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Roentgenographic Indicators of Skeletal Maturity in Marine Mammals (*Cetacea*)

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Abstract. A new roentgenographic classification (grading) scheme is presented for utilization in studies of skeletal development and maturation in marine mammals, particularly cetaceans. This is based on adequate description of the extent of development and maturation of the various secondary ossification centers, their eventual patterns of fusion, and subsequent remodeling with the metaphysis. The six stages are illustrated schematically and roentgenographically. This scheme may be applied to any cetacean longitudinal bone developing proximal and distal epiphyseal ossification centers.

Key words: Physis – Epiphyseal ossification – Physiologic epiphyseodesis – Skeletal maturation – Comparative anatomy

Estimation of mammalian chondro-osseous growth patterns is often quite difficult [8, 9]. Certainly in the human it has been shown that skeletal age often differs from chronological age. In many disease conditions, such as Legg-Perthes, there may be a two to three year disparity. However, despite such differences, skeletal aging has proven to be an extremely important tool in the evaluation of normal children, as well as those with various developmental disorders, both physiologic and skeletal.

Its application in other mammalian species has been limited. Knight [5] ascertained developmental details of the distal radius and ulna in elk, basing the findings on gross specimens dissected free of soft tissue; no roentgenographic correlation was undertaken. Sullivan [16] attempted to radiograph the distal radius and ulna of wild and captive foxes, finding that the physis closed at approximately eight to nine months. Other authors have studied epiphyseal roentgenographic appearances in the black bear [7], deer [6], and various dogs [3, 13–15, 17]. In most of these species physiologic epiphyseodesis was present within nine to twelve months following birth for major epiphyses, although some areas (e.g., greater trochanter, iliac crest) stayed open longer.

Work with marine mammals is even more limited. Wingate and Todd [19] described gross findings in *Sirenia*. Sumner-Smith et al. [18] roentgenographed four harp seal (*Pagophilus groenlandicus*) pups serially. Hui [4] has recently developed an epiphyseal maturation index for the common dolphin (*Tursiops truncatus*). Perrin [12] discussed fusion of the different bones in *Stenella*. Other than these latter two studies, neither of which considers the detailed stages of maturation of the secondary (epiphyseal) ossification center, there is no work on an easily reproducible, non-invasive technique, such as radiologic bone aging, that would prove efficacious in studying stranded and captive marine mammals. Such a method would appear, a priori, much simpler to utilize than estimating animal age by removal of and sectioning of teeth.

Based on our findings in large population samples of Dall's porpoise (*Phocoenoides dalli dalli*), and the short-finned pilot whale (*Globicephala macrorhynchus*), we have devised a method of grading epiphyseal and physeal maturation primarily of the distal radius and ulna. However, this method may be applied to all cetacean longitudinal bones developing secondary (epiphyseal) ossification centers. This paper describes the basic maturation scheme, as visualized roentgenographically, and does not attempt to correlate degree of maturation with chronologic animal age. Such correlation will be ascertained for individual species subsequently, since it appears that skeletal and biologic maturation takes longer than terrestrial mammals, and may differ significantly among the different cetacean species.

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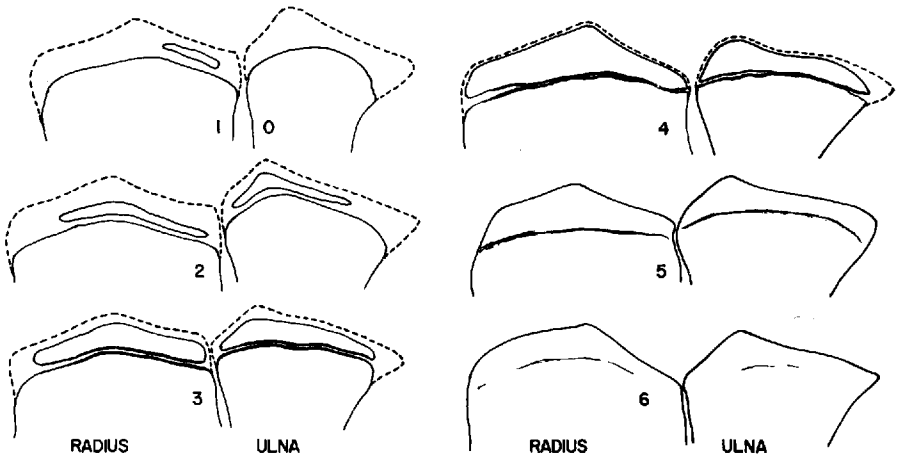


Fig. 1. Schematic of six stages of chondro-osseous transformation and maturation, as exemplified by the distal radius and ulna. See text for details of each stage. The outline of the epiphyseal cartilage is indicated by the *broken lines*. Such cartilage is always radiolucent. This pattern may be applied to the epiphyseal region of any longitudinal bone

Materials and Methods

Phocoenoides dalli dalli

Flippers were removed from 114 animals collected by one of the authors (GJC) during a collaborative investigation with NOAA(NMFS) and the Japanese salmon fisheries service. These animals were primarily collected for parasitology studies [1]. In 27 cases both flippers were removed; in the remaining 87 animals only one flipper was removed. In animals in which both flippers were available there was no significant incidence of variation in ossification patterns, with the exception of phalangeal ossification, where there was an occasional difference in timing of appearance of one of the phalangeal secondary ossification centers.

All flippers were disarticulated at the glenohumeral joint, kept frozen, and returned to the developmental laboratories at Yale, where each was radiographed on either Kodak KTL or Sakura Type C film using 55 KVP and 250 mAs. The details of these techniques for cetaceans and pinnipeds are described [2].

Globicephala macrorhynchus

Flippers were obtained from 154 animals collected primarily by James Mead and Charles Potter of the Smithsonian Institution. The majority of these were from a large herd stranded in Florida in 1977. Additional specimens obtained directly by the authors through the auspices of the Yale Marine Mammal Stranding and Study Center were also included.

As in the Dall's porpoises, significant asymmetry of the carpal region, especially the distal radius and ulna, was not observed. All flippers had been disarticulated at the glenohumeral joint, fixed in formalin (10%) or kept frozen, and roentgenographed using the aforementioned techniques.

Results

Based on the roentgenographic appearance of the secondary (epiphyseal) ossification center of these flippers a new scheme of classification has been devised. This is schematically depicted in Fig. 1. Grading is as follows:

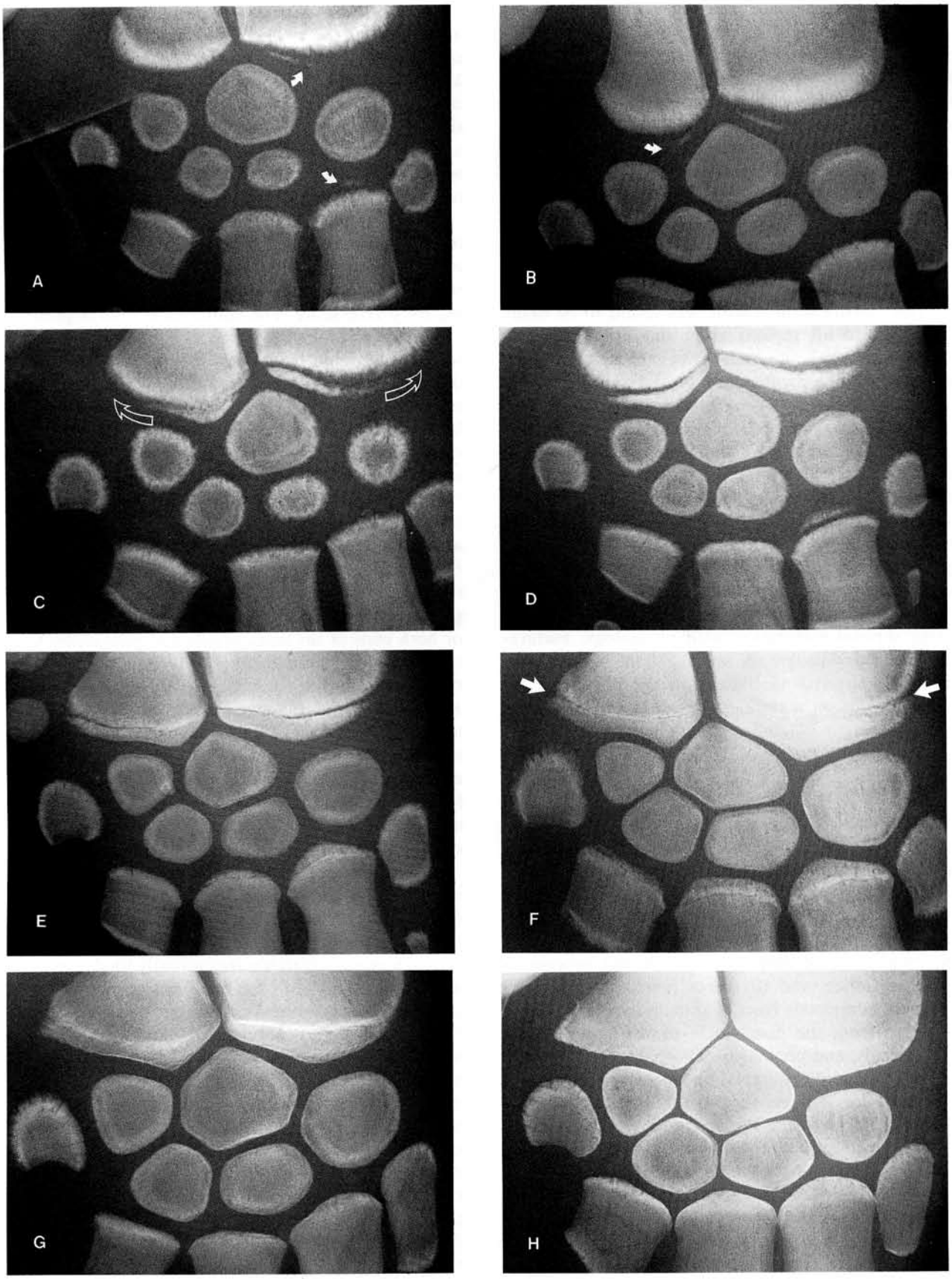
Stage 0: No secondary ossification center is present.

Stage 1: The epiphyseal ossification center has appeared, but is less than 50% of the latitudinal width of the adjacent metaphysis (Fig. 2A and B).

Stage 2. The secondary ossification center is well established, and ranges from 50% to the full width of the metaphysis. The physis is evident as a distinct, radiolucent line between the secondary center and the metaphysis (Fig. 2C and D).

Stage 3. There is thinning of the radiolucent physis, with formation of more dense juxtaphyseal osseous plates in the metaphysis and secondary ossification center (Fig. 2E).

Fig. 2A–H. Representative examples of the different stages in epiphyseal ossification center development in the wrist of the Dall's porpoise (*Phocoenoides dalli*). In each stage note the difference between the fifth metacarpal (longitudinal epiphyseal bracketed) and the other four metacarpals, which follow the characteristic development of longitudinal bones with epiphyseal ossification centers at each end. **A** Stage 1 development of distal radius and proximal second metacarpal (*arrows*). There is no epiphyseal ossification center in the distal ulna (Stage 0). **B** Onset of Stage 1 development in distal ulna. A larger epiphyseal ossification center is present in the distal radius (but is still Stage 1). Notice there is no secondary metacarpal ossification, reflecting the variability of ossification in this region. **C** Stage 2 ossification of the distal radius and ulna. Notice the irregularity of the less mature regions of the secondary ossification center, despite the increased radioulnar maturation, as they progressively mature in a mid-line to lateral fashion (*arrows*). **D** Later Stage 2 in the distal radius and ulna. In this animal metacarpal II exhibits Stage 2 ossification, and metacarpal III is just starting Stage 1. **E** Stage 3 with thinning of physeal region near the distal radioulnar articulation, but a more normal width further away. Metacarpal II exhibits Stage 3 and metacarpal III Stage 2. **F** Stage 4 with formation of osseous bridges. This process starts near the radioulnar joint, and proceeds away from this region, so that more lateral regions are still open (*arrows*). Metacarpals II and III are also Stage 4, although metacarpal IV exhibits Stage 3. **G** Stage 5 showing complete closure with a physeal "ghost", the remnant of the fused transverse juxtaphyseal osseous plates. **H** Stage 6 with remodeling of physeal "ghost". This process is almost complete in the ulna. Similar remodeling has occurred in the metacarpals


A
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Stage 4. There is evidence of closure of the physis, with the formation of trabecular osseous bridges between the secondary ossification center and the metaphysis. This varies considerably in this stage, but there need only be evidence of beginning closure to fit this stage (Fig. 2F).

Stage 5. There is complete closure of the physis, with a dense physeal "ghost" traversing the entire latitudinal width of the bone (Fig. 2G).

Stage 6. Remodeling has commenced with removal of portions of the "ghost" present in Stage 5. In this stage there is less than 50% to no evidence of the transverse physeal remnant (Fig. 2H).

This classification scheme was found to be easily applicable to all regions from the distal humerus through the phalanges. All areas exhibited the same stages of development, although different areas obviously went through these stages at different times and rates. It was not possible to routinely assess the proximal humerus, since the plane of the humeral head that best illustrates the entire contour of the physis is ninety degrees from the plane of all the remaining physes and, therefore, is very difficult to visualize by the current technique. It would be similarly difficult to visualize in the live animal. Furthermore, since it appears to close before distal radioulnar closure, it is not useful as a major indicator for correlating skeletal maturation with physiologic maturation (i.e., reproductive age onset). All the regions from the distal humerus to the tips of the phalanges are easily evident on a radiographic film, which can be taken by simply placing it under the flipper.

Discussion

We have chosen to develop this more detailed roentgenographic grading scheme because of discrepancies in Hui's methodology, which was as follows: (a) no points were scored if the epiphysis had not formed, (b) one point if the epiphysis had formed but fusion to the diaphysis had not started, (c) two points if the epiphysis and diaphysis were in the process of fusing together, and (d) three points if the physis had been completely fused. Furthermore, scores were obtained from the distal ends of the radius, ulna, metacarpals, and phalanges of each flipper. The sum of the individual epiphyseal fusion scores constituted the index for a given flipper and the sum of both flippers comprised the Flipper Index (FI) for a given animal. In cases where one of the flippers was damaged, the score for the undamaged flipper was doubled to obtain the FI for that animal.

Certainly our data shows a high degree of bilateral symmetry in the development and maturation of the secondary ossification centers in *Phocoenoides* and *Globicephala*, so Hui's assumption of doubling the value when one of the flippers was damaged was certainly valid. However, in our studies of *Globicephala* we have found an approximately 1% incidence of unilateral developmental (congenital) abnormalities. If such an abnormal flipper were to appear in any species being studied and the opposite flipper were lost through damage or not obtained, then misleading maturational data might result.

More important to our feeling of a need for a new classification methodology was the lack of adherence to appropriate biologic terminology in Hui's scheme. First, he talks about the epiphysis not forming for a zero score. The epiphysis is *always* present, but it is in a cartilaginous phase (Fig. 1). By standard roentgenographic techniques, because of the similar density of cartilage to surrounding tissues, this structure is radiolucent [9]. The true description should be the appearance of the secondary (epiphyseal) ossification center. Second, the epiphysis does not fuse to the diaphysis, but instead the secondary ossification center will eventually fuse to the metaphysis through the formation of osseous bridges through a process termed physiologic epiphyseodesis [8, 9]. Finally, Hui mentions scoring at the distal ends of the radius and ulna followed by the metacarpus and phalanges, but it is unclear whether he grades one or both ends of the metacarpals and phalanges. Certainly marine mammals appear to be unique in that they form discrete secondary ossification centers at *both* ends of the metacarpals and phalanges, unlike terrestrial mammals and semiaquatic marine mammals (e.g., pinnipeds), both of which groups (also under study in our Skeletal Development Laboratory) form a true epiphyseal ossification center in only one end. A pseudoepiphysis may form in the opposite end [8, 9].

Another area that is incomplete in Hui's study is reference to metacarpal five, or the ulnar-most metacarpal in cetaceans with less than five digital rays. In the Dall's porpoise and in several other small and large cetaceans which we are currently analyzing, this metacarpal exhibited what has been termed a "delta phalanx", but is more appropriately termed a "longitudinal epiphyseal bracket" [10]. This particular structure is a triangular-shaped, combined metaphysis and diaphysis, which is eventually bracketed proximally, longitudinally (one side only), and distally by the cartilaginous epiphysis and subsequently the secondary ossification center. One would not expect two centers to form in this particular bone. In the human, this creates an abnormal situation which can lead to disparate growth in the affected digit. However, it seems to be a normal circumstance in many of these marine mammals (*Cetacea*). This pattern of me-

tacarpal development is not addressed at all in Hui's classification scheme.

The maturation sequence in a given epiphysis thus follows typical mammalian patterns, whether the animal is terrestrial, semi-aquatic, or fully aquatic [8, 9]. The chondroepiphysis is well vascularized by structures termed cartilage canals, containing artery, veins, and a complex capillary network. Such antecedent vascularization is necessary for the formation of the secondary ossification center within the chondroepiphysis. Once the ossification center expands to the periphery of the chondro-osseous epiphysis, a discrete "subchondral" bone plate forms along the juxtaposed physis (germinal cell region). This begins to thicken prior to closure (epiphyseodesis), and is then accompanied by similar thickening in the juxtaphyseal metaphysis. This combined process of trabecular thickening causes the appearance of the transverse plates in Stage 3. The physeal cartilage subsequently calcifies even in the germinal region, which then allows trabecular bone to cross between the subchondral plates, forming small osseous bridges which coalesce to completely replace the radiolucent physis (Stage 4). Once the physis is fused, the combined subchondral plates and ossified growth plate are evident as a transverse, radio-opaque plate (Stage 5), which is slowly replaced by remodeling (Stage 6), a phenomenon that is extremely slow in certain marine mammals (*Cetacea*).

We find that this rating scheme is more concise than that proposed by Hui, and allows a much better scheme of grading of the degree of skeletal maturation. Correlation with tooth aging and with reproductive maturation is being prepared for *Phocoenoides dalli dalli*. Preliminary data suggest that when the distal radius and ulna each reach Stage 4, the animal is sexually mature. This would be comparable to the applicability of iliac crest ossification patterns in the human to determine onset of biologic (sexual) maturity. The technique is also being applied currently to an extensive study of maturation of the flippers in *Globicephala macrorhynchus*.

This method also offers a way of studying the mysticetes (baleen whales), which do not have teeth which can be used for aging techniques. Most important is that this technique may be used in the live animal, and is therefore useful to ongoing biologic studies in aquaria.

The details of complete flipper skeletal development, sequential appearance of all the secondary ossification centers, especially subsequent maturation and fusion with the metaphysis, will be reported in detail later for *Phocoenoides dalli dalli* [11], *Globicephala macrorhynchus*, and several other cetacean species. The most important correlation will be between staging and onset of biologic (sexual) maturity, which may differ, in chronologic years, for different marine mammal species.

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