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# Application of dual energy X-ray absorptiometry method for small animals in measuring bone mineral density of the humerus of bottlenose dolphins (*Tursiops truncatus*) from the Adriatic Sea

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**ABSTRACT**

The measurement of bone mineral density (BMD) in dolphins was described in earlier investigations, but only by application of osteodensitometric methods used for man. The aim of this study was to test the possibilities of applying the method for small animals in the measurement of the bone mineral density in dolphins. The humeri of the right flippers of 24 bottlenose dolphins (11 males and 13 females) were analyzed using the dual energy x-ray absorptiometry (DEXA) method for measurement and analysis of the bone mineral density in small animals. The tested method can be successfully applied for measuring bone mineral density in dolphins, but the range of measured values was lower than values that resulted from using the method for man. Statistical analysis indicated positive linear correlations between bone mineral density of dolphin humerus and the total body length and age of the dolphins.

**Key words:** bottlenose dolphin, *Tursiops truncatus*, dolphin humerus, dual energy X-ray absorptiometry, bone mineral density

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## Introduction

Bone mineral density (BMD) is a clinical test for the diagnosis of bone pathology, and in animals it is mostly used in research of osteoporosis in laboratory animal models. Several investigations have been performed with the aim of determining changes to bone mineral density in a dolphin during its lifetime, and to develop alternative methods for age determination (GUGLIELMINI et al., 2002; LUCIĆ, 2006; BUTTI et al., 2007). All these investigations were carried out using the dual energy x-ray absorptiometry (DEXA) method for measuring human lumbar spines. In dolphins, no attempts were made to test other methods for measuring bone mineral density, up to now.

The macrostructure of the whale bone tissue is similar to that of terrestrial mammals, but has some typical features. All deep-diving whales have lower bone tissue density than terrestrial mammals (MAAS, 2002). The dolphin humerus is spongious bone, but some areas of denser bone tissue are also present. Bone density is lower in the central part of the bone than in the marginal parts (SEDMERA et al., 1997). Macroscopic porosity is visible even in areas of maximum bone density and it is characteristic of bones which are not important for supporting animal body mass (FELTS and SPURREL, 1965; ZYLBERBERG et al., 1998). Spongious bone has a more intensive process of bone remodeling than cortical bone. The loss of bone mass and lower bone density is characteristic of spongious bone and it can be best measured by the DEXA device (FATAYERJI et al., 1999). GUGLIELMINI et al. (2002) investigated bone mineral density in 15 striped dolphins and identified differences in bone mineral density, which depended on the age of animals. During growth, increase in body mass is in strong correlation with the increase in bone mineral density, both in humans and animals (BLAKE et al., 2000). Reduction of bone mass and bone density as a consequence of aging was found in humans by all methods applied. The process is more intensive and quicker in women than in men (ZIOUPOS and CURREY, 1998; EBBESEN et al., 1999). Similar phenomena were described in some monkeys (POPE et al., 1989) and in beagle dogs (MARTIN et al., 1981).

General guidelines for measuring bone mineral density in animals were given by GRIER et al. (1996) and TURNER (2001). The anatomical positions of bones in animals are different than in humans. Because of this, correct body position towards the source of radiation and proper selection of the search area (ROI, region of interest) are very important for all measurement techniques. In the osteodensitometry of small animals a modification of the method has been developed, which regulates the strength and direction of X-ray radiation. A corresponding ultrahigh resolution computer program has also been developed too, and is applicable for rats, rabbits, cats, dogs and some reptiles (NAGY and CLAIRE, 2000; ZOTTI et al., 2004).

Since different methods and devices are designed for osteodensitometry, in this research we describe the differences in protocols and results (bone mineral content and density) that can be expected when different methods are applied for measuring bone mineral density in dolphins. The primary objective of this research was to test the possibilities of applying the DEXA osteodenzitometric method for small animals in measuring bone mineral density in dolphins.

## Materials and methods

Bone preparations from the right flipper of 24 bottlenose dolphins (*Tursiops truncatus*), 11 males and 13 females, were used in this study. All the dolphins investigated were found dead on the Croatian part of the Adriatic coast during the period from 1997 to 2002. Bottlenose dolphins are strictly protected by Croatian law, and their number in the Adriatic Sea is estimated at over 220 individuals (GOMERČIĆ et al., 1998).Total body length was measured between the top of the upper jaw and the bottom of the median incisure of the tail flukes (PERRIN, 1975). Dolphin age was determined by counting annual layers of tooth dentin (MYRICK, 1988; MOLINA and OPORTO 1993). Bone mineral density of the right flipper was measured by a Hologic QDR-4000 osteodensitometer (S/N 55428; Hologic Inc., Waltham, MA, USA). The osteodensitometer was equipped with computer programs for measuring and analyzing bone density of the human lumbar spine, human femoral bone, human shoulder, a child's spine, human femoral prosthesis, and bones of small animals (laboratory animals, dogs and cats). The last was the computer program "Rat Whole Body®" (Hologic Inc., Waltham, MA, USA), which allows high-resolution densitometry of bones of small animals. The soft tissues of the flippers were simulated by using a specially designed plate, called a lexan-plate (Acrylic Scan Platform, Hologic Inc., Waltham, MA, USA) made of pure acrylic with the same absorption intensity of X-rays as soft tissues. The bone preparation (Fig. 1) in the dorsopalmar position was placed on the lexan-plate before measuring. The area of measurement was marked at the osteodensitometric image of a fin, as the region of interest, R1 (Fig. 2). Bone mineral density (BMD) and bone mineral content (BMC) were measured in the selected area. The data were analyzed using standard statistical software, "Statistica for Windows 7.1". Descriptive statistical analysis was conducted and age, total body length, sex, BMC and BMD of the humerus were taken as statistical variables. Correlative statistical analysis was performed separately for male and female bottlenose dolphins.


Fig. 1. Bones of the right flipper with marked humerus of a bottlenose dolphin (D072, male, 10 years old)

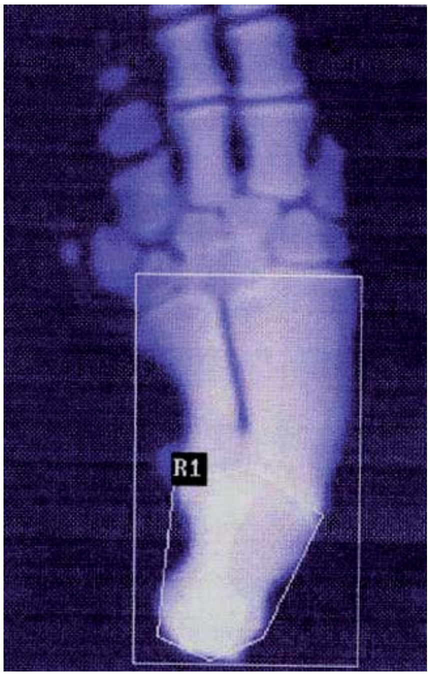


Fig. 2. Osteodensitometric image of the right flipper in the dorsopalmar projection of a bottlenose dolphin (D072, male, 10 years old) (R1, region of interest, humerus)

## Results

Total body length of 11 male dolphins was in the range from 208 cm to 312 cm, while that of 13 females was in the range from 200 cm to 288 cm. Male dolphins were estimated to be from three to 17 years old, and six of them were older than ten years. Female dolphins were estimated to be from two to 21 years old, and three of those animals were older than 20 years (Table 1).

Table 1. Bone mineral content (BMC) and bone mineral density (BMD) of male (M) and female (F) bottlenose dolphins together with their total body length and age estimated by the tooth dentine technique.

| Dolphin number* | Sex (M/F) | Age (years) | Total body length (cm) | BMC (g) | BMD (g/cm$^2$) |
|---|---|---|---|---|---|
| 22 | M | 3 | 234 | 18.49 | 0.801 |
| 32 | M | 3 | 208 | 16.77 | 0.812 |
| 72 | M | 10 | 235 | 21.81 | 0.912 |
| 19 | M | 7 | 240 | 24.01 | 0.938 |
| 40 | M | 13 | 288 | 34.99 | 0.985 |
| 23 | M | 11 | 291 | 34.21 | 0.999 |
| 64 | M | 17 | 312 | 36.45 | 1.049 |
| 80 | M | 17 | 294 | 37.00 | 1.081 |
| 36 | M | 10 | 282 | 35.61 | 1.117 |
| 62 | M | 14 | 290 | 42.13 | 1.140 |
| 28 | M | 16 | 312 | 41.92 | 1.173 |
| | | | | | |
| 16 | F | 7 | 275 | 16.45 | 0.649 |
| 63 | F | 2 | 200 | 11.94 | 0.679 |
| 57 | F | 7 | 246 | 21.47 | 0.883 |
| 54 | F | 17 | 281 | 27.07 | 0.893 |
| 35 | F | 14 | 258 | 25.05 | 0.918 |
| 51 | F | 21 | 275 | 24.46 | 0.919 |
| 20 | F | 21 | 288 | 30.97 | 0.939 |
| 38 | F | 21 | 286 | 28.72 | 0.981 |
| 41 | F | 12 | 261 | 27.03 | 1.003 |
| 66 | F | 11 | 283 | 33.45 | 1.043 |
| 39 | F | 17 | 276 | 33.74 | 1.085 |
| 25 | F | 12 | 278 | 34.35 | 1.093 |
| 17 | F | 16 | 279 | 31.50 | 1.153 |

*Individual dolphin number in the dolphin research project collection

The results of the descriptive and correlative statistical analyses are shown below. Mean values as well as the range of BMC and BMD were higher in male than in female dolphins (Fig. 3).

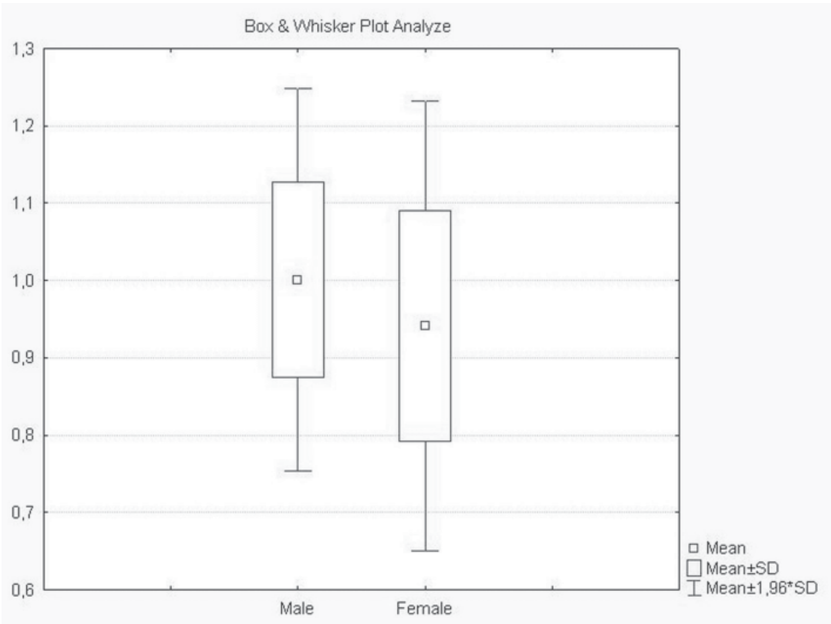


Fig. 3. Mean values, standard deviation and standard error of the bone mineral density (BMD) in males (1) and females (2) of the bottlenosed dolphins

Both estimated age and total body length of male bottlenose dolphins were positively correlated to the BMC of their humeri (correlation coefficients r = 0.88 and r = 0.95, respecively) (Table 2). Furthermore, both estimated age and total body length of male dolphins were positively correlated to BMD as well (correlation coefficients r = 0.84, Fig. 4 and r = 0.87, respectively) (Table 2).

Table 2. Correlation coefficients between all tested variables in male and female investigated bottlenose dolphins

| | Male dolphins (11) | | Female dolphins (13) | |
|---|---|---|---|---|
| | age | total body lengt | age | total body lengt |
| BMC | 0.88 | 0.95 | 0.63 | 0.75 |
| BMD | 0.84 | 0.87 | 0.53 | 0.55 |

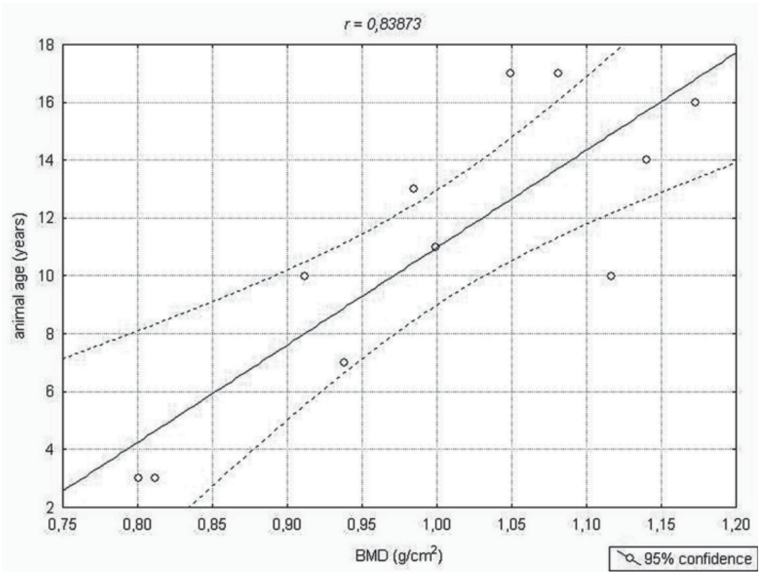


Fig. 4. Correlation between bone mineral density (BMD) and age in 11 male bottlenose dolphins

Both age and total body length of female dolphins were positively correlated with the BMC and BMD of their humeri, but the correlation coefficients were lower than in male dolphins: the correlation coefficient between the BMC of dolphin humerus and female dolphin age was 0.63, that of BMC of humerus and total body length of female dolphins was 0.75, while the correlation coefficient between BMD and total body length was 0.54 (Table 2).

The same statistical correlation test was performed in female dolphins, excluding the three oldest animals (above 21 years of age). In that case, the correlation coefficients were higher than before when all the females were included in the analysis. After this analysis, the correlation coefficient between BMC and the age of the female dolphins was 0.79 (Fig. 5), while the correlation coefficient between BMD and dolphin age was 0.73.

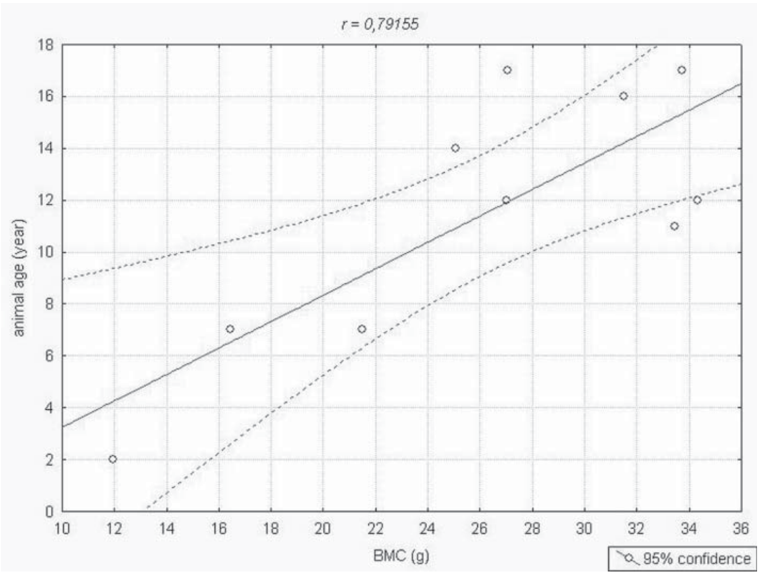


Fig. 5. Correlation between bone mineral content (BMC) and age in 10 female bottlenose dolphins (three oldest females were excluded from the group)

**Discussion**

The osteodensitometric method for small animals was developed because of the weaker resolution (especially at the edges of the bones) that was recorded when applying the human method to the small bones of animals. The difference between bone density and the density of surrounding tissue in small animals is much smaller than in man (GRIER et al., 1996). Human osteodensitometry is routinely applied mostly in humans and people of an older age. All human osteodensitometric methods were developed for those people, as well as all osteodensitometric devices, which were constructed for the adult human population. Therefore, osteodensitometry of small animals includes a small beam of X-rays given by special device, a collimator which focuses on smaller areas of measurement (GRIER et al., 1996). This method results in the higher resolution of images, but the measured values of the bone mineral density are smaller than those obtained when using the method for humans (GALA PANIAGUA et al., 1998). Unlike in people where osteodensitometry is routinely applied in the adult population, osteodensitometry in our research was applied for dolphins at different ages, which varied from only two years of age to very old animals of more than 20 years. Therefore, the range of the bone density in the investigated samples was very wide. The procedure for measuring bone mineral density in dolphins does not differ from the methods for small animals and for humans.

However, when measuring dolphin bone mineral density, only bone preparations are used because the osteodensitometers are constructed for the human body shape.

A dolphin humerus is a relatively small bone and almost completely spongious in structure, which makes it very suitable for osteodensitometry. Furthermore, dolphin flippers are dorsopalmary flattened and can be easily placed in the position for measurement where very small irregularities in sample setting could appear. Compared with the method for humans, the duration of measuring is longer than the method for small animals because of the smaller X-ray beam. The time difference between these two methods is negligible because of the small size of the dolphin humerus. The values of BMD and BMC for the dolphin humeri were lower when applying the method for small animals than with the method for humans, which is a consequence of the different amount of x-rays directed at the bone.

Previous measurements of BMD in dolphins (GUGLIELMINI et al., 2002; LUCIC, 2006; BUTTI et al., 2007) were all performed on an identical device or on devices with the similar physical characteristics, which resulted in the same or similar range of measured values. On the other hand our measurements performed by applying the method for small animals, resulted in lower measured values. In all previous investigations (GUGLIELMINI et al., 2002; LUCIC, 2006; BUTTI et al., 2007) statistical analyzes were conducted with the aim of establishing the relationship between BMD parameters and the biological properties of animals, namely the age and gender of animals, as well as with the morphometrical characteristics of animals. Similarly, in this research BMD parameters were correlated to the total body length and to the estimated age of the animals. A strong correlation between BMC or BMD and animal age or total body length was found in male dolphins. Our results are comparable with the data obtained by osteodensitometric methods for humans, which were applied on dolphins in investigations by GUGLIELMINI et al. (2002), LUCIĆ (2006) and BUTTI et al. (2007). In male dolphins, the increase in the BMC and BMD of humeri was correlated with the ageing of the animals and the increase in their total body length. Growth and ageing of dolphins increased the disposal of minerals in the bones, and therefore increased values of BMC and BMD in dolphin humeri (GUGLIELMINI et al., 2002; LUCIĆ, 2006).

Statistical analysis indicated lower correlation coefficients between the tested variables in the female dolphins compared with the males. Correlation of BMD and BMC with the total body length and animal age was lower in female dolphins than in male dolphins. The same statistical differences between males and females of bottlenose dolphins were described in the aforementioned studies (GUGLIELMINI et al., 2002; LUCIC, 2006; BUTTI et al., 2007). The gender-related impact on statistical values was probably the result of the relatively small statistical sample consisting of only 13 female dolphins. The BMC and BMD of humeri decreased in the oldest female dolphins, which was not the case in the male dolphins. That was the reason for excluding the three oldest females from the statistical correlation analysis. A stronger correlation was detected when the oldest three animals were excluded from the tested group. These data indicate a reduction in the BMD in female dolphins, which is similar to that described in women of postmenopausal age and in some other species of animals (GRYNPAS et al., 1993; LIM et al., 2004). Furthermore, results like these were also described when a much larger sample of female dolphins was included in the analysis after application of the osteodensitometric method for humans (LUCIĆ, 2006).

Statistical values and gender-based differences found in the investigated group of dolphins are completely comparable with the results of previous investigations where methods for humans were used for measuring BMC and BMD in dolphins. The method for small animals used for measuring BMD in dolphins in this investigation could be applied with the same efficacy as the method for humans. The investigation described confirmed the comparability of the statistical values regardless of the method used for measuring bone mineral density.

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**SAŽETAK**

Mjerenje mineralne gustoće kostiju dupina opisano je u ranijim istraživanjima, ali samo primjenom osteodenzitometrijske metode za čovjeka. Cilj ovog istraživanja bio je utvrditi mogućnosti za primjenu metode za male životinje u mjerenju mineralne gustoće kosti u dupina. Nadlaktične kosti desnih prsnih peraja od 24 dobra dupina (11 mužjaka i 13 ženki) istražene su DEXA (apsorpciometrija dvostrukih X-zraka) metodom za mjerenje mineralne gustoće kosti malih životinja. Istražena metoda može se uspješno primijeniti na dupinima, ali je raspon izmjerenih vrijednosti niži nego što je utvrđen primjenom metode za čovjeka na kostima dupina. Statistička je analiza pokazala pozitivnu linearnu korelaciju između mineralne gustoće nadlaktične kosti dupina i ukupne dužine tijela i dobi dupina.

**Ključne riječi:** dobri dupin, *Tursiops truncatus*, nadlaktična kost, apsorpciometrija dvostrukih rengenskih zraka, mineralna gustoća