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ARTICLE

Bone mineral density of the common bottlenose dolphin radius: A primary skeletal site for clinical bone densitometry and preliminary descriptive data set using archival specimens

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Abstract

We examined bone mineral density (BMD) in the pectoral flipper of the common bottlenose dolphin, *Tursiops truncatus*. These data addressed the need to define a comprehensive target site for osteodensitometric assessment and to provide a descriptive bone density data set for this species. We analyzed 388 radii from 279 bottlenose dolphins using dual energy X-ray absorptiometry (DXA), the accepted standard in human medical studies. Radii were examined for differences based on sex, age, total body length, handedness, geographical affinity, and nutritional status at death. BMD increased with age and body length (R^2 = 0.58, $p < .05$). No statistically significant differences were observed in BMD measurements for male and female dolphins ($t = -1.60$, $p > .05$) or right and left flippers ($t = -1.76$, $p > .05$). Additionally, no statistically significant differences were observed based on geographical region ($t = -0.190$, $p > .05$) or nutritional status ($F = 0.83$, $p > .05$). These results support the use of these findings as a preliminary descriptive data set for BMD in bottlenose dolphins and detail a primary skeletal site for clinical assessment of bone density for the species. As this study relies on archived museum specimens collected from dolphins at time of death, further studies regarding bone density may be better addressed using live dolphins with known

health status.

KEYWORDS

bone mineral density, BMD, dual energy X-ray absorptiometry, DXA, pectoral flipper, descriptive dataset, common bottlenose dolphin, *Tursiops truncatus*

1 | INTRODUCTION

Assessing marine mammal health is a fundamental aspect of understanding and monitoring marine ecosystems, particularly regarding anthropogenic impacts. Health assessments of common bottlenose dolphins, *Tursiops truncatus*, are critical in areas where populations show signs of epidemic disease, high mortality, and/or where ecosystems are being altered or impacted by human activities (Wells et al., 2004). Because bone mineral density (BMD) may be affected by malnutrition and limited access to food (Swift et al., 2012; Talbot et al., 1998), as well as exposure to environmental contaminants (Sonne et al., 2004; Staessen et al., 1999), the capability to determine BMD and understand how those values fit within ranges observed in the species could be an important tool for dolphin health assessments.

The research presented herein seeks to develop assessment capabilities through the comprehensive evaluation of bottlenose dolphin BMD and to provide a preliminary descriptive BMD data set for this species. There exists little knowledge about bone health, and specifically about BMD, of the bottlenose dolphin, and no reference data sets are available as there are for humans. Reference data sets used in human clinical settings establish a context by which an individual is compared against a normative distribution of values observed within appropriate demographic classifications in order to diagnose a patient within a range of osteoporotic categories (Bhudhikanok et al., 1996).

This study focuses on the bones of the pectoral flipper, specifically the radius, as it is the best target for diagnostic assessment on intact, live animals and is the skeletal site used in previous dolphin studies (Butti et al., 2007; Guglielmini et al., 2002). By establishing a descriptive data set using this bone, translation of findings from analyses of disarticulated specimens collected from stranded, beachcast animals to applications on live animals can be readily facilitated. Pectoral flippers are favorable targets for BMD studies as there is minimal overlying soft tissue, spongious bone composition, and dorsopalmarly flattened orientation (Lucić et al., 2010). Of the three bones of the pectoral flipper, the radius is best for osteodensitometry due to its comparatively large surface area, more regular geometry, and morphometrically identifiable location within the intact flipper (Figure 1).

Morphometrically identifiable regions of interest (ROIs) are defined as target skeletal sites used for clinical assessment of a patient. For example, a common clinical BMD measurement for the human forearm is the distal third radius (i.e., one-third radius or 33% radius), and this is defined as an ROI centered at a distance equal to one-third of the forearm length measured from the distal end of the radius (Shepherd et al., 2002). Measurements at ROIs allow clinicians to facilitate diagnostics by establishing readily identifiable locations by which to compare individuals within a population or demographic subgroup. Targeted ROIs should, most importantly, have BMD values indicative of the entire assessed bone.

An extensive set of archived bottlenose dolphin radii was used to establish BMD patterns at multiple loci within the radius to support the selection of a single, target ROI for clinical osteological assessment. Intra-individual differences in BMD from paired left and right radii; differences in BMD in male and female individuals; and differences associated with residency patterns and varying nutritional status at time of death were assessed at the selected skeletal site.

2 | METHODS

2.1 | Specimens

Bone specimens were obtained from extensive collections maintained by the National Ocean Service's Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) in Charleston, South Carolina, and Mote Marine Laboratory (Mote) in Sarasota, Florida. Specimens were collected from dead, stranded bottlenose dolphins under Letters of Authorization from the National Marine Fisheries Service then macerated for soft tissue removal and archived as dry skeletal specimens. Associated data including sex, total body length, residency patterns, and nutritional status at time of death were available for subsets of the specimens. Routine necropsy procedures followed by both organizations (CCHEBR and Mote) included the collection and archival of at least one pectoral flipper from each stranded animal. Ages of individual dolphins were known either from photo-identification records from local research teams, which track an individual animal from birth (e.g., Wells, 2009), or were estimated based on examination of dentinal layers in teeth, each set of growth layers representing 1 year of life (Hohn et al., 1989).

Radii (n = 388) from 279 individual bottlenose dolphins were analyzed. BMD of the whole radius and at multiple loci within the radius was measured to establish BMD distribution patterns within the bone and to facilitate selection of an ROI indicative of the overall bone BMD. A subset of radii (n = 274) with an approximately even male-female distribution was used to investigate differences in sex. Paired left-right radii (n = 218) were available to assess bilateral variation in dolphin radius BMD (i.e., handedness). The 279 dolphins included in the data set represented animals that stranded in two distinct geographical regions, the Atlantic coastline and inland waters of South Carolina (*n* = 214) and the Gulf of Mexico coastline and inland waters near Sarasota, Florida (*n* = 66). Of these individuals, residency patterns were known for 39 individuals (*n* = 24 in Charleston, South Carolina; *n* = 15 in Sarasota Bay, Florida). Nutritional status at time of death (i.e., robust, undernourished, or emaciated) was available for 116 dolphins. Nutritional status for stranded dolphins is a qualitative assessment of body condition based on morphological observations such as depression caudal to blowhole, concavity ventrolateral to dorsal fin, and visibility of ribs (Joblon et al., 2008). Ages and total body lengths were available for 205 dolphins and used for developing descriptive BMD curves.

2.2 | DXA (dual-energy X-ray absorptiometry)

All BMD measurements were conducted on a Norland Sabre pDEXA (peripheral dual-energy X-ray absorptiometry) densitometer and analyzed with Norland Sabre Research software (Version 3.9.2; Norland Medical Systems, Fort Atkinson, WI). The Norland pDEXA unit was developed for use on the human forearm with a radiographic template to accommodate osteological research applications. Bone density measurements were made following established protocols that calculate BMD by measuring absorption of two X-ray wavelengths as they pass through the bone generating a two-dimensional areal measurement of density in g/cm^2. The software interface allows the analysis of up to five ROIs of adjustable size and shape at user-specified loci. Whole bone BMD was measured for each radius using user-defined, adjustable polygons surrounding the entire radius. BMD values were also measured in each radius at four 1-cm^2 ROIs that were readily and repeatably identifiable based on morphometric landmarks and included: the geometric center of the radius, distal-third radius along the central midline of the bone, maximum BMD value across the width at the distal-third of the radius, and at the center of the maximum distal width of the radius (Figure 2).

2.3 | Statistical analysis

The relationship between BMD of each ROI and BMD of the whole radius was analyzed using ordinary least squares (OLS) linear regression analyses to establish the statistical significance of each ROI as a predictor for BMD of the whole radius. This both supported the selection of a single ROI for all subsequent tests and demonstrated the repeatability, accuracy, and precision with which ROIs were positioned during analyses. Radii from dolphins of known sex were compared to assess differences in male and female BMD using a Welch’s two sample *t*-test. Paired left-right radii were tested for bilateral differences using a paired *t*-test.

Bone mineral density measurements cannot be interpreted in isolation. Since the same BMD measurement on a 1-year-old and 30-year-old dolphin would indicate problematic BMD in the older dolphin, but be deemed normal for the younger dolphin, BMD needs to be interpreted in context with age and total body length. To provide an age- and length-corrected BMD, Principal Component Analysis (PCA) was used to reduce the dimensions of the three biological variables (age, total body length, and BMD) to a synthetic variable (PCI). Age and total length corrected BMD values were established using PCA to investigate differences in BMD based on geographical residency patterns and nutritional status at time of death.

To evaluate the effects of confounding variables associated with ecology, ecotype, and environment, age and total length corrected BMD for individuals with established residency in two distinct geographic regions was established using principal component analysis and a Welch's *t*-test was performed to determine whether differences were observed. An analysis of variance (ANOVA) was performed to examine differences in the PCI scores for each of three categories of health status at time of death (*i.e.,* robust, undernourished, or emaciated). A post hoc

Tukey honestly significant difference (HSD) test was used to examine for any difference in means observed in pairwise comparisons amongst the three nutritional status categories to determine if decreased nutritional health at time of death is associated with decreased BMD.

As body size and maturation are major determinants of BMD (Zemel et al., 2010), the univariate relationship of BMD values and total body length, independent of age, for all dolphins in this study was examined.

3 | RESULTS

OLS regression models for pair-wise correlations between whole radius BMD and BMD at each ROI showed strong, positive linear relationships (R^2 values from 0.94 to 0.97, $p < .001$; Figure 3). The strong correlations observed support the selection of any of the ROIs as a valid skeletal target site. All subsequent analyses utilized the BMD value measured at an ROI located in the geometric center of the radius as the BMD for each respective animal. Bone density values and life history metrics for all dolphins in this study are openly available in Open Science Framework (DOI 10.17605/OSF.IO/XWF93) as an open-source descriptive reference data set for *T. truncatus* radii.

Dolphins used to study the relationship between age and BMD ranged in age from 0 to 50 years. BMD values of animals included in this study ranged from 0.3436 to 1.406 g/cm^2, with a mean of 0.8269 g/cm^2 and a standard deviation of ±0.23 g/cm^2. The best-fit line to represent the relationship between dolphin age and radius BMD is curvilinear with BMD following a rapid, nearly linear increase up to approximately 20 years of age before plateauing over the remainder of the dolphins' lifespan (Figure 4). The observed osteogenetic pattern, positive correlation of BMD with age and body size, has also been reported in humans and other mammals (Blake et al., 2000).

Differences in BMD of radii from male and female dolphins across all ages were not found to be statistically significant ($p > .05$). Mean radial BMD of the male subset was 0.8367 (SD = 0.2676 g/cm^2), vs. the female subset mean of 0.7925 (SD = 0.1770 g/cm^2).

The range of BMD values for paired left and right radii was similar (0.337–1.357 g/cm^2 and 0.319–1.406 g/cm^2, respectively). Mean values were also very similar in left and right radii (0.816 g/cm^2 and 0.807 g/cm^2, respectively). Bone mineral density was found to not differ significantly between the left and right radii ($p > .05$).

Age and total length corrected BMD values (i.e., PCI) measured in radii from dolphins with established residency patterns were compared. Median values are very similar between the two groups, with considerable overlap in the range. The two geographic group means were found to not differ statistically (*t* = −0.190, *p* > .05).

Median PCI values relative to qualitative nutritional status at time of death data were found to not differ significantly among the three groups, but there was considerable overlap. An ANOVA performed on these PCI scores failed to find significant differences among the three nutritional status conditions (*F* = 0.83, *p* > .05). A post hoc Tukey HSD test failed to show that the difference in means between the three conditions was significantly different from zero (Robust vs. Emaciated, *p* = .41; Emaciated vs. Undernourished *p* = .64; Robust vs. Undernourished *p* = .88).

Bone mineral density values were plotted against total body length to establish normative trends in bone density in relation to skeletal maturation, independent of age (Figure 5). The statistical relationship between total body length and BMD can best be described by a polynomial regression (R^2 = 0.79) where BMD increases with age until peak bone mass is reached.

4 | DISCUSSION

Using a robust set of archived skeletal specimens, we aimed to establish preliminary descriptive bone density values and a primary skeletal site for clinical bone densitometry in bottlenose dolphins. Because very little research has been conducted on BMD in dolphins, this study effectively serves as the foundation for osteodensitometry in the species. Human clinical bone density assessment relies on a vast baseline of reference data. Reference data are used to assess bone health and disease status including osteoporosis and establish age-based normative distributions of BMD for males and females of various populations (Bhudhikanok et al., 1996). These applications target specific loci in skeletal sites and cover an array of body regions to address various clinical constraints and medical contexts. At each skeletal site, specific morphometrically identified regions of interest (ROI) are targeted. Considerable effort has been devoted to characterizing the most suitable bone and ROI for skeletal analysis in a manner that will facilitate application to live bottlenose dolphins rather than being limited to the assessment of archived skeletal specimens and bones collected during postmortem examination.

Establishing a descriptive BMD data set based on a readily identifiable ROI using morphometrics fosters a smooth transition into next generation diagnostics. This approach, and the selection of any of the targeted ROIs, is justified by the strong statistical significance of the relationship between the BMD of each ROI and the BMD of the whole radius. The decision to select the geometric center of the radius as the definitive BMD target was multifaceted: (1) the geometric center ROI is more easily located within a fully intact flipper, facilitating ease of field-based assessments while maximizing accuracy and precision of ROI placement; (2) proposed next generation ultrasonic bone density assessment is a through-transmission technology that requires access to both sides of the bone; and (3) bone densitometry accuracy is enhanced in a region of higher BMD compared to a region of lower BMD.

Much interest has been generated in utilizing BMD to estimate age in bottlenose dolphins (Butti et al., 2007; Guglielmini et al., 2002). The practical application of this technique would have great value in various marine mammal research settings, but it would rely heavily on BMD values at any given age having a very limited variance. Using a much larger data set than the aforementioned studies, an attempt to confirm a usable correlation between BMD and age as a predictive tool for estimating age in bottlenose dolphins, especially for adults, was unsuccessful (Powell et al., 2019). The authors noted that variation in BMD values observed at any given age may represent natural variation in the species but also could be an indicator of altered skeletal health due to factors including overall health, nutritive status, contaminant exposure, body condition, or metabolic and endocrine related disorders.

To establish a descriptive BMD data set, potentially confounding life history variables needed to be examined to justify the inclusion of all specimens. Based on information available about dolphins in the study, subsets of the total specimens were used to address specific questions. In the coalesced specimens from 279 dolphins, no statistically significant differences were observed between male and female dolphins, left and right flipper bones, dolphins from different geographical regions, or dolphins with different nutritional body condition at time of death.

In human development, well-characterized differences in males and females increase in magnitude with increasing age (Lim et al., 2004). The loss of estrogen production in menopausal women results in increased bone resorption, driven by an increase in osteoclastic activity, and consequently decreased bone mass in comparison to males of the same age (Civitelli et al., 1988). Bottlenose dolphins are long-lived marine mammals that can live to more than 63 years (Wells, 2014), an age where osteological changes are regularly observed in humans (Sözen et al., 2017). The individuals included in this study that are older than 40 years old exhibit lower than expected BMD if no late age bone loss was occurring. Specifically, four of the five oldest dolphins are females and may be exhibiting age-related bone loss, low bone density, or osteoporosis. Low sample numbers in this age class reduce the statistical power necessary to address this topic in a robust manner, but there is an apparent trend in decreased BMD observed in the oldest females compared to the oldest males in the data set. Further analysis of male/female differences in BMD exceeds the limits of the available specimens used in this study. Continued and expanded acquisition of specimens, particularly from older dolphins, may help to address this deficiency and foster investigation of age-related metabolic bone disorders in marine mammals. As we found no statistically significant difference in BMD observed in male and female dolphins in the skeletal specimens used in this study, it may not be necessary to separate these specimens by sex or to establish sex-specific descriptive curves. The lack of differences observed may in part be due to the multitude of variants inherent in postmortem collected specimens as health issues associated with the individuals' deaths may result in values that deviate from the values of healthy individuals. Future studies targeting healthy, free-ranging dolphins should investigate potential differences in males and females across all ages.

The intraindividual bilateral symmetry observed in BMD provides support for the use of either pectoral flipper to assess BMD for an individual dolphin and lends support to the use of all radii available, regardless of whether they are left or right pectoral flippers. Similarly, no difference in bone density was observed between left and right thoracic limbs in a study of Guiana dolphins, *Sotalia guianensis* (Azevedo et al., 2015). Bilateral symmetry is beneficial in a practical sense because, due to spatial constraints, routine necropsies and tissue archival protocols at many institutions include retention of partial skeletons, and under field conditions it is not always possible to access the same flipper for every animal. In future clinical applications, BMD assessments of live dolphins may be able to be conducted on either the left or right flipper, however serial measurements on a specific individual over time should be performed on the same flipper.

As long-lived apex predators, bottlenose dolphins serve as indicators of ecosystem health (Wells et al., 2004). The specimens utilized in this reference data set come from two disparate geographical regions and would thereby be impacted by different ecological and environmental factors. Comparisons between animals known to be year-round residents of two locations revealed no statistically significant differences in BMD, providing support for the total inclusion of specimens from both regions into the descriptive BMD data set.

Dolphins that were deemed emaciated at time of death were found to not differ significantly from dolphins deemed robust or undernourished at time of death. Because postmortem nutritional status assessment is a qualitative metric that describes the animal’s body condition at time of death and does not reflect the longevity of the underlying malnourishment, the data here may not be an accurate reflection of actual differences that are expected to be seen between live animals of robust and emaciated body condition. Additionally, there is the potential for introduced error as these qualitative determinations were made by multiple stranding response personnel and may have not been characterized in a standardized method. With the specimens available for the current study, it is not possible to know if the emaciated or undernourished conditions observed at time of death were acute, where changes in body condition were rapid enough that osteological changes had not occurred, or chronic, where osteological changes would be expected due to prolonged malnutrition. Given that no statistically significant differences were observed across the three postmortem nutritional status categories, all specimens regardless of body condition were included in the normative reference data set. Differences may be delineated in the future from photo-identification records where long-term undernourishment has been recorded for specific individuals in field studies (e.g., Hart et al., 2013) or where body condition can be scored quantitatively. Clinical assessments of live dolphins exhibiting varying body conditions in future studies, particularly studies of live dolphins, may elucidate an association between BMD and nutritive health status.

To the authors’ knowledge, the 389 radii utilized in this study represent the largest data set published on bone density in any wildlife or marine mammal species to date. An approximately even distribution of male and female dolphins from birth to 50 years of age is included, representing a range of life history classifications. Additionally, the data set includes individuals from two distinct geographical regions of the southeastern United States.

Diagnosis of metabolic bone disorders such as osteoporosis and low bone density (i.e., osteopenia) in humans is based on a statistical comparison to the average BMD values for a healthy, young adult at peak bone mass. The descriptive bone density reference data set established in this study will facilitate a similar diagnostic approach for bottlenose dolphins. Future studies to establish BMD values for a normal, healthy population, perhaps from live, free-ranging dolphins rather than from dead-stranded animals, would enhance the utility of such a reference data set. With a reference standard by which to compare and diagnose skeletal health in individuals, bone densitometry can be incorporated into health assessment studies of free-ranging bottlenose dolphins and on museum-archived specimens collected by marine mammal stranding response programs.

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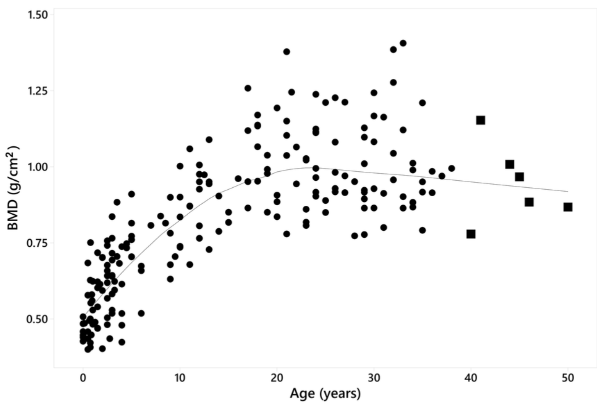
FIGURE 1 (Left to right) typical bottlenose dolphin flipper specimen; standard digital radiograph of a bottlenose dolphin flipper; and museum pectoral flipper bone specimen showing humerus (H), radius (R), and ulna (U).

FIGURE 2 Bottlenose dolphin radius (left) and pDEXA software screen capture of a typical radius BMD scan (right). pDEXA software allows for multiple user-defined ROIs on the same scan. The 5 ROIs defined for each radius pDEXA scan include: whole radius (defined by the outer margin of the entire bone), geometric center of the radius (GC), distal-third radius (D1/3), max BMD across the width at the distal-third of the radius (DMAX), and an ROI set in the center of the maximum distal width of the radius (DML).

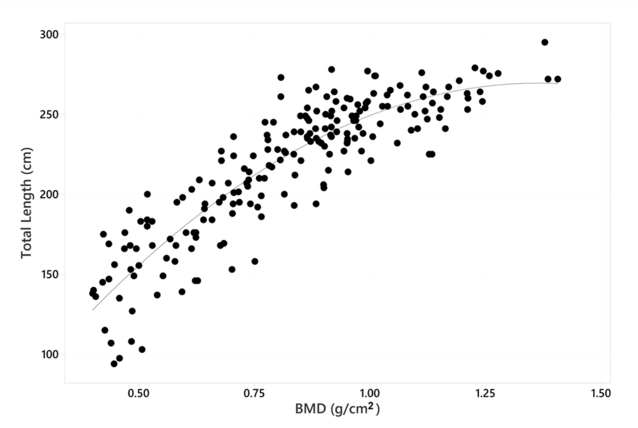
FIGURE 3 BMD values at four selected ROIs (described in Figure 2) and the whole radius BMD (RadBMD). The R^2 values range from 0.94 to 0.97 ($p < .001$), thereby supporting the selection of any ROI as a robust representation of whole radius BMD. All subsequent analyses in this study were conducted using an ROI at the geometric center of the radius, depicted in graph A.

FIGURE 4 Distribution of BMD values from 279 bottlenose dolphins. The lowess curve (locally weighted scatterplot smoother) is provided to assist in visualizing the nonparametric relationship between BMD and age as BMD increases with age up to approximately 25 years of age before slowly declining with increasing age. Dolphins 40 years old and older are highlighted (■) for emphasis.

FIGURE 5 Bone density (BMD) vs. total body length for 279 bottlenose dolphins. The polynomial equation $y = -152.76x^2 + 416.68x - 14.673$ ($R^2 = 0.7959$) represents the best fit of the series of data points and demonstrates how BMD increases with total body length as the skeleton develops during growth and maturation.



MMS_12769_4903_Fig4rev.tif



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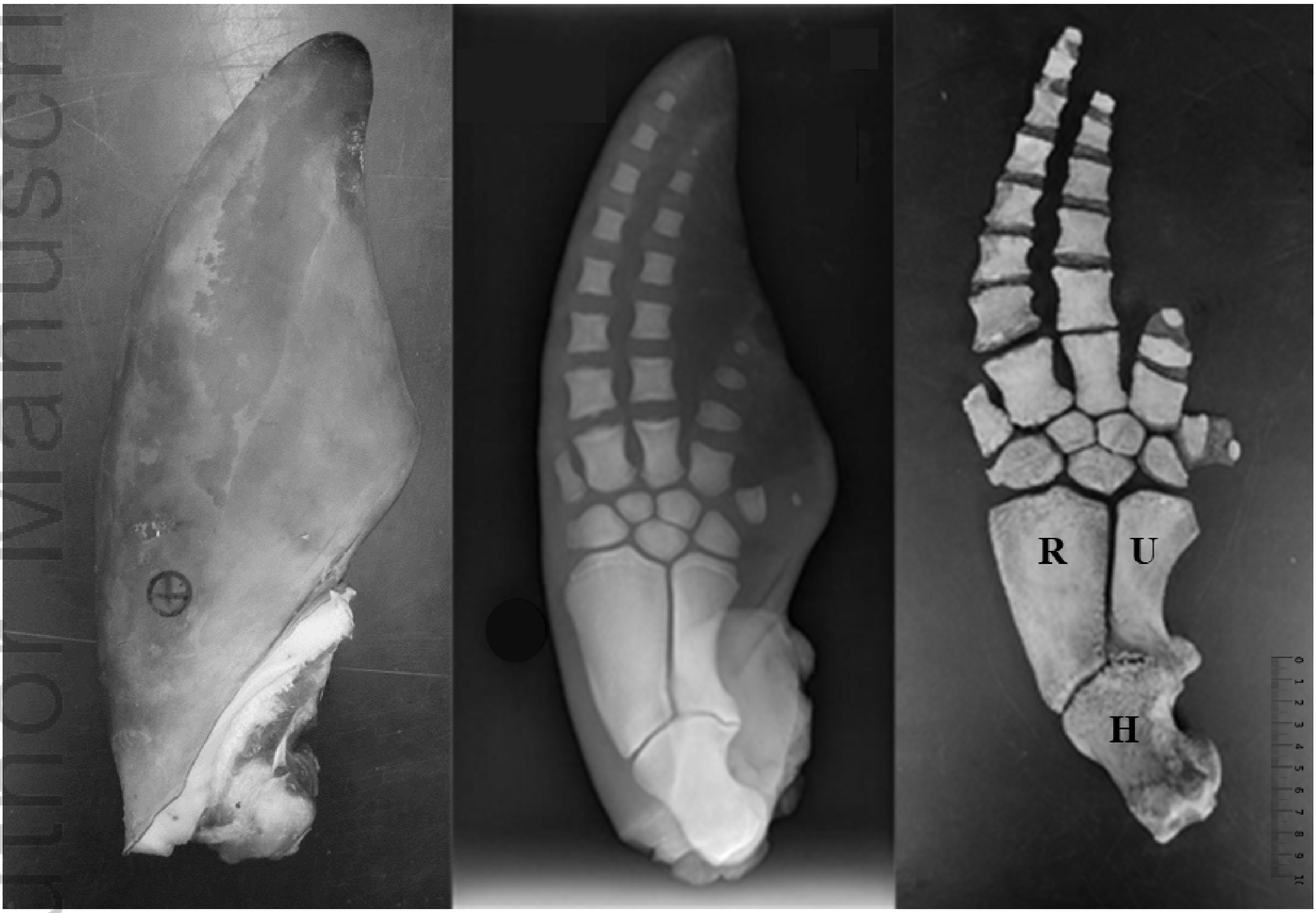
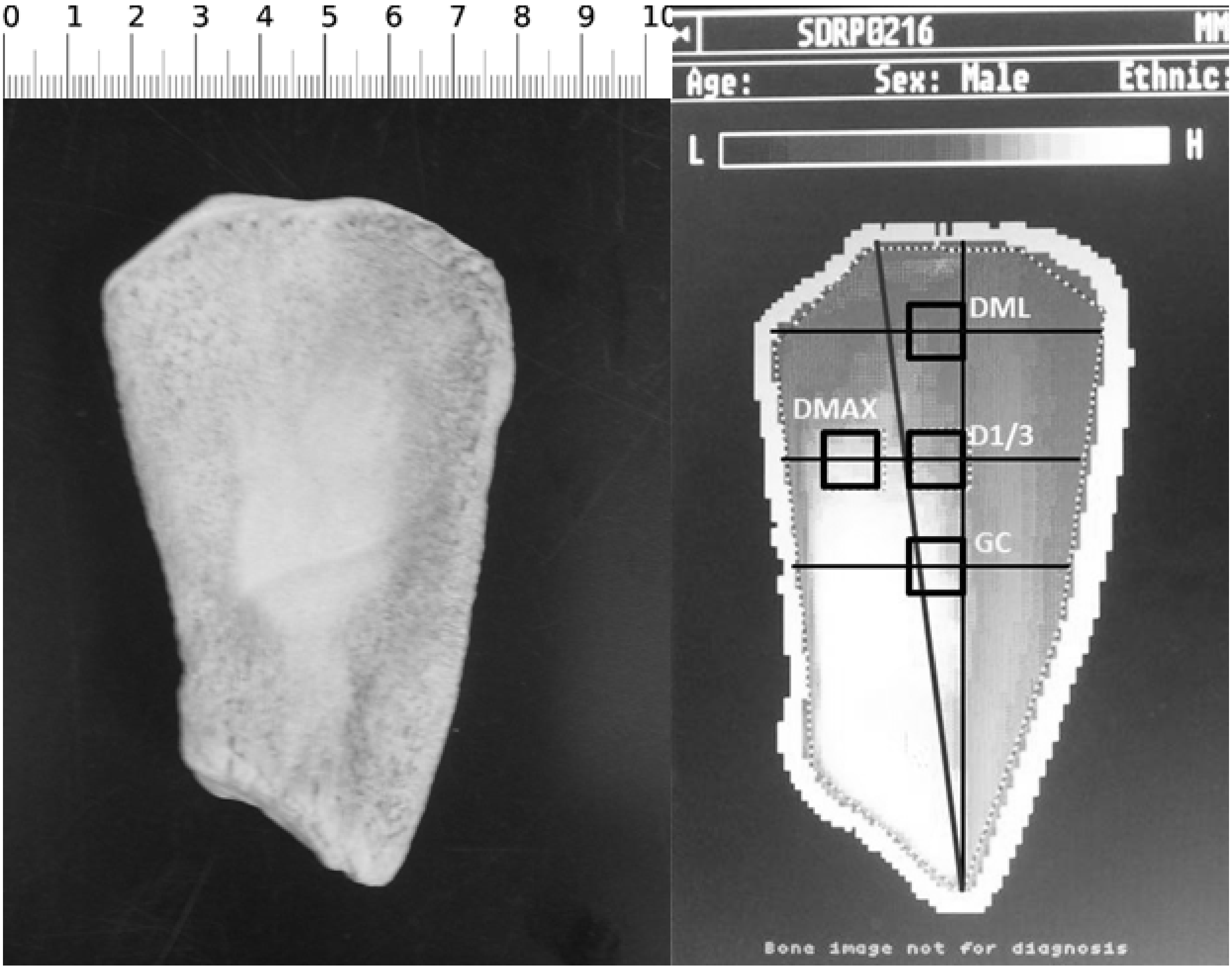


MMS_12769_4903-Figure1.tif



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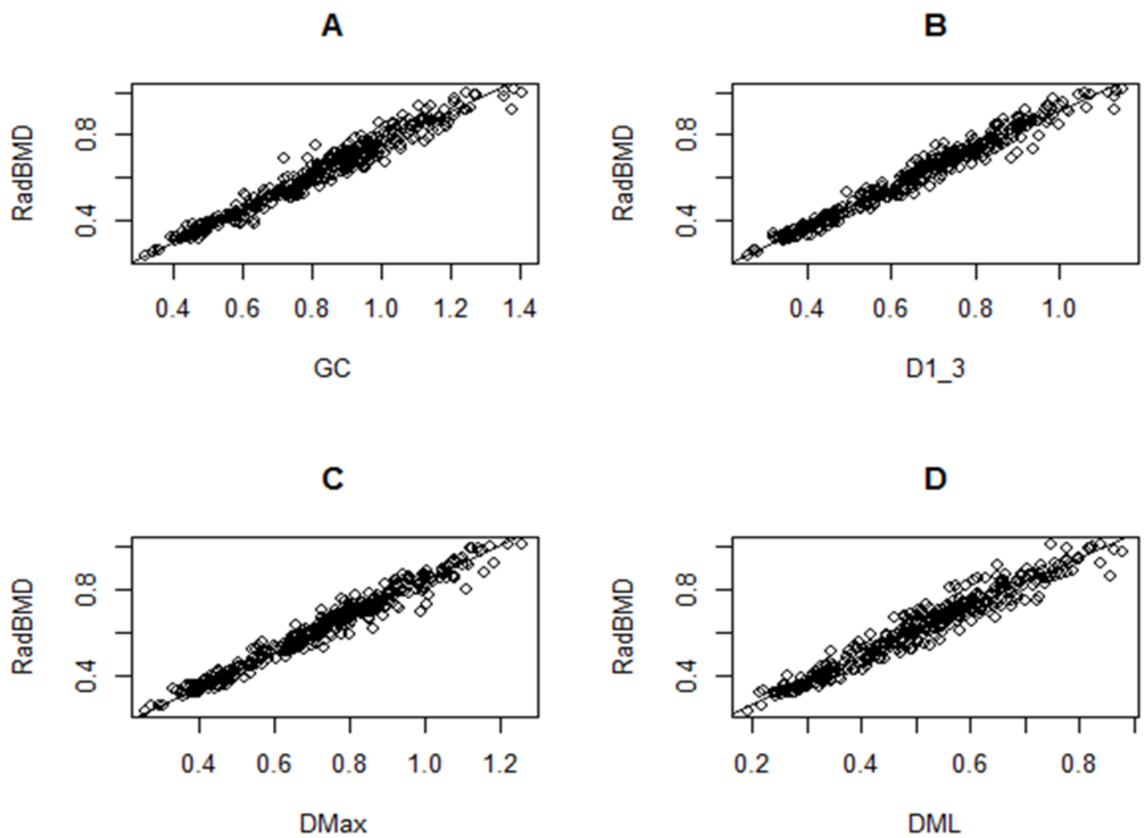

MMS_12769_4903-Figure3.tif