers that differed in the number of carpal bones, with four bones in the left carpus and five bones in the right carpus.

In another study of Gulf of Mexico bottlenose dolphins, radiographs of 35 limbs from 20 dolphins ($\geq$2.0 m) were examined to determine the number of carpal bones (Marshall et al. 1992). The number of bones ranged from five to seven, with the most common number being six (60%, n = 21), and the next most common being seven (29%, n = 10). The remainder (11%, n = 4) had five bones as has been reported for the western Atlantic bottlenose dolphin (Rommel 1990).

Summary

This brief review reveals a carpal pattern common to odontocete cetaceans: five bones, arranged into two rows. The proximal row consistently contains three bones (Flower 1868), the radiale, intermedium, and ulnare, and the distal row contains two bones which have traditionally been designated as carpal two and carpal four-plus-five (Flower 1868), although some now call these bones carpal three and carpal four-plus-five (Rommel 1990). These five bones were present in five of the nine species reviewed, including the three delphinid species - spinner dolphin, false killer whale, bottlenose dolphin. Variations due to additional bones and apparent fusion among bones are common, however, and were reported for each of the delphinid species reviewed. The accessory carpal bone is present in many

species (Flower 1868), and is considered a canonical element in cetaceans (Stepaniuk and Watson 1995).

Additional bones may be present. In river dolphins (Pilleri and Gihr 1976a), spinner dolphin (Calzada and Aguilar 1996) and bottlenose dolphin (Rommel 1990, Watson et al. 1994, Fitzgerald 1970), two bones are sometimes found in the space occupied by the radiale in other odontocetes. Additional bones can be found in the distal row of the carpus in the narwhal (Eales 1954). The distal carpal row of the bottlenose whale contains five bones (Turner 1909). A centrale may be located between the proximal and distal rows of narwhal (Eales 1954) and beluga (Yablakov 1974). Fusions appear to occur between the radiale and intermedium and between the ulna and ulnare in river dolphins (Pilleri and Gihr 1976a), between carpals four and five in the narwhal (Eales 1954), and between the carpal bones and the metacarpal bones in the narwhal (Eales 1954) and spinner dolphins (Calzada and Aguilar 1996). Some bones may not develop (Yablakov 1974).

Selective pressures within the aquatic environment have streamlined the cetacean limb, but have not maintained the topographical relationships of the skeletal elements as in the ancestral carpus. In addition there is a high degree of variation among individuals within a species and between the thoracic limbs of an individual. Few studies have used large numbers of specimens and reports based upon small numbers of animals are inadequate for describing the carpus of a cetacean population. "It may be premised that

every species appears liable to certain individual variations, and that sometimes the different sides of the same animal were not precisely alike either in the arrangement or even the number of carpal ossifications" (Flower 1868). The number of bones in the carpus of bottlenose dolphins varies, and thus, this common species is an ideal subject for a study to determine the amount of variation in number of carpal bones. This variation will be examined by using a larger number of individuals than in previous studies of this species.

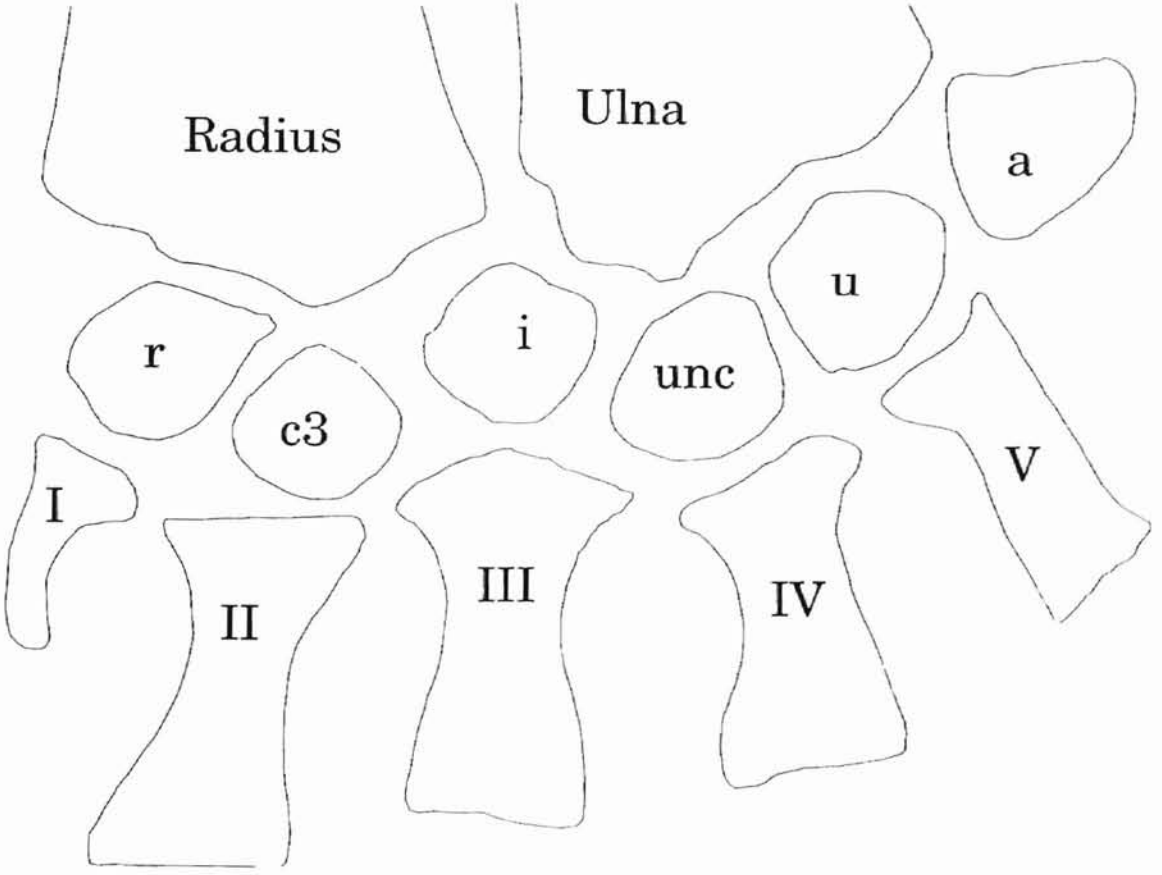

Figure 1. The cleaned, dried carpal bones of an adolescent sperm whale, Physeter macrocephalus, in left lateral view, redrawn from Flower (1868). unc – unciform.

(All figures and radiographs printed with the radius to the left)

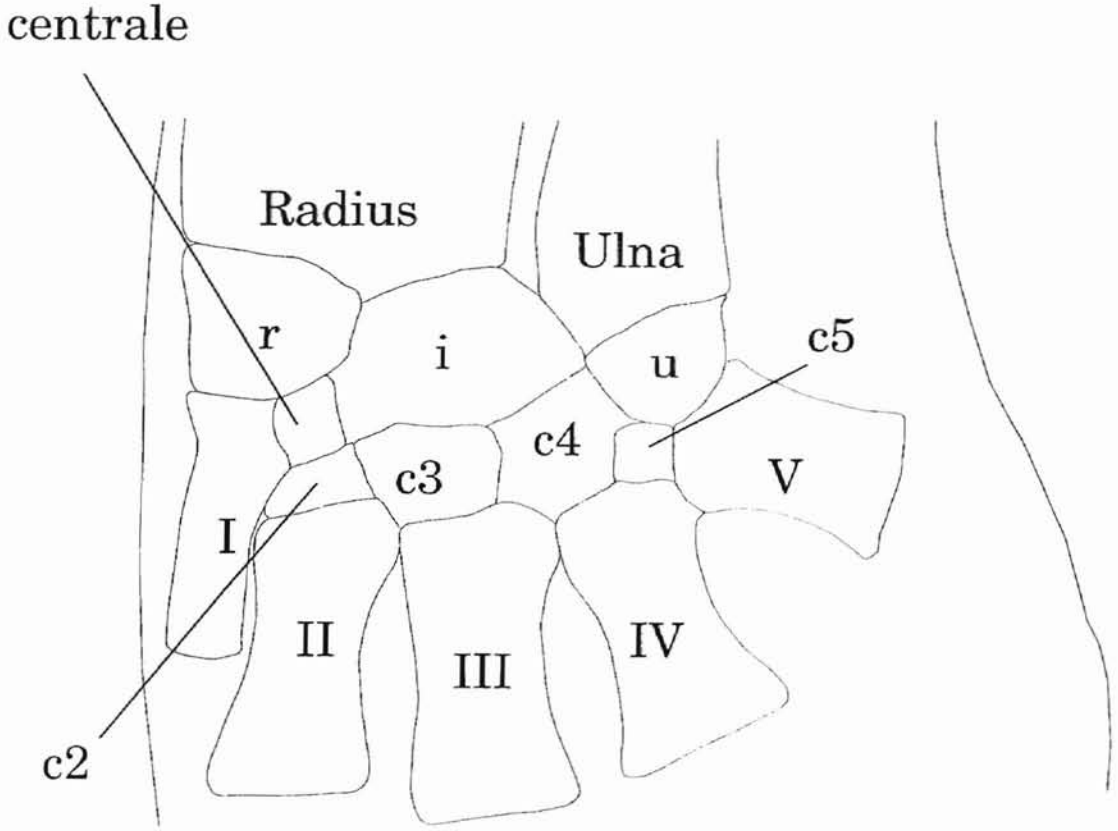

Figure 2-a. The dissected cartilaginous carpal elements of a fetal narwhal, Monodon monoceros, in left lateral view, redrawn from Eales (1954).

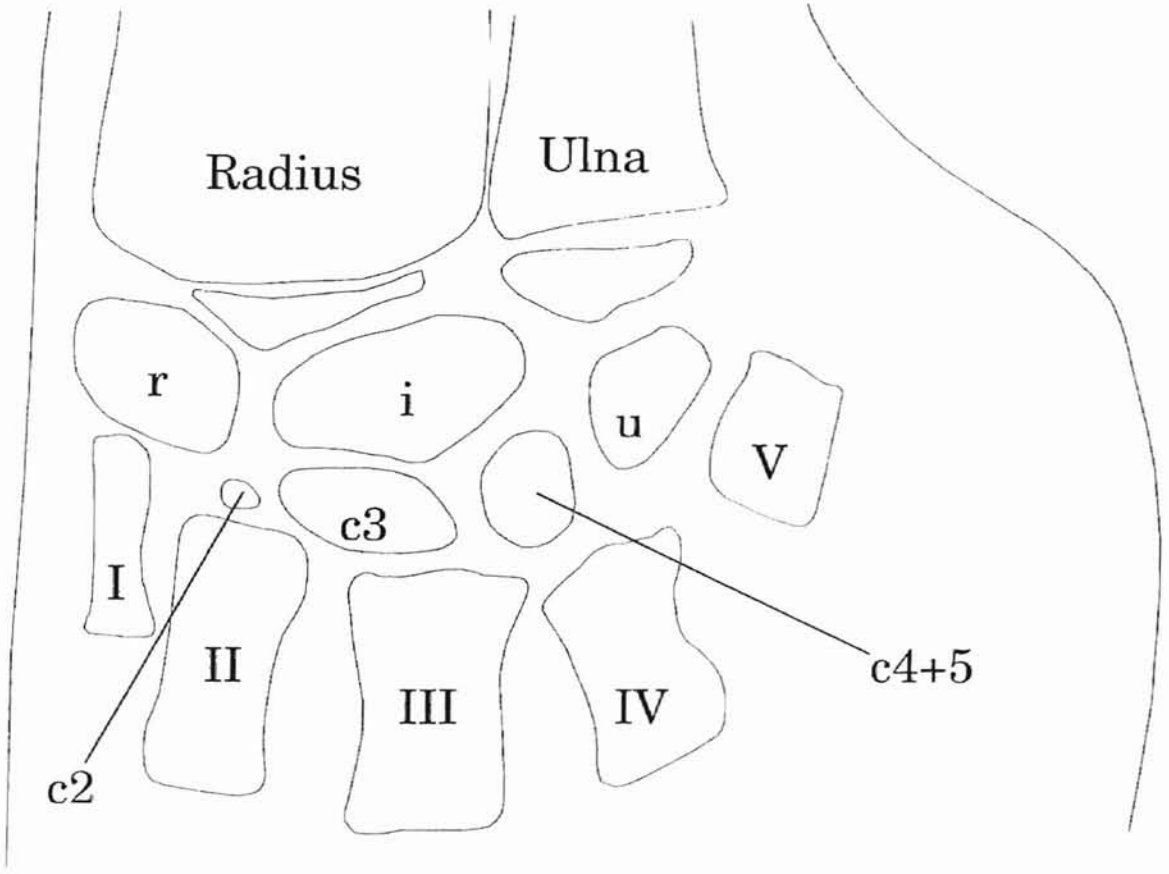

Figure 2-b. The carpal bones of a sub-adult narwhal, Monodon monoceros, in left lateral view as revealed by a radiograph. A separate distal epiphysis was present for both the radius and the ulna. Redrawn from Eales (1954).

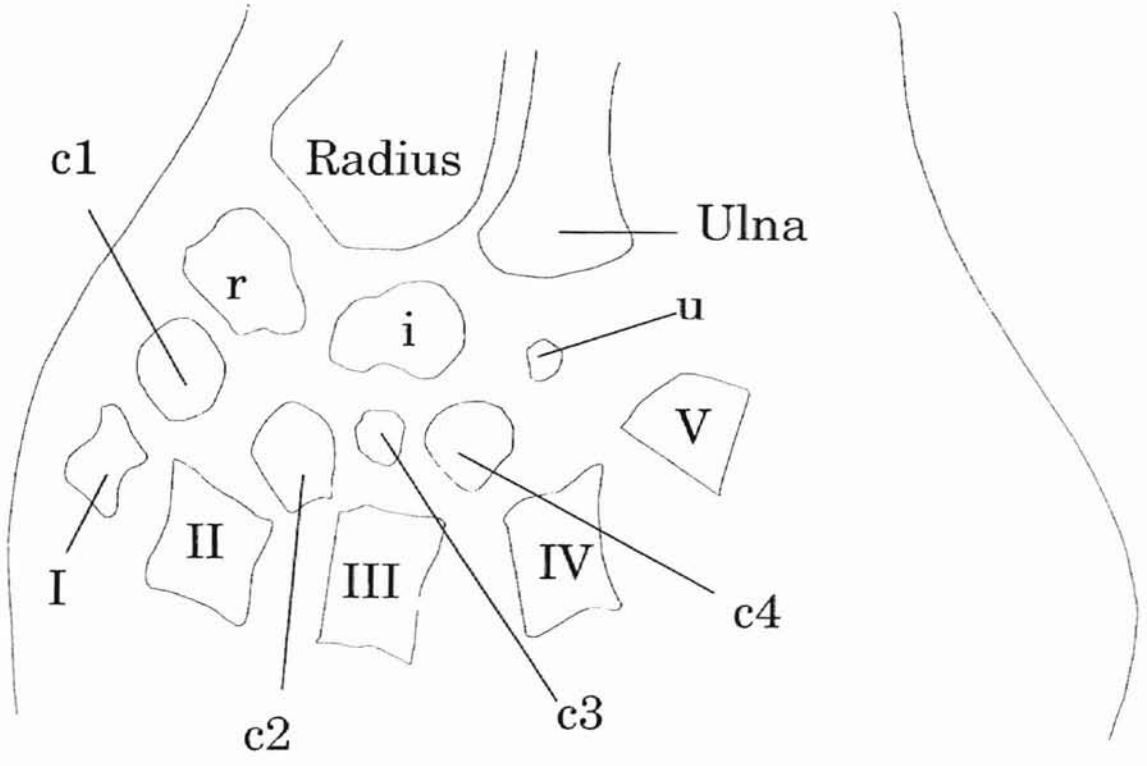

Figure 3. Sketch from radiograph of the carpal bones of an adult female beluga, Delphinapterus leucas, in left lateral view. Redrawn from Vladykov (1943).

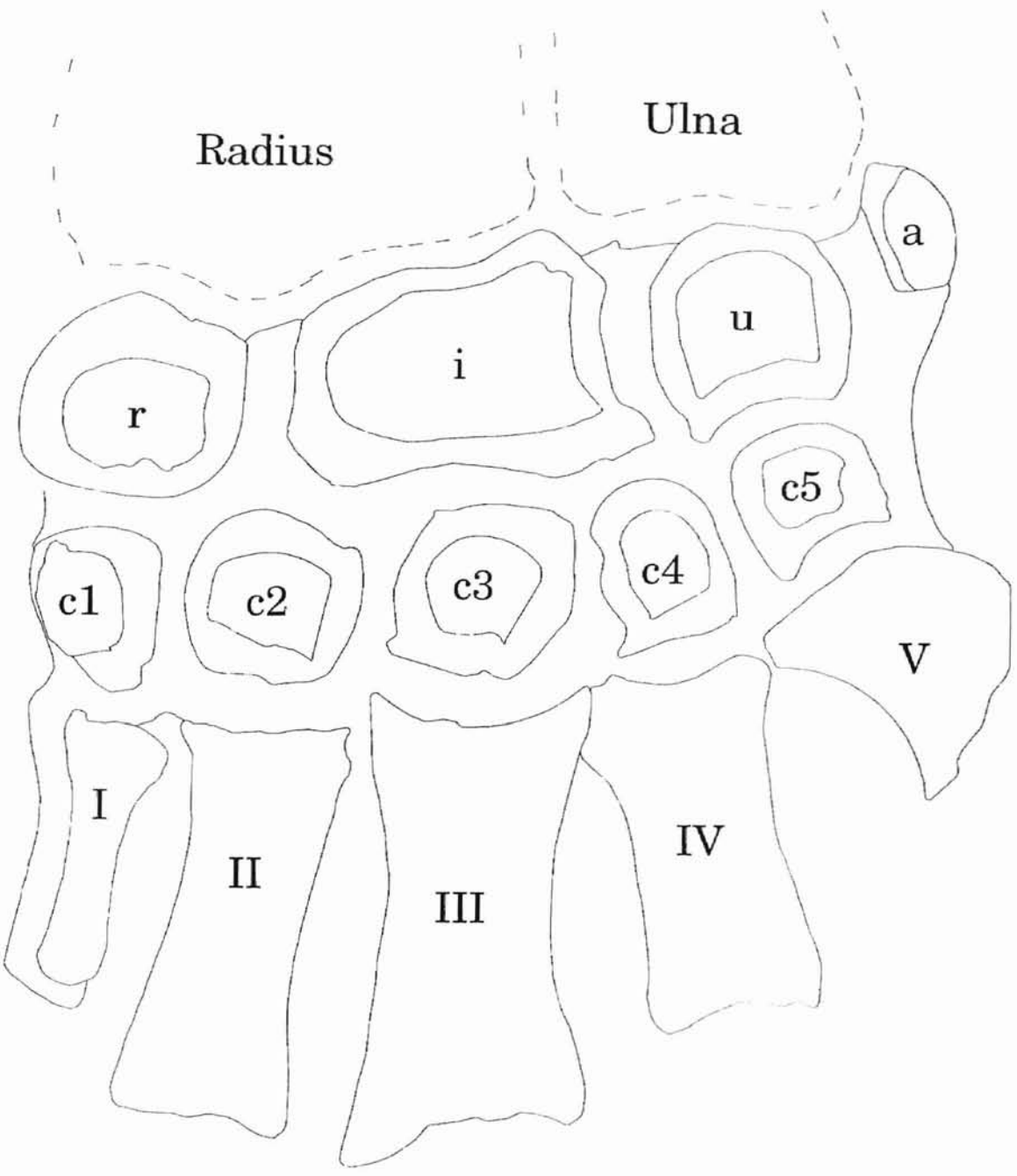

Figure 4. The dissected carpal bones of a sub-adult bottlenose whale, Hyperoodon ampullatus, in left lateral view, redrawn from Turner (1909). (Radius and ulna - represented by dashed lines - added. Ossified nodules of bone are surrounded by cartilage.)

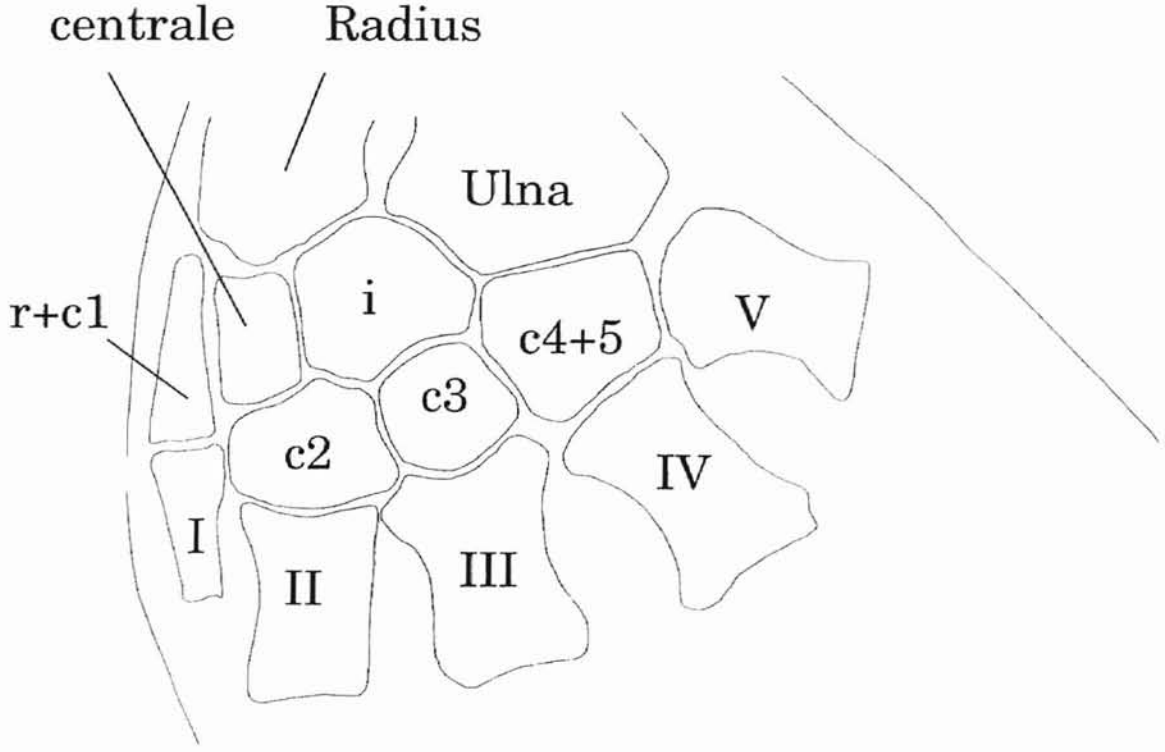

Figure 5-a. The carpal bones of an sub-adult Indus susu, Platanista minor, in left lateral view, as revealed by radiography. Redrawn from a sketch based upon radiographs from Pilleri and Gihr (1976a).

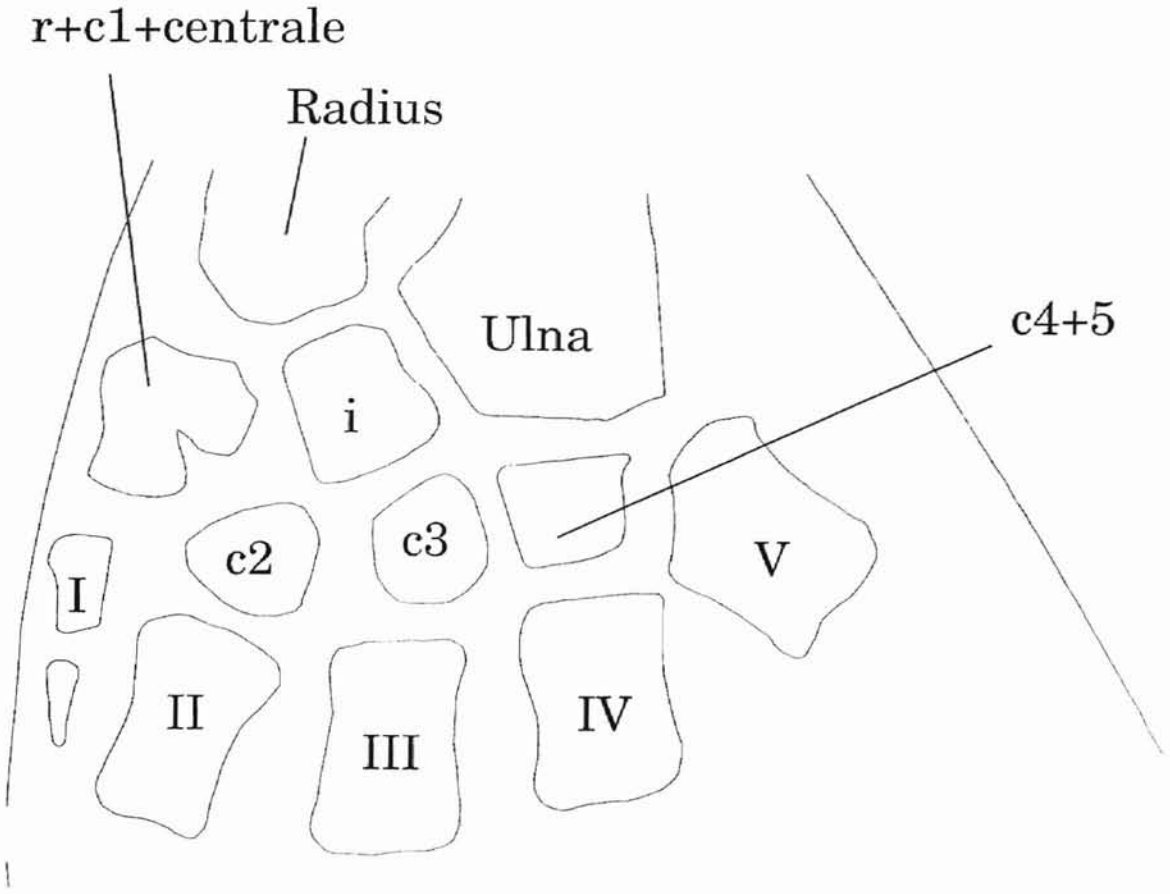

Figure 5-b. The carpal bones of an adult Ganges susu, Platanista gangetica, in left lateral view, as revealed by radiography. Redrawn from a sketch based upon radiographs from Pilleri and Gihr (1976a).

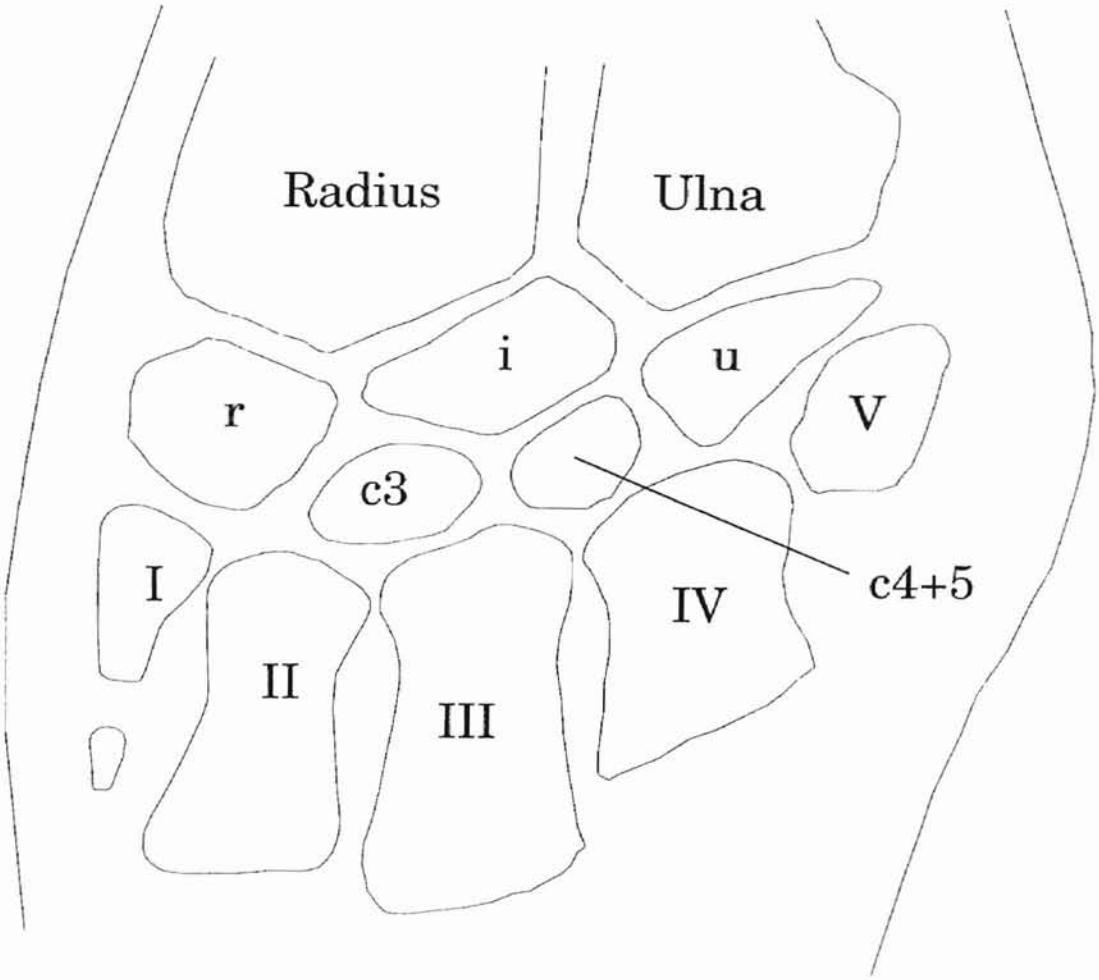

Figure 6. The carpal bones of a striped dolphin, Stenella coeruleoalba, in left lateral view, redrawn from composite sketch from radiographs from Calzada and Aguilar (1996).

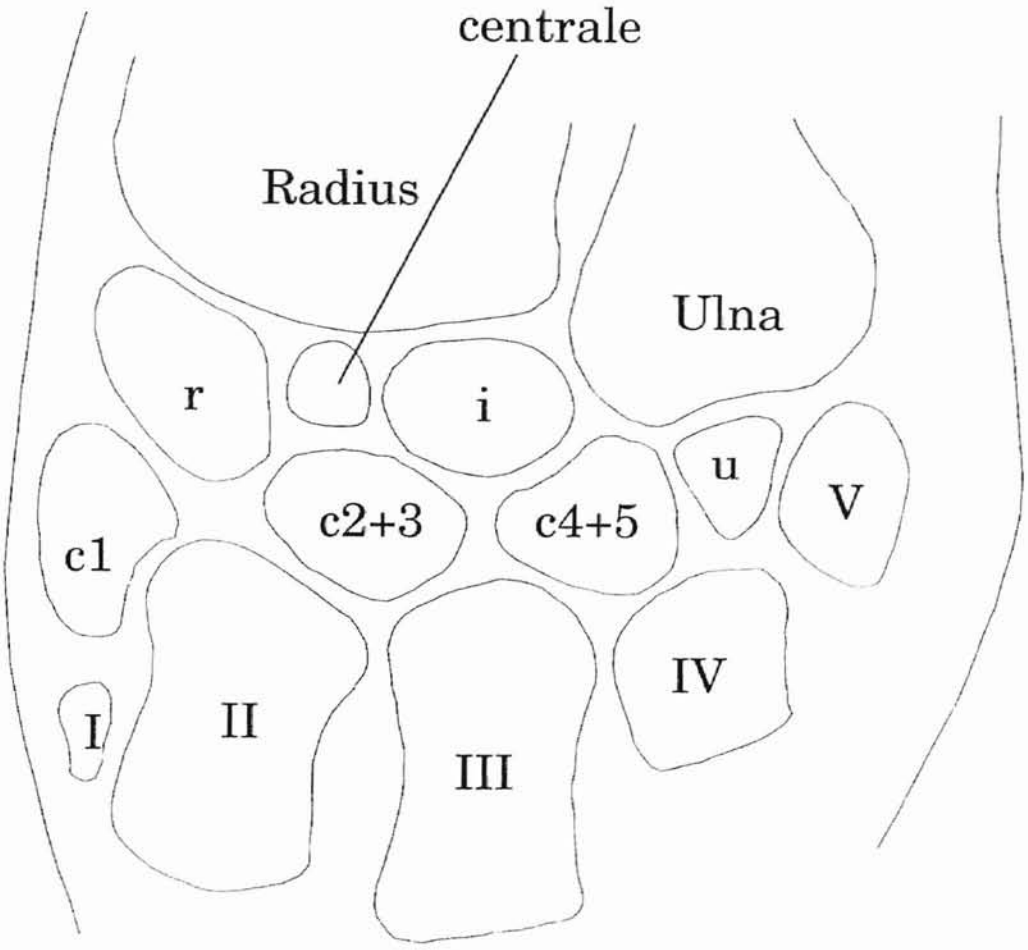

Figure 7. The carpal bones of a false killer whale, Pseudorca crassidens, in left lateral view. Redrawn from composite sketch of carpus, as revealed by radiographs from Gihr et al. (1982).

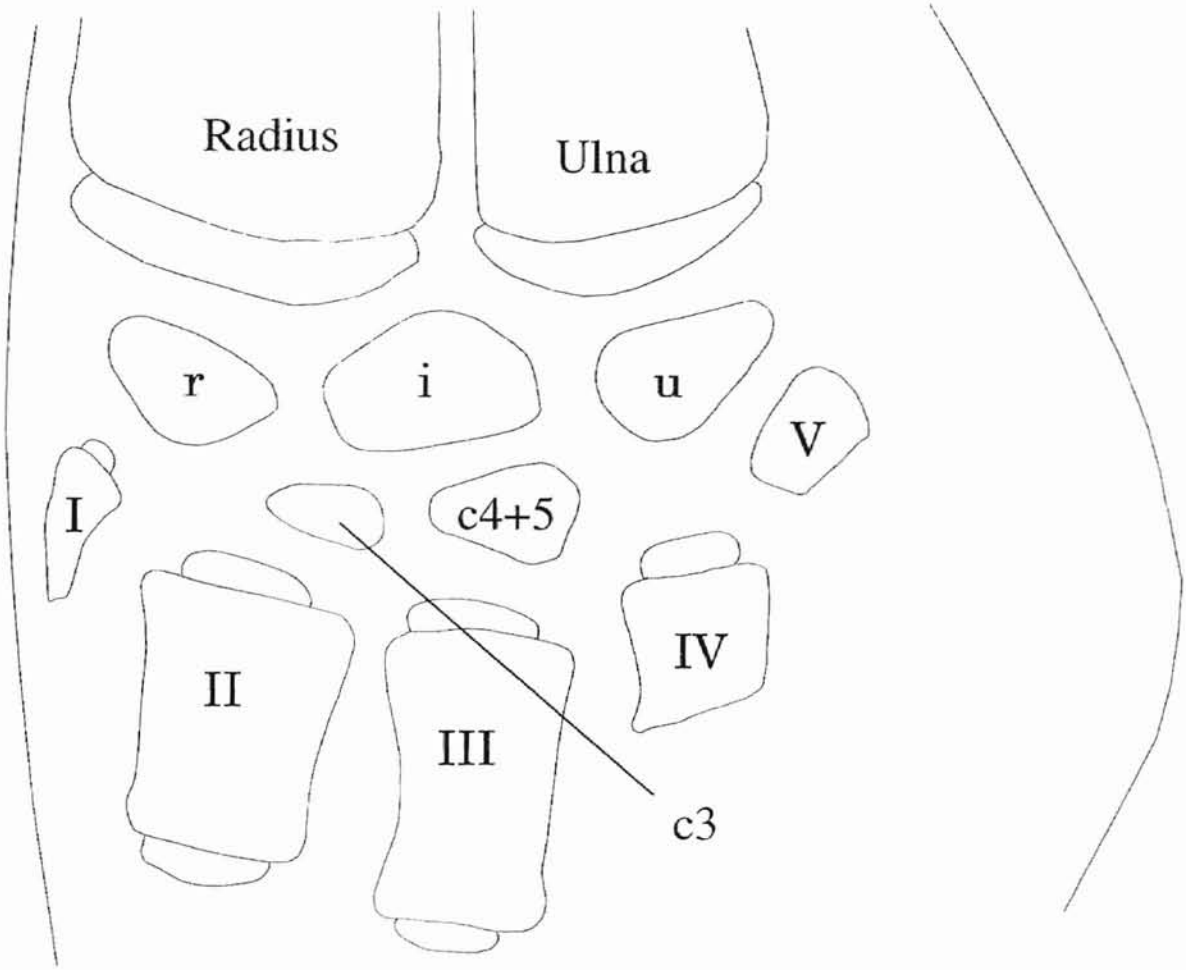

Figure 8. The carpal bones of a bottlenose dolphin, Tursiops truncatus, in left lateral view, as revealed by radiography. Separate unfused bony epiphyses are present on the distal radius and ulna, as well as metacarpal bones one through four. Redrawn from composite sketch of radiographs from Rommel (1990).

Chapter 3

MATERIALS AND METHODS

Specimens

Disarticulated left and right flippers were collected and frozen from 27 mature bottlenose dolphins, Tursiops truncatus, stranded on the Texas coast (1990-1995). Standard measurements (Norris 1961) were recorded for the carcass of each of these dead dolphins. Stranding records from the Texas Marine Mammal Stranding Network (Galveston, TX) provided the field number, total body length (TL in meters, m), sex, side of body of flipper, stranding location and date, and descriptive comments. This sample consisted of 27 left and 27 right flippers from 14 adult male and 13 adult female dolphins that ranged from 2.01 m to 2.94 m TL (Table 1).

Radiography

Flippers were thawed, cleaned, and standard plain-film (Ultra-vision G film, Dupont) mediolateral radiographs were taken of all flippers at the College of Veterinary Medicine, Oklahoma State University, Stillwater, OK.

Each prepared flipper was placed on top of a 36 x 43 cm non-grid cassette (Ultra-vision detail screen, Dupont) covered with a thin plastic sheet. Film-to-source distance was 102 cm. These flippers were radiographed at 54 kVp and 200 mA for 1/40 second and film was processed in an automatic processor. Each radiograph was examined two times by the naked eye on a standard radiographic illuminator to determine the presence, location and number of bones in the carpus.

Dissection

The carpi of four flippers (Table 1) were dissected to establish the borders of carpal elements by location of the inter-element soft tissue separations that could be used to determine the identity of the carpal bones. Dissection was aided by partial bacterial maceration. Dry cleaned bones were then prepared after bacterial maceration to determine the morphology of fully developed carpal bones.

Data Analysis

Statistical software (SPSS for Windows, Release 6.0, SPSS Inc., Chicago, 1993) was used to compare, via independent sample t-tests, the relationships between sex, side of body, and frequency of occurrence of individual carpal bones.

Definitions

The bones of the carpus were identified by topographical location in mediolateral radiographs of mature dolphin flippers (Fig. 9). For the purpose of this report, carpal bones articulating with the distal ends of the radius or ulna are herein defined as belonging to the "proximal row" of the carpus. The bone of the proximal row located distal to the cranial half of the radius is identified as the "radiale." The bone of the proximal row distal to the caudal half of the radius and the cranial half of the ulna is herein identified as the "intermedium." The bone of the proximal row distal to the caudal half of the ulna is herein identified as the "ulnare." The most caudal bone in the proximal row articulates with the distocaudal surface of the ulna, and is called the "accessory carpal bone."

Carpal bones that articulate with metacarpal bones, but not with the distal ends of the radius and the ulna are herein defined as belonging to the "distal row" of the carpus. The two bones which consistently appear in the distal row are named in accordance with Rommel (1990). Thus, the bone that lies primarily caudal to the axis of metacarpal two and cranial to the axis of metacarpal three, and articulates with metacarpals two and three, is herein identified as "carpal three." Likewise, the bone that lies primarily caudal to the axis of metacarpal three and cranial to the axis of metacarpal four, and articulates with metacarpals three and four, is herein identified as "carpal four-plus-five."

Moreover, the homology of individual carpal bones was evaluated based upon topographical relationships of the individual bones in the adult carpus as defined above.

Hypothesis

The majority of mature bottlenose dolphins from the Gulf of Mexico have a carpus composed of a proximal row with three bones (radiale, intermedium, and ulnare) and a distal row with two bones (carpal three and carpal four-plus-five).

Table 1. Field collection data and number of carpal bones for 27 Gulf of Mexico bottlenose dolphins, Tursiops truncatus, with paired flippers radiographed in this study. Arranged in ascending total body length (TL - in meters, m).

Field #	TL (m)	Sex	Side of body	Carpal Bones Total	Proximal row	Distal Row
GA 289	2.01	M	L	5	3	2
			R	5	3	2
GA 407	2.06	F	L	5	3	2
			R	5	3	2
GA 417	2.06	M	L	5	3	2
			R	5	3	2
GA 408	2.09	M	L	5	3	2
			R	5	3	2
GA 420	2.10	M	L	5	3	2
			R	5	3	2
PA 196	2.10	M	L	5	3	2
			R	5	3	2
GA 409	2.14	F	L	5	3	2
			R	5	3	2
GA 423	2.15	M	L	5	3	2
			R	5	3	2
PA 236	2.30	F	L	5	3	2
			R	5	3	2
PO 158	2.32	F	L [a]	5	3	2
			R	5	3	2
GA 381	2.33	F	L	5	3	2
			R	5	3	2
PO 180	2.36	F	L	5	3	2
			R	5	3	2
GA 366	2.37	F	L	7	5	2
			R	7	5	2
PO 159	2.37	F	L	5	3	2
			R	5	3	2
GA 571	2.38	M	L	5	3	2
			R	5	3	2

F = female, M = male, L = left, R = right, * flipper also dissected

a) The left flipper of PO 158 had a single bone in the location of the small radiale (r') and carpal three.

Continued...

(Table 1 continued)

Field #	TL (m)	Sex	Side of body		Carpal Bones Total	Proximal row	Distal Row
GA 720	2.40	M	L		5	3	2
			R		5	3	2
GA 412	2.41	F	L		7	5	2
			R		7	5	2
CC 110	2.42	F	L		6	4	2
			R		6	4	2
PA 229	2.47	F	L	*	5	3	2
			R		5	3	2
GA 363	2.49	F	L		7	5	2
			R	*	7	5	2
SP 257	2.51	M	L		5	3	2
			R		5	3	2
GA 374	2.54	F	L		5	3	2
			R	*	5	3	2
CC 132	2.57	M	L		5	3	2
			R		5	3	2
GA 715	2.60	M	L		6	4	2
			R		6	4	2
PA 211	2.63	M	L		5	3	2
			R		5	3	2
PO 169	2.78	M	L		7	4	3
			R	*	6	3	3
PO 331	2.94	M	L		6	4	2
			R		6	4	2

F = female, M = male, L = left, R = right, * flipper also dissected

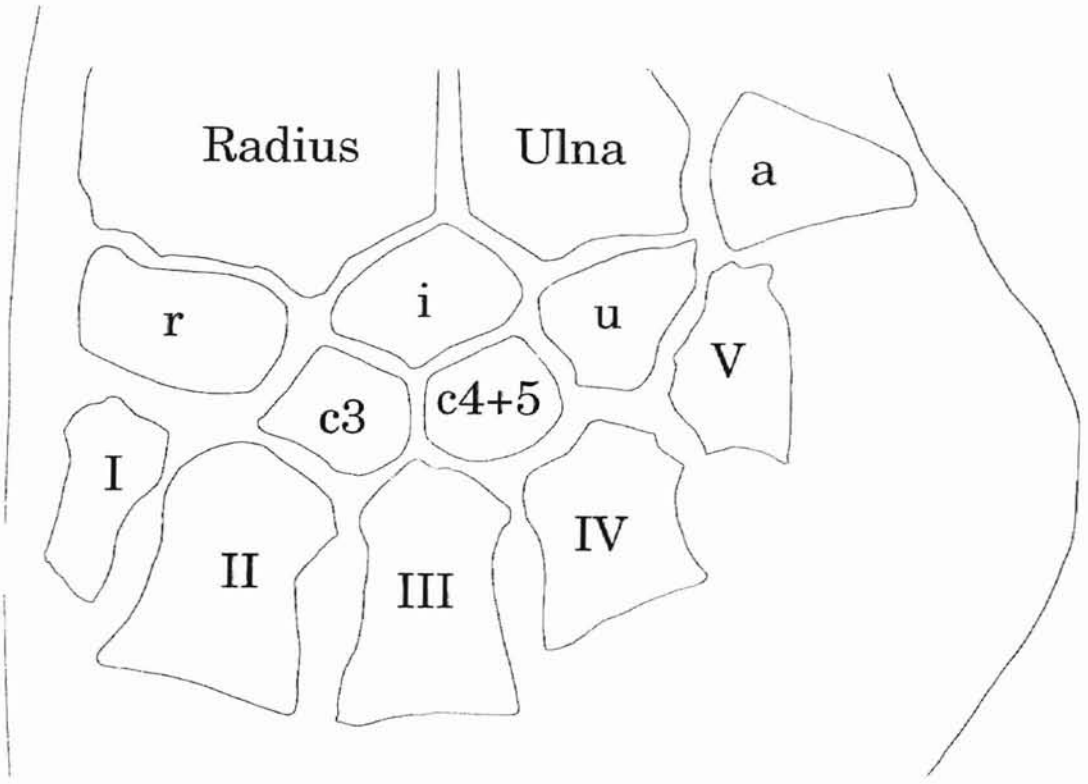

Figure 9. Lateral sketch demonstrating the topography and the naming of bones in the left carpus of the mature bottlenose dolphin: r - radiale, i - intermedium, u - ulnare, c3 - carpal three, c4+5 - carpal four-plus-five, I - metacarpal one, II - metacarpal two, III - metacarpal three, IV - metacarpal four, V - metacarpal five. The accessory carpal element (a) is included (Stepaniuk and Watson 1995).

Chapter 4

RESULTS

Topography of carpal bones

The most common carpal type observed radiographically contained five bones (74%, n = 40) (Fig. 10). In this pattern, three bones - the radiale, intermedium and ulnare - formed the proximal row. These three bones articulated proximally with the radius and ulna. The radiale and ulnare also spanned the carpus to articulate distally with the metacarpals one and two, and metacarpals four and five, respectively. The two remaining carpal bones, identified in this study as carpal three and carpal four-plus-five, articulated proximally with the bones of the proximal row, and distally with metacarpals two, three and four.

From observations on the radiographs, the distal epiphyses of the radius and ulna each had a prominent eminence that projected distally to form a ridge between the lateral and medial surfaces and thus divided the distal end of each bone into two articular surfaces. This ridge is herein defined as the "distal epiphyseal eminence" (DEE) (Fig. 10). The articular

surface of the radial epiphysis caudal to the DEE had a single articular facet which is defined as the "caudal articular facet" of the distal radial epiphysis, and which sloped proximocaudally from the DEE to the caudal edge of the radius (Fig. 10). The articular surface of the radial epiphysis located cranial to the DEE had two facets which are defined as the "middle articular facet" and "cranial articular facet" of the distal radial epiphysis. The size of these two facets was variable; i.e., the location where they meet, a rounded, oblique angle that is referred to as the "distal junction" (DJ) (Fig. 10), was located approximately one third to one half of the distance between the cranial edge of the radius and the DEE. The middle articular facet of the epiphysis was nearly perpendicular to the axis of the flipper, and occupied the space between the DEE and the DJ, thus forming, approximately, between one half and two thirds of the distal radius's surface cranial to the DEE (Fig. 10). The cranial articular facet of the distal radial epiphysis slanted proximocranially from the DJ to the cranial margin of the radial epiphysis, occupying approximately one third to one half of the space cranial to the DEE.

Like the radius, the distal epiphysis of the ulna was divided into two articular surfaces and three articular facets by a DEE and DJ. These facets are herein defined as the "cranial articular facet," "middle articular facet," and "caudal articular facet" of the distal ulnar epiphysis. The DJ of the ulna was located caudal to the DEE and separated the middle and caudal articular facets of the caudal surface of the epiphysis. The articular surface of the ulna

cranial to the DEE contained only one facet, the cranial articular facet, which sloped proximocranially from the ulna's DEE to its cranial edge. The middle articular facet of the ulna was nearly perpendicular to the axis of the flipper and occupied the space between the DEE and the DJ. The DJ of the ulna was more acute than in the radius; in some specimens, the angle between the caudal and middle articular facets of the ulna approached 90°. The caudal articular facet of the ulna was flat or slightly concave and sloped proximocaudally from the DJ to the caudal edge of the ulna.

Each proximal row carpal bone articulated with the radius or ulna cranial or caudal to a DEE; no carpal bone extended across a DEE to articulate with two articular surfaces. The carpal bone of the proximal row located cranial to the DEE of the radius was identified as the "radiale." The carpal bone of the proximal row located between the DEE of the radius and the DEE of the ulna was identified as the "intermedium." The "ulnare" lay caudal to the DEE of the ulna, and articulated with the middle articular facet of the ulna.

Carpal three was centered between metacarpals two and three, articulating with the caudal and cranial surfaces, respectively, of the proximal ends of these bones. It was roughly lozenge shaped with a proximal surface composed of two facets, the cranial of which articulated with the radiale, and the caudal with the intermedium. The caudal surface of carpal three articulated with carpal four-plus-five.

Carpal four-plus-five was similarly centered between the proximal ends of metacarpals three and four. It articulated with the proximocaudal surface of metacarpal three and the proximocranial surface of metacarpal four. It did not articulate with metacarpal five. The cranial surface of carpal four-plus-five articulated with the caudal surface of carpal three. The proximal surface had a cranial facet that articulated with the intermedium, and a caudal facet that articulated with the ulnare.

Additional carpal bones

Twenty seven percent (n = 14) of the carpi radiographed contained more than five bones, and 40% (n = 22) of the carpi contained one of the following additional carpal bones: second radiale, carpal two, accessory carpal (Table 2). Fifty-four percent (n = 29) of the carpi contained bones which appeared to arise from the fusion of one of these additional carpal bones with another bone.

The radiale was present in a wide spectrum of shapes and sizes. This bone was located in the space bordered by metacarpals one and two distally, carpal three distocaudally, the intermedium proximocaudally, and proximally by the radius's DEE, and middle and cranial articular facets. In 35% (n = 19) of the flippers a large radiale (r) (Figs. 10, 13), often trapezoidal or triangular in shape, filled the region between the DEE and the DJ of the radius, and extended beyond the DJ towards the cranial margin of the radius and

metacarpal one. Thirty-one percent (n = 17) of the flippers contained a small radiale (r') (Fig. 11) which did not extend cranially beyond the DJ of the radius, thus articulating proximally with only the middle articular facet and DEE of the distal radius. In 14 of these 17 flippers, a prominent additional bone, the "second radiale" (r"), was located immediately cranial to a small radiale (r') (Figs. 11, 14). The other three of these 17 carpi had a non-osseous gap cranial to the small radiale (r') in the location of the second radiale (r"). Eighteen other flippers contained a "dumbbell" shaped bone, which looked like a small radiale (r') caudally joined to the second radiale (r") cranially (Fig. 12). The constriction in this bone was adjacent to the DJ of the radius. In none of these cases did the second radiale or the cranial lobe of the dumbbell extend cranial to the cranial border of the radius or metacarpal one.

In one dolphin (2.78 m TL, male) an additional bone was observed between the small radiale (r') and metacarpal one in both flippers (Fig. 11). It was located caudal to the axis of metacarpal one. Thirteen additional flippers contained a metacarpal one with a proximocaudal extension located caudal to its long axis. In four of these, the extension was finger-like. In the other nine, the extension was expanded proximocaudally but constricted at its junction with metacarpal one (Fig. 12). Topographically this additional bony element, free or apparently fused with metacarpal one, was located in a position relative to metacarpal one that was comparable to the position of

carpal three relative to metacarpal two: this bone was thus tentatively identified as carpal two.

An ossified accessory carpal bone was present in six flippers, all of which were from female dolphins longer than 2.3 m (Fig. 9). Two of these flippers contained a thick, slightly curved bar-like ossification that paralleled the distal articular facet of the ulna. In the other four flippers, the accessory carpal bone was present as a tear-drop or an apostrophe-shaped ossification at the distal end of the caudal articular epiphysis of the ulna. No evidence was found of fusions between the accessory carpal bone and the ulna.

Evidence of a centrale was located in the left flipper of a 2.37 m female dolphin (Fig. 12). This skeletal element was represented by a stout, squarish ossified extension of the proximal third of the cranial surface of the ulnare: there was a comparable reduction in the intermedium. In all other flippers the cranial surface of the ulnare was relatively smooth.

A possible carpal five was identified in the left flipper of a 2.38 m male dolphin (Fig. 13). In this flipper, carpal four-plus-five had an additional caudal extension. The caudal osseous projection of this bone was smaller than the main cranial portion. Although there was no narrowing or constriction between the two portions of this bone, the larger cranial portion occupied the entire area filled by carpal four-plus-five in other dolphins. The ulnare had undergone a complementary reduction in its size, losing its articulation with metacarpal four seen in other flippers.

In addition to the composite bones described above, the left flipper of a 2.32 m female contained a large bone which filled the space occupied by carpal three and the small radiale (r') in other flippers (Fig. 14). Altogether, 54% (n = 29) of the flippers in this study contained large, composite bones which appeared to be the result of coalescence of adjacent ossifications.

Dissection

In the dissection of four flippers (Table 1), each carpal bone was surrounded by a smooth layer of cartilage. These surrounding cartilages were clearly delineated from one another by distinct grooves and layers of fibrous tissue, and less commonly, by a distinct gap between the smooth cartilage surfaces. In the majority of the carpal joints, the two adjacent cartilages were knit together by a thin layer of fibrous tissue. The surfaces of the second carpal and the cranial facet of the proximal surface of metacarpal two were separated by a gap. In the left flipper of one dolphin individual fibers were especially apparent between the intermedium and the adjacent carpal bones. The borders of the ulnare and carpal four-plus-five were emphasized by slight depressions in the fibrous tissue between the cartilage surfaces of the carpals. These depressions did not extend through the flipper from the medial to the lateral surface. Separation of the cartilage surfaces by placing a scalpel in these depressions revealed densely packed fibrous tissue.

Manual manipulation revealed a relatively rigid carpus with little joint motion.

One of the four flippers dissected contained a small radiale bone (r') and the second radiale bone (r") separated by a fibro-cartilage joint that passed completely through the carpus from the lateral to the medial surface. Two flippers contained a dumbbell-shaped radiale in which a bony isthmus connected two larger ossified areas that lay in the locations of the small radiale (r') and second radiale (r"). This bony union extended all the way through the flipper, being visible on both the lateral and medial surfaces. One flipper contained a large radiale (r), with its cranial edge between the DJ and the cranial edge of the radius and metacarpal one. The space cranial to the radiale (r), that appeared in a radiograph as a non-osseous gap, contained cartilage attatched to its cranial edge.

Three of the four flippers dissected contained a carpal two caudal to the axis of metacarpal one. This element was most pronounced in the right flipper of a 2.78 m male dolphin, where it was clearly identifiable on the radiograph, as a 15 mm x 10 mm bone (Fig. 11). In dissection, this carpal two was prominent and extended through the full width of the flipper from lateral to medial. It was smaller than the other carpal bones of the distal row, and resembled the lozenge shape of carpal three. The cartilage layer around this bone was slightly thicker than the cartilage layer around the other bones of the carpus. It articulated proximally with the caudal section of a dumbbell

shaped radiale, the proximocaudal surface of metacarpal one, the proximocranial surface of metacarpal two, and its thin caudal end just reached carpal three.

A cartilaginous carpal two was seen in each of the left flippers of two female dolphins (2.49 - 2.49 m). It was roughly triangular in shape, and was much smaller than the carpal two described above, but with similar topographical relationships.

The accessory carpal bone was present in all four of the flippers dissected. This skeletal element was present as a cartilaginous structure that was roughly triangular with rounded corners. The cranial edge of this skeletal element was slightly convex and matched the orientation of the caudal articular facet of the ulna, with which it articulated (Fig. 9). The distal surface of the accessory carpal bone was nearly perpendicular to the axis of the flipper, extending caudally from the caudal edge of the ulna to near the caudal edge of the flipper. The proximal surface of the accessory carpal bone sloped proximocranially from its caudal end to the caudal articular facet of the ulna.

Statistical analysis of the number of carpal bones

The majority of the flippers (74%, n = 40) contained five carpal bones, 13% (n = 7) of the flippers had six bones, and 13% (n = 7) had seven carpal bones. There was no difference between the number of bones in the left and

right flippers in this sample, although in one 2.78 m male, the right flipper contained six bones (including a dumbbell radiale), and the left flipper contained seven bones (including a separate small radiale (r') and second radiale (r")). Female dolphins had a significantly ($p = 0.001$) higher number of carpal bones (mean = 5.54) than male dolphins (mean = 5.25).

Table 2. Frequency of occurrence of individual carpal bones, seen on radiographs, by total body length and sex

Length (m)*	2.0 - 2.09	2.1 - 2.19	2.3 - 2.39	2.4 - 2.49	2.5 - 2.59	2.6 - 2.69	2.7 - 2.79	2.9-2.99	Total
# of flippers	6 M, 2 F	6 M, 2 F	2 M, 12 F	2 M, 8 F	4 M, 2 F	4 M, 0 F	2 M, 0 F	2 M, 0 F	28 M, 26 F
Bone									
radiale									
large (r)	3 M, 0 F	4 M, 0 F	2 M, 4 F	0 M, 0 F	4 M, 2 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	13 M, 6 F
dumbbell	0 M, 2 F	2 M, 2 F	0 M, 5 F	2 M, 2 F	0 M, 0 F	2 M, 0 F	1 M, 0 F	0 M, 0 F	7 M, 11 F
r' and r"	0 M, 0 F	0 M, 0 F	0 M, 3 F[b]	0 M, 6 F	0 M, 0 F	2 M, 0 F	1 M, 0 F	2 M, 0 F	5 M, 9 F[b]
small (r')	3 M, 0 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	3 M, 0 F
ulnare	6 M, 2 F	6 M, 2 F	2 M[a], 12 F[c]	2 M, 8 F	4 M, 2 F	4 M, 0 F	2 M, 0 F	2 M, 0 F	28 M[a], 26 F[c]
intermedium	6 M, 2 F	6 M, 2 F	2 M[a], 12 F	2 M, 8 F	4 M, 2 F	4 M, 0 F	2 M, 0 F	2 M, 0 F	28 M[a], 26 F
accessory	0 M, 0 F	0 M, 0 F	0 M, 2 F	0 M, 4 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	0 M, 6 F
carpal 2									
free	0 M, 0 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	0 M, 0 F	2 M, 0 F	0 M, 0 F	2 M, 0 F
+ Mc I	0 M, 0 F	1 M, 0 F	0 M, 4 F	0 M, 4 F	1 M, 0 F	2 M, 0 F	0 F, 0 F	0 M, 0 F	5 M, 8 F
carpal 3	6 M, 2 F	6 M, 2 F	2 M, 12 F[b]	2 M, 8 F	4 M, 2 F	4 M, 0 F	2 M, 0 F	2 M, 0 F	28 M, 26 F[b]
carpal 4+5	6 M, 2 F	6 M, 2 F	2 M[a], 12 F	2 M, 8 F	4 M, 2 F	4 M, 0 F	2 M, 0 F	2 M, 0 F	28 M[a], 26 F

*No specimens were available in the 2.2-2.29 m or 2.8-2.89 m length classes. m = meter, M = male, F = female

a) The left flipper of GA 571 had a reduced ulnare, a caudally expanded intermedium, and a carpal four-plus-five with an additional caudal extension (tentatively identified as carpal five).

b) The left flipper of PO 158 had a single bone in the location of the small radiale (r') and carpal three.

c) The left flipper of PO 180 contained an ulnare with a finger-like extension on its proximal edge (tentatively identified as a centrale).

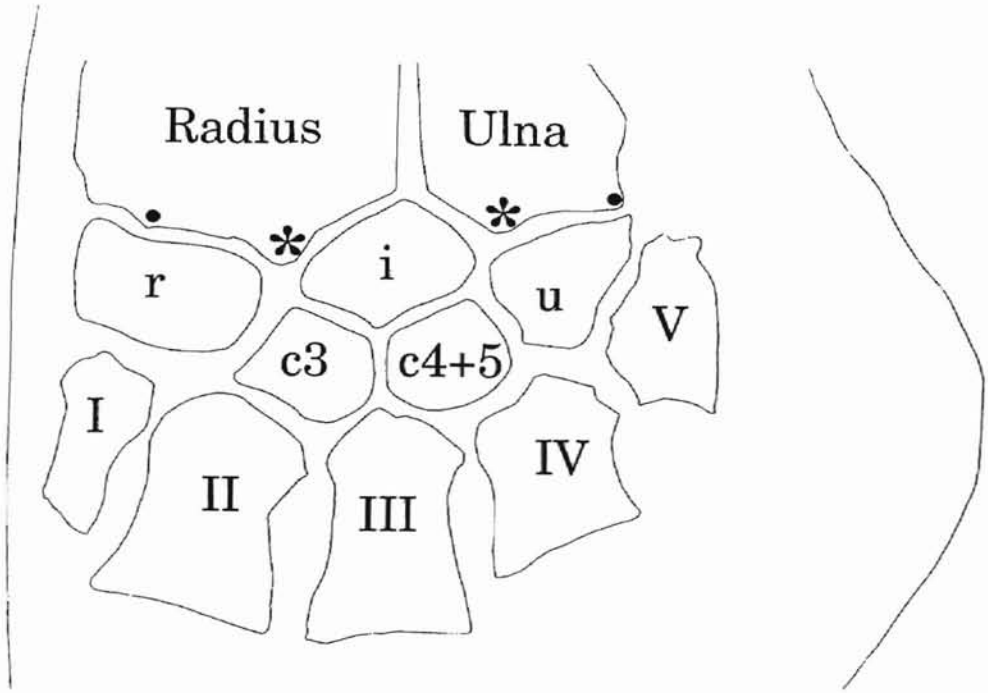

Figure 10. Lateral sketch from radiograph of the left carpus of a mature male bottlenose dolphin (Tursiops truncatus, 2.7 m TL) showing the arrangement of the bones in a five-bone carpus. The distal epiphysis of both the radius and the ulna is divided by: * - the distal epiphyseal eminence (DEE) and • - the distal junction the (DJ), which together divide the distal articular surface into three facets.

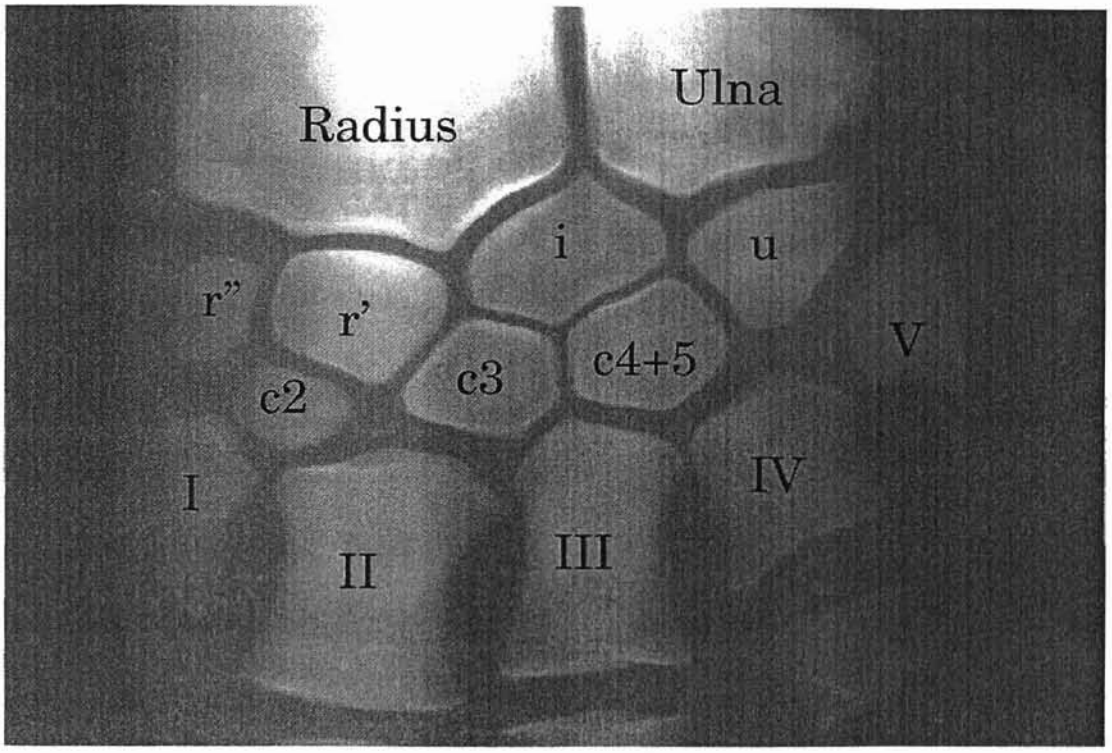

Figure 11. Mediolateral radiograph of the seven carpal bones in the left carpus of a mature male bottlenose dolphin (Tursiops truncatus, 2.78 m TL). The additional carpal bones in this individual were: r" – second radiale and c2 – carpal two.

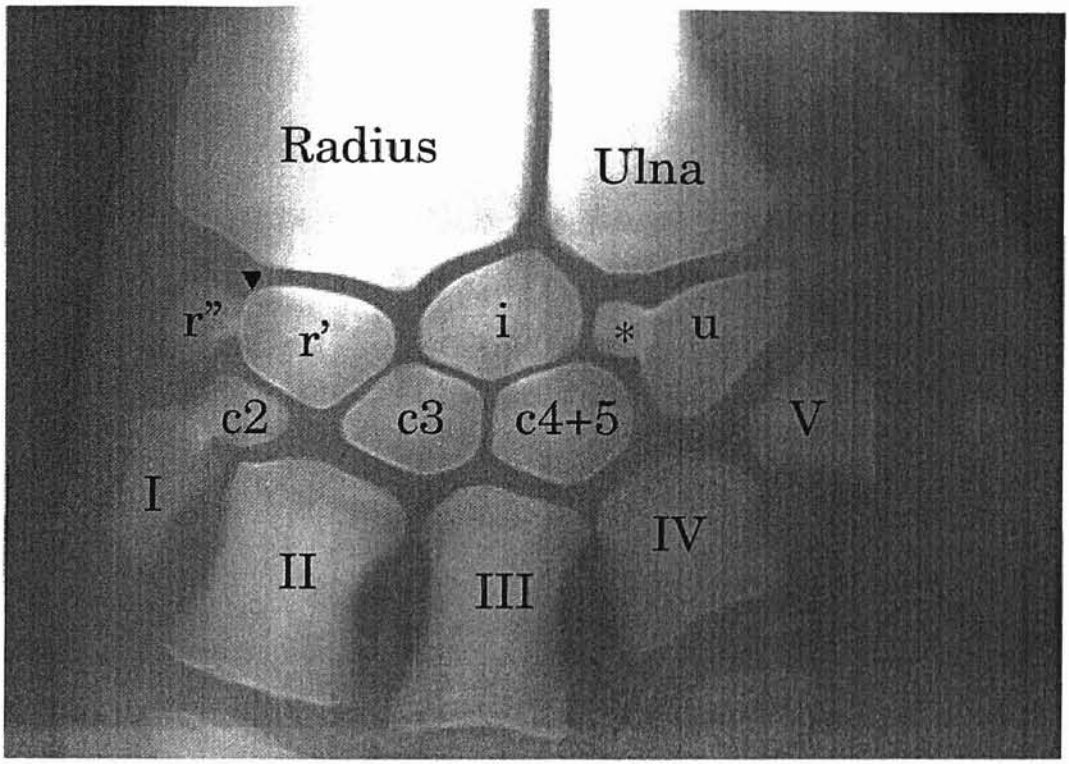

Figure 12. Mediolateral radiograph of the five carpal bones in the left carpus of a mature female bottlenose dolphin (Tursiops truncatus, 2.37 m TL): r'/r" – dumbbell-shaped bone with isthmus (arrow head), i - reduced intermedium, c2 - carpal two portion of composite bone, I - metacarpal one portion of composite bone, * - osseous cranial extension to ulnare.

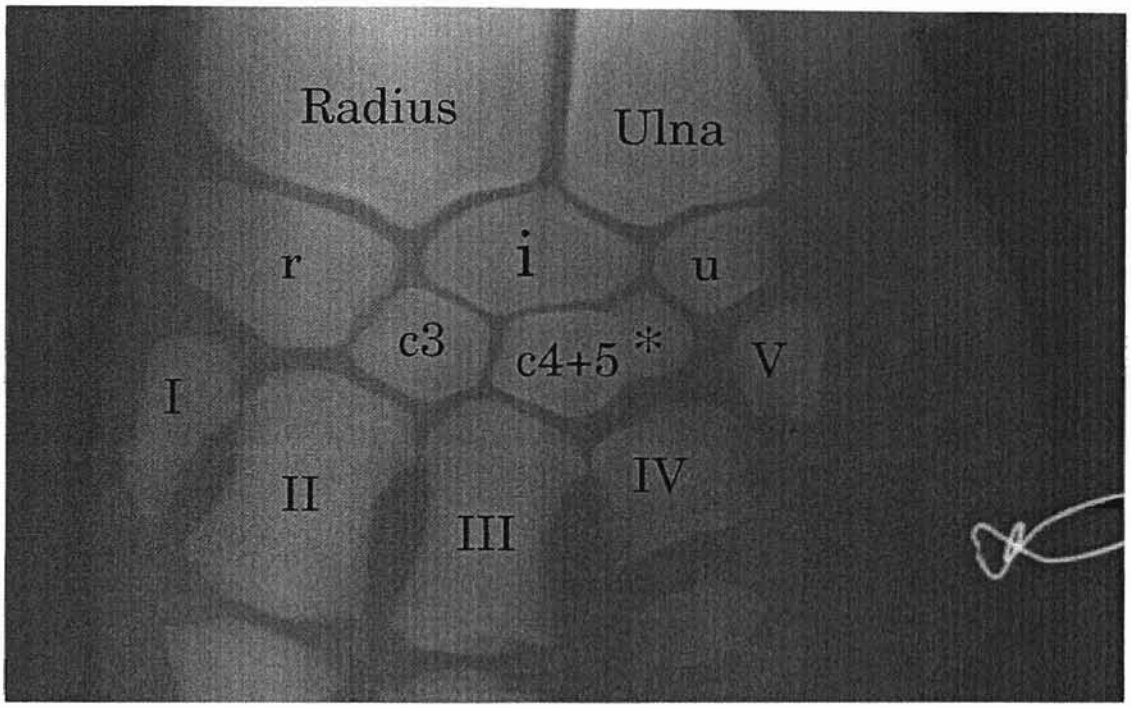

Figure 13. Mediolateral radiograph of the five bones in the left carpus of a mature male bottlenose dolphin (Tursiops truncatus, 2.38 m TL): i - caudally extended intermedium, u - reduced ulnare, c4+5* - carpal four-plus-five with caudal extension.

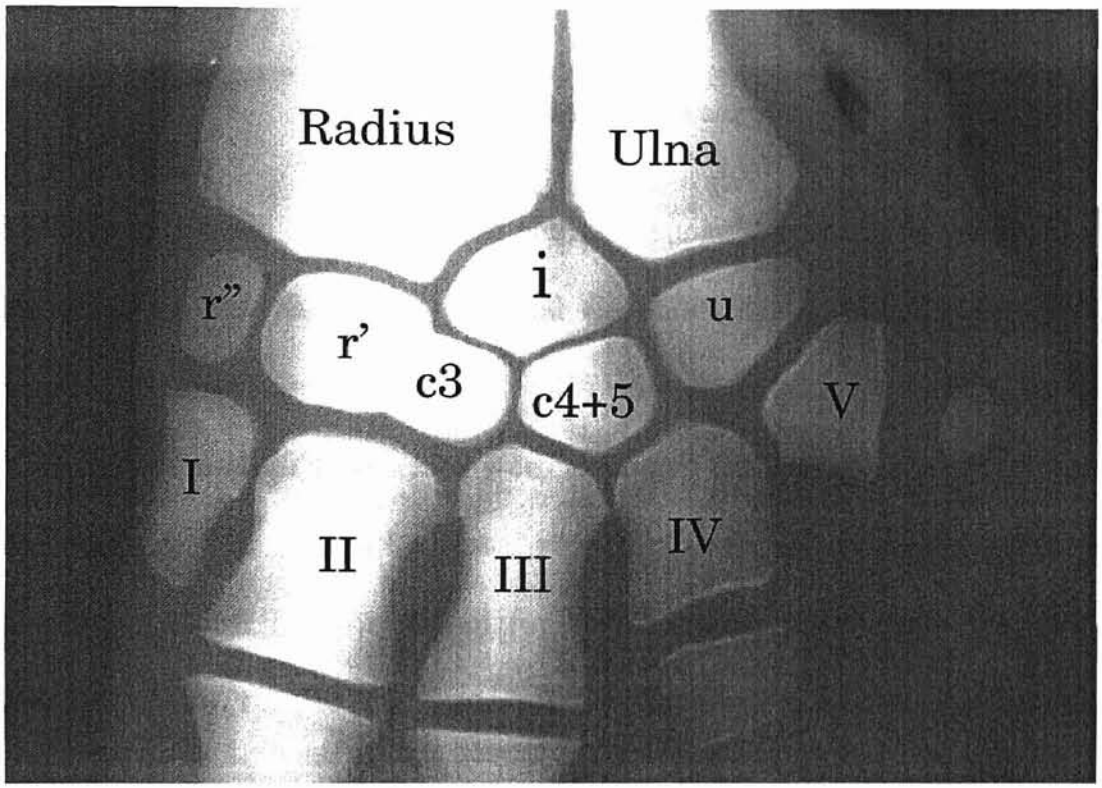

Figure 14. Mediolateral radiograph of the five bones in the left carpus of a mature female bottlenose dolphin (Tursiops truncatus, 2.32 m TL) with a composite bone arising from fusion between the proximal and distal row: r" - second radiale, r' - radiale portion of composite bone, c3 - carpal three portion of composite bone.

Chapter 5

DISCUSSION

Number of bones in the carpus

In this study, the carpus of the bottlenose dolphin was variable, with the number of carpal bones observed via radiography ranging from five to seven. The most common (74%, n = 40) number of bones was five, as stated in the hypothesis. These bones were arranged into a proximal row (radiale, intermedium, ulnare), and a distal row (carpal three, carpal four-plus-five), as has been previously reported for bottlenose dolphins from the Atlantic (Rommel 1990), Pacific (Fitzgerald 1970) and Gulf of Mexico (Schwab 1988, Holmes 1993) coasts of the United States. These are the same five carpal bones, arranged in the same topography, as found in other delphinids such as the false killer whale (Gihr et al. 1982) and striped dolphin (Calzada and Aguilar 1996), and in non-delphinid odontocetes such as the sperm whale (Flower 1868).

Twenty-six percent (n = 14) of the flippers examined in this study contained additional bones. This variation appeared to be due to two conditions: the ossification of other canonical carpal bones, and the

ossification of additional carpal bones that were variable in their appearance.

Seven carpi contained six bones. In one of these six-bone carpi, the additional bone was carpal two. This carpal pattern has previously been reported in bottlenose dolphins from the western Atlantic (Rommel 1990) and the Gulf of Mexico (Marshall et al. 1991). Delphinids such as the spinner dolphin (Calzada and Aguilar, 1996) and other odontocetes such as the La Plata dolphin, Pontoporia blainvillei, (Pilleri and Gihr 1976b) have also been reported with these six carpal bones. In the other six of the six-bone carpi from this current study, the additional bone was the second radiale (r"). This carpal bone has previously been reported in bottlenose dolphins from the Gulf of Mexico (Marshall et al. 1991, Watson et al. 1994) and from specimens in European museums (Leboucq 1889). This pattern is sometimes seen in false killer whales from Scotland (Pilleri and Gihr 1982), the Ganges susu (Pilleri and Gihr 1976a), and is the most common carpal pattern in the Indus susu (Pilleri and Gihr 1976a).

Seven carpi contained seven bones. In one of these seven-bone carpi, the two additional bones were the second radiale (r") and carpal two. A bottlenose dolphin from the western Atlantic with these two additional ossifications has previously been reported (Rommel 1990), but the ossification of carpal two was fused to metacarpal one. In the other six of the seven-bone carpi in the current study, the two additional bones were the second radiale (r") and an ossified accessory carpal bone. This pattern has not been

previously reported.

Bottlenose dolphins with four carpal bones have been collected from the Gulf of Mexico (Schwab 1988) and the western Atlantic (Rommel 1990). No dolphins with four carpal bones were seen in this study.

Additional bones in the carpus

In addition to the five bones described above in the most common carpal pattern, three other carpal bones were also identified – the second radiale (r"), carpal two, and the accessory carpal bone. The second radiale (r") was found cranial to the small radiale (r') and caudal to the cranial edge of the radius and metacarpal one. Fifty-nine percent (n = 32) of the flippers in this study had either a separate bone (26%, n = 14) or bony tissue (33%, n = 18) (cranial lobe of dumbbell) in the region of the second radiale (r"). Previous studies have identified a separate un-named bone, comparable to this second radiale (r"), in bottlenose dolphins from the Gulf of Mexico (Marshall et al. 1991, Watson et al. 1994) and in European museums (Leboucq 1889). Dumbbell-shaped radiale have been described in previous reports, in which it was concluded that the dumbbell-shaped bone arose from a fusion of the radiale and an additional bone (herein identified as the second radiale (r")) (Fitzgerald 1970, Schwab 1988, Marshall et al. 1991). Two bones in the region of the radiale (r), which sometimes fuse, also occur in other delphinids such as the spinner dolphin (Calzada and Aguilar 1996) and the

false killer whale (Pilleri et al. 1982), and in non-delphinid odontocetes such as the Ganges susu (Pilleri and Gihr 1976a).

The second radiale of the bottlenose dolphin has been identified as the radial sesamoid bone (Fitzgerald 1970), a "secondary ossification" of the radiale (Rommel 1990), and the carpal bone of a now non-existent digital ray (prepollex) (Leboucq 1889). The intracarpal location of the second radiale (r"), caudal to the cranial edge of the radius and metacarpal one, is not consistent with that of the intratendinous radial sesamoid bone in terrestrial mammals (Evans 1993). In addition, multiple ossifications in carpal bones are uncommon in mammals. One exception is the accessory carpal bone, which often bears a separate bony epiphysis on its free end in terrestrial mammals (Dyce et al. 1996). The second radiale observed in this study does not resemble the epiphyseal-like osseous rings surrounding the carpal bones in the sperm whale (Flower 1868).

Carpal two was identified as an additional carpal element in 15 flippers, but only in two of these as a free bone (both flippers from one individual). This carpal two was in a similar topographical location to the carpal two of a fetal narwhal (Eales 1954). In the La Plata dolphin (Pilleri and Gihr 1976b) and the bottlenose whale (Turner 1909) on the other hand, a distinct carpal one and a distinct carpal two were found directly proximal to their respective metacarpals. In the other 13 flippers, carpal two was present as a bony extension of metacarpal one, similar to that reported in two (18%)

Atlantic bottlenose dolphins (Rommel 1990).

In pentadactylous terrestrial mammals the distal carpal bones are named according to the metacarpal bone they are proximal to (Dyce et al. 1996). This topographical arrangement is also seen in the bottlenose whale (Turner 1909), which has five distal carpal bones, each one located immediately proximal to the metacarpal bone of the same number. In delphinids and some other odontocetes, however, the bone which is traditionally referred to as carpal three (Flower 1868, Eales 1954, Rommel 1990, Calzada and Aguilar 1996) articulates with both metacarpal three and metacarpal two. Based upon topography, it is equally likely that this carpal bone is either carpal two or carpal three. It could also be a composite bone arising from a fusion of carpals two and three. Similarly, the carpal two described in this study is located between metacarpal one and metacarpal two, and could be either carpal one, carpal two, or a composite bone containing components of both. It is also possible that these bones are correctly named according to homology, but that they have been displaced from their ancestral topographies as the cetacean carpus expanded during adaptation to the aquatic environment. Embryologic and paleontologic study would be necessary to analyze this hypothesis.

The accessory carpal bone was radiographically visible in this study as an ossification in six flippers from large dolphins (2.37 – 2.49 m), all female (and not seen in males of similar length), and all four of the flippers dissected

contained a cartilaginous accessory carpal bone. This structure, which is a canonical element in the cetacean carpus, is cartilaginous in bottlenose dolphins less than 2.3 m in length (Stepaniuk et al. 1995). In other odontocetes, this carpal element has been reported as either cartilaginous (Holmes 1993, Stepaniuk et al. 1995) or ossified (Flower 1868, Stepaniuk et al. 1995).

A single flipper in this study showed an unusual caudal extension of the bone identified as carpal four-plus-five, along with a corresponding reduction in the size and distal elongation of the ulnare. It is possible that the so-called ulnare is actually a composite bone arising from a fusion of the true ulnare and carpal five, and that the carpus of this specimen displayed a fusion of the true carpal five with the more caudal of the two bones in the distal row of the carpus. If this were true, the so-called carpal four-plus-five might be carpal four, carpal three, or a fusion of the two elements.

This study also identified one flipper with a stout finger-like extension on the cranial surface of the ulnare. This structure was tentatively identified as an ossification representing a centrale. A previous study of bottlenose dolphins (origin unknown) found three dolphins with a separate bone in this region (Fitzgerald 1970). The author explained these separate bones as a secondary ossification of the intermedium. The ulnare in these animals appeared to be less elongate than normal, a situation that contrasts with the reduced intermedium of the specimen in the current study. The "secondary

ossifications" of the ulnare (no description available) reported for bottlenose dolphins from the Atlantic coast of the United States (Rommel 1990) might also reflect this bone. It is possible that this ossification center represents a centrale which was proximally displaced as the carpus expanded during adapatation to the aquatic environment.

Dissection revealed that most of the articulations of the carpus were not synovial. Instead, the individual carpal bones were demarcated by fibrous tissue that adhered tightly to a ring of cartilage surrounding each carpal bone. This resulted in the carpus being relatively inflexible, as might be expected for a limb adapted to function as a rudder-like control surface (Felts 1966). These findings concur with a previous examination of fetal cetaceans (Leboucq 1889). Histologic examination could help to determine the tissue type(s) of the joints in the adult carpus.

The appearance of an ossification center does not, however, necessarily indicate that this structure was derived from a recognized phylogenetic skeletal element. Double loci of initial ossification centers have been detected radiographically in the developing ulnar epiphyses of a few dogs (Hare 1961). Perhaps the two ossifications in the region of the radiale in bottlenose dolphins represent a single skeletal element that is homologous to the radiale of other animals. The stiffening of the carpus as the thoracic limb adapted to the aquatic environment may have resulted in carpal cartilages being large,

and multiple ossification centers could be a physiological by-product of this adaptation.

This study identified ten possible ossification centers (Fig. 15) that may develop in the carpus of the bottlenose dolphin. Five of these ossifications (radiale, intermedium, ulnare, carpal three, and carpal four) are consistent in their appearance. The other five (accessory, second radiale, carpal two, carpal five, and centrale) are variable in their appearance.

Conclusion

In this radiographic study the flippers of female bottlenose dolphins contained a significantly ($p = 0.001$) higher number of carpal bones (mean = 5.54) than the flippers of male dolphins (mean = 5.25). There was no difference between left and right carpi in the number of bones present. The most common carpal pattern contained five carpal bones arranged in a proximal row (radiale, intermedium, ulnare) and a distal row (carpal three, carpal four-plus-five), as stated in the hypothesis for this study. This five-bone carpus was also found as a common pattern in published descriptions of many adult odontocetes. Variations from this pattern were due to the appearance of additional bones – the second radiale (r") and carpal two – or the ossification of the accessory carpal bone (the latter process only occurred in large, mature female dolphins). Some carpi had large bones which suggests that carpal bones may have undergone fusion with adjacent bones.

Study of immature specimens will be needed to help determine if the bones in the distal row of the carpus are appropriately named, and whether they have been displaced due to the adaptation of the thoracic limb to the aquatic environment.

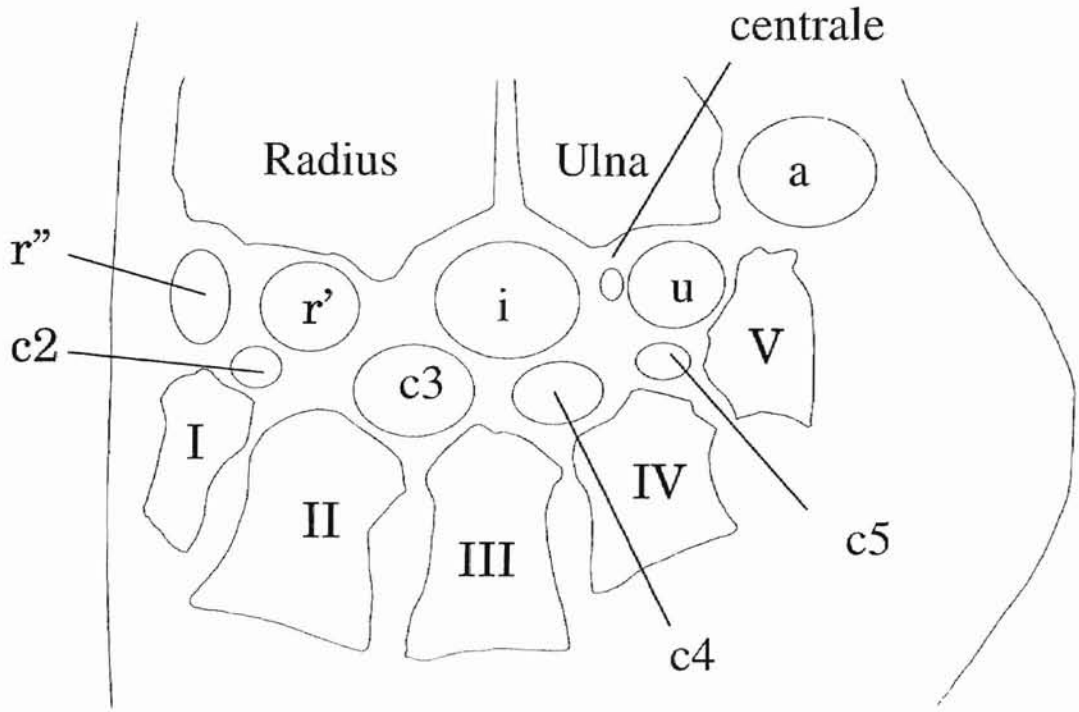

Figure 15. Sketch of the carpus of the bottlenose dolphin showing the locations of the ten possible ossification centers identified in this study.

Chapter 6

SUMMARY OF REPORT

Radiographs of 27 pairs of flippers were used to test the hypothesis that the majority of carpi in mature (≥2 m TL) Gulf of Mexico bottlenose dolphins, Tursiops truncatus, contain a proximal row with three carpal bones (radiale, intermedium, and ulnare) and a distal row with two bones (carpal three and carpal four-plus-five). The information gained from radiography was supplemented with observations from the dissection of four of these flippers.

Analysis of these radiographs revealed the most common pattern of five carpal bones. These bones were arranged into a proximal row of three bones and a distal row of two bones, as stated in the hypothesis. These same five bones, in similar topograpy, were found as a common carpal pattern in other odontocetes reported in the literature. In this study, variation from the five-bone carpal pattern was due to the ossification of additional carpal bones. Traditionally, the radiale lies in the proximal row, in the area adjacent to the cranial half of the distal radial epiphysis. In this study, four different patterns were found: 1) a single large radiale occupying this space, 2) a small radiale in the caudal two-thirds of the space, 3) the second radiale

in the cranial third and a small radiale caudally, and 4) a dumbbell-shaped bone which looked like a joining of the two radialia occupying this space. The other additional bone of the proximal row was the accessory carpal bone, which was present as a cartilaginous element in four dissected flippers, and observable as a separate carpal bone in six of the 54 flippers radiographed.

Carpal two of the distal row was identified as an additional bone immediately proximal to metacarpals one and two. It was variable in its degree of ossification and shape. It was most commonly found attached to metacarpal one.

The flippers of the female dolphins in this study contained a significantly ($p = 0.001$) higher number of carpal bones (mean = 5.54) than the flippers of male dolphins (mean = 5.25). There was no difference between left and right carpi in the number of bones present.

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VITA

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