*J. Anat.* (2007) **211**, pp639–646

doi: 10.1111/j.1469-7580.2007.00805.x

# Age estimation in the Mediterranean bottlenose dolphin *Tursiops truncatus* (Montagu 1821) by bone density of the thoracic limb

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## Abstract

The determination of age is an important step in defining the life history traits of individuals and populations. Age determination of odontocetes is mainly based on counting annual growth layer groups in the teeth. However, this useful method is always invasive, requiring the cutting of at least one tooth, and sometimes the results are difficult to interpret. Based on the concept that bone matrix is constantly deposited throughout life, we analysed the bone mineral density of the arm and forearm of a series of bottlenose dolphins (*Tursiops truncatus*, Montagu 1821) stranded along the Italian coast of the Adriatic Sea or maintained in confined waters. The bone mineral density values we obtained were evaluated as possible age predictors of the Mediterranean population of this species, considering age as determined by counting growth layer groups in sections of the teeth and the total body length of the animal as references. Comparisons between left and right flipper showed no difference. Our results show that bone mineral density values of the thoracic limb are indeed reliable age predictors in *Tursiops truncatus*. Further investigations in additional odontocete species are necessary to provide strong evidence of the reliability of bone mineral density as an indicator of growth and chronological wear and tear in toothed-whales.

**Key words** age determination; bone density; bottlenose dolphin; growth; thoracic limb.

## Introduction

Stranded cetaceans yield useful information on the natural history of the species and the medical condition of the single individual or that of their pod. Age is one of the key parameters that must be established, even approximately, in stranded specimens, to get a reliable insight into the causes of death and eventually into the whole biology of the species in the wild. Up to now, the standard and most accurate technique for age determination in odontocete cetaceans is the count of dentine layers in the teeth (for review see Perrin & Myrick, 1980). Although dentine (and cement) growth layers have been calibrated in only a few cetacean species, and most information comes from studies on bottlenose dolphins, the count of dentine growth layers has allowed a sharper insight into the biology of several odontocetes. However, this procedure requires expensive laboratory techniques and operator experience and often results in misleading age estimates (Hui, 1980; Kimura, 1980). The use of this method in dolphins is further limited in specimens older than 13–14 years due to the overlapping of the new dentine layers with the older ones (Perrin & Myrick, 1980). Any bias in age determination will affect subsequent estimates of life history parameters. Thus the development of new techniques, complementary or alternative to dentinal layer examination, would lead to a more accurate age determination of single individuals and improve our knowledge of aging in populations and species.

In the past, several methods besides the growth layer groups (GLGs) count have been used to evaluate age in dolphins. Such methods include histological examination of gonads, and biochemical analysis of aspartic acid racemization (Bada et al. 1980). Most of the above mentioned methods can be performed only if the animal body is in good conditions, a detailed necropsy is feasible and they all require further laboratory investigations. The evaluation of the degree of fusion of ephiphysial plates by

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Accepted for publication *8 August 2007*

means of X-ray examination or the degree of fusion of the hyoid complex have been used in the past to estimate the age of stranded cetaceans (Ogden et al. 1981; Galatius et al. 2006) and especially in the striped dolphin *Stenella coeruleoalba* (Calzada & Aguilar, 1996; Calzada et al. 1997; DiGiancamillo et al. 1998). Unfortunately, although radiographic examination is a common clinical practice and is easy to perform even in decomposed animals or skeletal remains, this method only allows one to ascribe a specimen to a general age class without any evidence of chronological age. In fact X-rays are indicative of bony development, rather than age in years.

The structure of the bones is among the most interesting features of cetacean limb anatomy since their modifications are associated with a complete adaptation to the aquatic environment (Benke, 1993). Cetacean bones have been reported to have a moderate-to-low overall density on the base of a *whole bone dry weight/volume* measurement by Felts & Spurrell (1965). Based on this consideration, our group (Guglielmini et al. 2002) carried out the first study to report a measurement of the bone mineral density (BMD) in cetaceans using the dual-energy X-ray absorptiometry (DXA) technology, the 'gold standard method' for measuring bone density in human and veterinary clinical practice. The latter study reported a positive correlation between values of measured BMD, age as determined by GLGs count, and total body length (Guglielmini et al. 2002). According to this model, age in *Stenella coeruleoalba* can be accurately predicted on the basis of bone density and total body length of the stranded animals.

In the present paper we describe the mineral density features of the right and left arm (humerus) and forearm (radius and ulna) bones of a series of specimens of *Tursiops truncatus* stranded along the Italian coast by means of DXA technique. We also report BMD values collected from a series of bottlenose dolphins of known age maintained in a confined environment. BMD values thus obtained are discussed in relation to the age of the animals estimated by examination of GLGs in tooth sections and to the total body length. Values are also examined in relation to possible differences between the right and the left forelimb, and – within the limits imposed by the number of captive specimens – between wild and captive animals. The aim of this paper was to contribute to a better understanding of bone density pattern in cetaceans and to improve the criteria for age estimation in *Tursiops truncatus.*

## Materials and methods

### Animals

The present study was based on 24 bottlenose dolphins (*Tursiops truncatus*), either stranded along the Italian coasts of the Adriatic Sea between September 2003 and September 2006 ($n = 21$) or maintained in a confined environment ($n = 3$). For the analysis of BMD, the flippers were detached from the gleno-humeral joint and stored frozen. We analyzed the right flipper of 18 animals (except specimens 1, 3 and 6) and the left from all but one animal (specimen 24) for a total of 41 flippers used for comparative right-left analysis of BMD values.

Dentine layers were counted using the four central mandibular teeth sampled from each specimen. Once removed, the teeth were stored in 10% buffered formaldehyde until sectioning. Teeth from five animals did not allow the GLGs count because of structural damage. Estimated age of the animals by examination of GLGs in available tooth sections varied between 0 (a newborn animal) and 30 years (sample mean 7.7, SD 6.8).

The total body length (from the tip of the snout to the notch between the flukes) was measured in 23 out of 24 dolphins. Data obtained varied between 136 and 305 cm (sample mean 238.0, SD 51.8; for details of each animal see Table 1).

### Bone densitometry

Flippers were scanned after removal of soft tissue. The bone mineral density (g $\mathrm{cm}^{-2}$) of the flippers' bone was measured using a DXA device with a double X-rays level source (70 kVp and 140 kVp) and a resolution of 0.76 mm × 1.5 mm. Before each scan session the stability of the device (Hologic QDR-1000™, Hologic Inc., Waltham, MA, USA) was checked using the standard calibration tool (Hologic Calibration Phantom™, Hologic Inc.). Flippers were scanned in dorso-palmar projection with bones laying on a Lexan™ support (Acrylic Scan Platform, Hologic Inc.), a device used to simulate soft tissue covering and to improve the application of the protocol to small samples (Ammann et al. 1992). The analysis of the scan images was always performed by the same person (A.Z.) using the Subregion Analysis Lumbar Spine™ Software (version 6.20 D, Hologic Inc., Waltham, MA).

We considered two different regions of interest (ROI) of the thoracic limb: (1) the global region of interest (GROI) between the proximal epiphysis of the humerus and the distal epiphysis of the radius and ulna; and (2) the subregion of interest (SROI), the whole humerus.

Table 1 displays all available data of specimens, including sex, length and age determination. Table 1 also reports the BMD values of the right and left global region of interest (*r*GROI and *l*GROI respectively) and of the right and left subregion of interest (*r*SROI and *l*SROI respectively).

### Preparation of teeth and age determination

The least worn and straightest tooth from each dolphin was sectioned sagitally in a central 3-mm-thick wafer using a diamond blade (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, USA). The wafer was then fixed overnight in

**Table 1** Body length, captivity, sex, age, BMD of the right (*r*GROI) and left (*l*GROI) global region of interest and of the right (*r*SROI) and left subregion of interest (*l*SROI) of the dolphins examined in the present study

| Dolphin# | Body length (cm) | Captivity | Sex | Age (y) | *r*GROI BMD (gr cm$^{-2}$) | *r*SROI BMD (gr cm$^{-2}$) | *l*GROI BMD (gr cm$^{-2}$) | *l*SROI BMD (gr cm$^{-2}$) |
|---|---|---|---|---|---|---|---|---|
| 1 | 220 | NO | M | | | | 0.735 | 0.735 |
| 2 | 281 | NO | M | | 1.003 | 1.280 | 1.036 | 1.036 |
| 3 | 272 | NO | F | 10 | | | 0.911 | 0.911 |
| 4 | 207 | NO | F | | 0.804 | 1.046 | 0.803 | 0.803 |
| 5 | 290 | NO | | 8 | 0.946 | 1.302 | 0.942 | 0.942 |
| 6 | 295 | NO | M | 12 | | | 1.259 | 1.259 |
| 7 | 200 | NO | | 6 | 0.797 | 1.021 | 0.789 | 0.789 |
| 8 | 277 | NO | F | 6.5 | 0.959 | 1.367 | 0.959 | 0.959 |
| 9 | 270 | NO | M | 10 | 0.936 | 1.240 | 1.018 | 1.018 |
| 10 | 177 | NO | M | 2 | 0.571 | 0.743 | 0.577 | 0.577 |
| 11 | 274 | NO | F | 10 | 0.821 | 1.094 | 0.841 | 0.841 |
| 12 | 160 | NO | | 1 | 0.608 | 0.768 | 0.627 | 0.627 |
| 13 | 272 | NO | F | 11 | 0.927 | 1.240 | 0.902 | 0.902 |
| 14 | 282 | NO | | | 0.973 | 1.362 | 0.961 | 0.961 |
| 15 | 272 | NO | F | 11 | 1.006 | 1.343 | 0.997 | 0.997 |
| 16 | 160 | NO | F | 1 | 0.647 | 0.839 | 0.648 | 0.648 |
| 17 | 180 | NO | | uncertain results | 0.562 | 0.693 | 0.567 | 0.567 |
| 18 | 219 | YES | M | 5 | 0.780 | 1.035 | 0.799 | 0.799 |
| 19 | 285 | NO | F | 10.5 | 0.854 | 1.112 | 0.862 | 0.862 |
| 20 | 305 | NO | M | | 1.088 | 1.417 | 1.090 | 1.090 |
| 21 | 136 | NO | M | newborn | 0.421 | 0.513 | 0.423 | 0.423 |
| 22 | 250 | YES | M | 9 | 0.796 | 0.964 | 0.772 | 0.772 |
| 23 | 190 | NO | M | 3 | 0.734 | 0.946 | 0.743 | 0.743 |
| 24 | | YES | F | 30 | 0.597 | 0.750 | | |

10% buffered formaldehyde, rinsed in running water, decalcified in RDO (Apex Engineering Products Corporation, Aurora, IL, USA), thin-sectioned using a cryostat, toluidine blue stained, and mounted on microscope slides. GLGs reading was performed by optical microscopy, using 10× magnification on 20–25 µm teeth sections. During the reading the total number of GLGs in each of 19 tooth sections was determined independently by two different readers (C.B. and M.P.). Each reading was made without reference to previous readings or additional information on animals (e.g. total length, sex etc.) and sections were read in random order.

Growth layers groups were identified as successive pairs of light and dark stained layers in the post-natal dentine. This pattern of dentine deposition was previously defined as a countable unit in *Tursiops truncatus* teeth sections (Perrin & Myrick, 1980).

### Statistical analysis

All statistical analysis was performed using the statistical software Minitab (State College, PA, USA), release 13.20. First, paired *t*-tests (Daniel, 2005) were used to investigate possible significant statistical differences between the following pair of densitometry variables: (1) BMD of the right GROI (*r*GROI) and the left GROI (*l*GROI); (2) BMD of the right SROI (*r*SROI) and the left SROI (*l*SROI); (3) BMD of the right GROI (*r*GROI) and the right SROI (*r*SROI); (4) BMD of the left GROI (*l*GROI) and the left SROI (*l*SROI).

Then, to estimate a possible predictive model for the age of the subjects, we fitted our data to a linear model (Daniel, 2005) where the response variable was the age (determined by dentine layers) and the possible predictors were the total body length and the four BMD variables. Sex and captivity were also included in the linear model as potential confounding factors for age estimation.

As a final statistical analysis we compared the four BMD variables between captive and free ranging animals by using the *t*-test for two independent samples (Daniel, 2005). Given the really unbalanced number of dolphins belonging to the captive group (only three subjects), in comparison with those referred to the free group (21 subjects), we emphasize that these results should be carefully considered.

## Results

The anatomy of the bones of the arm and foream is represented in Fig. 1, together with the areas used for densitometry. Table 2 shows results of all paired comparisons of interest between paired BMD variables. The data indicate that there are no significant differences (at the significance level $\alpha$ equal to 0.05) when considering the

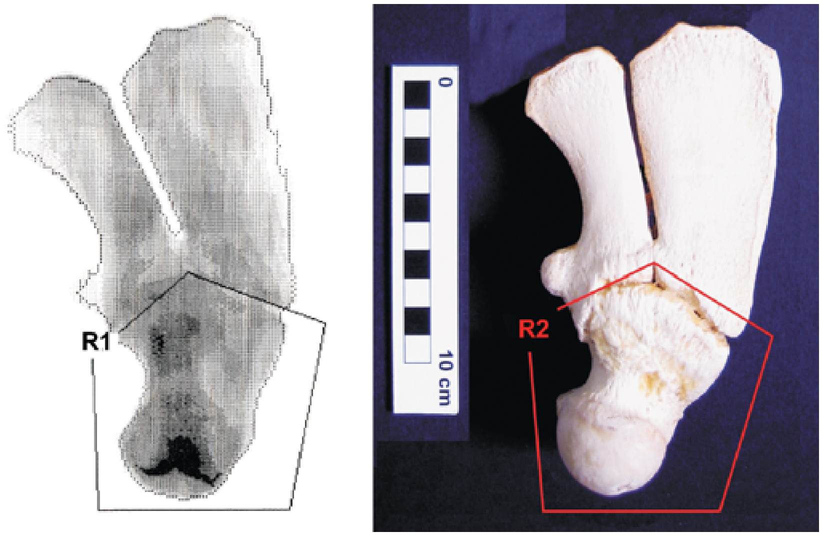

**Fig. 1** *Left*: dual-energy X-ray absorptiometry image of the right arm and forearm bones of a bottlenose dolphin showing the whole GROI (dotted line) and the SROI (solid line; in the humerus, i.e. R1). *Right*: humerus, radius and ulna of the right flipper of a bottlenose dolphin.

**Table 2** Results of paired *t*-tests for pairs of BMD densitometry variables

| Paired BMD densitometry variables | | | No. of cases | Difference of mean | *T*-value | *P*-value | Lower 95% CI for mean diff. | Upper 95% CI for mean diff. |
|---|---|---|---|---|---|---|---|---|
| *r*GROI | *vs* | *l*GROI | 20 | –0.01 | –1.20 | 0.243 | –0.017 | 0.005 |
| *r*SROI | *vs* | *l*SROI | 20 | –0.01 | –1.84 | 0.081 | –0.030 | 0.002 |
| *r*GROI | *vs* | *r*SROI | 21 | –0.25 | –13.11 | 0.000 | –0.290 | 0.210 |
| *l*GROI | *vs* | *l*SROI | 23 | –0.27 | –13.21 | 0.000 | –0.307 | –0.224 |

comparisons between right and left GROI and right and left SROI while there are significant differences when considering the comparisons between right GROI versus right SROI and left GROI versus left SROI. Specifically, both SROIs have greater densitometry mean value than the GROIs.

We replicated the paired *t*-tests by splitting our 24 subject sample by captivity status and by sex and obtained the same results. These latter findings represent an indirect demonstration that the density of the arm is higher than that of the ipsilateral forearm; in fact the BMD (g cm$^{-2}$) of the subregion is greater than that of the global region of analysis, showing that the whole area analysed (cm$^{2}$) and the global mineral content (g) do not increase equally.

Table 3 shows regression results of the linear model built to predict the age of the dolphins by using body length and BMD variables as predictors. Sex and captivity were also included in the linear model as potential confounding factors for age estimation. A regression model including all available predictors is simply the initial step to find a reliable model to predict age. Specifically, one should first consider possible high correlation between predictors (the so-called multicollinearity problem, Draper & Smith, 1998) then remove predictors that do not show a significant coefficient *t*-test *P*-value. Data reported in Table 4 (Pearson correlation coefficients between BMD variables) suggest that BMD variables are highly correlated, so we removed *l*GROI and *l*SROI from our model to avoid the problem of multicollinearity. Results of the second linear

**Table 3** Results of linear model for dolphin’s age prediction

| Predictor | Coef | SE Coef | *T*-value | *P*-value |
|---|---|---|---|---|
| Constant | –12.6 | 2.67 | –4.70 | 0.009 |
| Length | 0.1 | 0.02 | 3.00 | 0.040 |
| *r*GROI | 39.7 | 36.64 | 1.08 | 0.340 |
| *r*SROI | –29.9 | 25.33 | –1.18 | 0.304 |
| *l*GROI | 7.1 | 42.52 | 0.17 | 0.876 |
| *l*SROI | –1.3 | 29.36 | –0.05 | 0.966 |
| Captivity | –0.9 | 1.63 | –0.55 | 0.609 |
| Sex | –0.7 | 1.19 | –0.59 | 0.589 |

S = 1.32; R-Sq = 96.4%; R-Sq (adj) = 90.1%.
12 cases used; 7 cases contain missing values.

**Table 4** Pearson correlation coefficients between BMD densitometry variables

| | *r*GROI | *r*SROI | *l*GROI |
|---|---|---|---|
| *r*SROI | 0.989<br>(*P* = 0.000) | | |
| *l*GROI | 0.992<br>(*P* = 0.000) | 0.979<br>(*P* = 0.000) | |
| *l*SROI | 0.985<br>(*P* = 0.000) | 0.992<br>(*P* = 0.000) | 0.990<br>(*P* = 0.000) |

model are displayed in Table 5 (second linear model for dolphin's age prediction).

As a final step, we removed captivity and sex given that they were not significant predictors (i.e. they provided a *t*-test *P*-value greater than the adopted significance $\alpha$ level equal to 0.05), and applied the third final linear model to predict the age of the five dolphins whose age was unknown (Table 6). Results of predictions from our final model are also displayed in Table 6. Note that since right GROI and SROI were not available for dolphin #1 (see Table 2), a predicted age was not computable. To this effect we fitted our data to another model where *r*GROI and *r*SROI were replaced by *l*GROI and *l*SROI respectively. It is worth noting that the second final predictive model provided predicted ages whose values were very close to those computed by *r*GROI and *r*SROI final model.

We concluded our statistical analysis by comparing the four densitometry variables between captive and free animals by using the *t*-test for two independent samples. Results in Table 7 suggest that there is no significant difference (at the significance level $\alpha$ equal to 0.05) in the four BMD variables when comparing dolphins in captivity with those not in captivity.

**Table 5** Results of second linear model for dolphin's age prediction

| Predictor | Coef | SE coef | *T*-value | *P*-value |
|---|---|---|---|---|
| Constant | −12.7 | 2.04 | −6.24 | 0.001 |
| Length | 0.1 | 0.02 | 3.74 | 0.010 |
| *r*GROI | 44.5 | 15.81 | 2.82 | 0.030 |
| *r*SROI | −29.5 | 9.75 | −3.03 | 0.023 |
| Captivity | −1.0 | 1.30 | −0.73 | 0.491 |
| Sex | −0.5 | 0.81 | −0.62 | 0.559 |

S = 1.09; R-Sq = 96.3%; R-Sq (adj) = 93.3%.
12 cases used 7 cases contain missing values.

**Table 6** Results of final linear model and prediction for dolphins of unknown age

| Predictor | Coef | SE coef | *T*-value | *P*-value |
|---|---|---|---|---|
| Constant | −14.8 | 1.98 | −7.50 | 0.000 |
| Length | 0.1 | 0.02 | 3.75 | 0.003 |
| *r*GROI | 48.3 | 15.22 | 3.17 | 0.008 |
| *r*SROI | −29.4 | 9.73 | −3.02 | 0.011 |

S = 1.29; R-Sq = 92.4%; R-Sq (adj) = 90.5%.
16 cases used 3 cases contain missing values.

| Dolphin no. | Predicted age | SE Fit | 95% CI for fit | | 95% PI for fit | |
|---|---|---|---|---|---|---|
| | | | Lower | Upper | Lower | Upper |
| 1 | * | * | * | * | * | * |
| 2 | 12.4 | 0.9 | 10.4 | 14.4 | 8.9 | 15.9 |
| 5 | 5.3 | 0.6 | 4.1 | 6.6 | 2.3 | 8.4 |
| 16 | 8.6 | 0.7 | 7.0 | 10.2 | 5.4 | 11.8 |
| 23 | 13.9 | 0.9 | 11.8 | 15.9 | 10.4 | 17.4 |

| Predictor | Coef | SE Coef | T-Value | *P*-value |
|---|---|---|---|---|
| Constant | −13.7 | 2.07 | −6.63 | 0.000 |
| Length | 0.1 | 0.01 | 4.51 | 0.000 |
| *l*GROI | 34.2 | 16.59 | 2.06 | 0.059 |
| *l*SROI | −20.7 | 10.98 | −1.89 | 0.080 |

S = 1.43; R-Sq = 90.8%; R-Sq(adj) = 88.8%.
18 cases used 1 cases contain missing values.

| Dolphin no. | Predicted age | SE fit | 95% CI for fit | | 95% PI for fit | |
|---|---|---|---|---|---|---|
| | | | Lower | Upper | Lower | Upper |
| 1 | 7.0 | 0.9 | 5.0 | 8.9 | 3.3 | 10.6 |
| 2 | 11.4 | 0.8 | 9.8 | 13.0 | 7.9 | 14.9 |
| 5 | 5.2 | 0.5 | 4.1 | 6.2 | 1.9 | 8.4 |
| 16 | 8.7 | 0.9 | 6.8 | 10.6 | 5.1 | 12.3 |
| 23 | 13.7 | 1.1 | 11.4 | 15.9 | 9.9 | 17.5 |

## Discussion

The DXA technology is considered the 'gold standard' method to evaluate BMD in humans since it allows rapid and non-invasive measurements. During a scan, X-rays of two different energy levels originate from a source that moves over the subject together with a detector. X-rays are impeded differently by bone and flesh; therefore the type and amount of tissue scanned can be distinguished by the detector thus allowing the post-processing software to give a value of BMD (g $cm^{-2}$). Since in our study only defleshed specimens were analysed, the use of the Lexan specific for small samples densitometric protocols (Ammann et al. 1992) allowed the post-processing software of the DXA device to subtract automatically the mass of the platform from the measured mass of the animal plus the platform. The resulting difference is the bone mass of the animal. Thus, by using this platform, the filtration of the X-ray beam is optimized and beam-hardening effects are eliminated, resulting in improvement of the linearity of BMD results. Bone remodelling continues through life, and DXA is now widely used in bone research and applied to small (rodents, rabbit, cat) and large sized-mammals (dogs, swine, non-human primates, sheep and horse) (Grier et al. 1996; Zotti et al. 2004a, 2006; Isola et al. 2005), as well as to other vertebrates (turkey and iguana) (Zotti et al. 2003, 2004b). The first study that applied DXA to the study of cetacean (*Stenella coeruleoalba*) bone tissue, and specifically to the arm and forearm, was performed by Guglielmini et al. (2002). To the best of the authors' knowledge the present study is the first application of DXA to study the BMD in *Tursiops truncatus*.

**Table 7** Results of the comparison between captivity and free dolphins for the four BMD densitometry variables

| BMD densitometry variable | No. of captive subjects | No. of free subjects | Difference of mean | *T*-Value | *P*-Value |
|---|---|---|---|---|---|
| *r*GROI | 3 | 18 | –0.09 | –0.74 | 0.536 |
| *r*SROI | 3 | 18 | –0.10 | –0.63 | 0.590 |
| *l*GROI | 3 | 20 | –0.05 | –0.41 | 0.722 |
| *l*SROI | 3 | 20 | –0.04 | –0.27 | 0.805 |

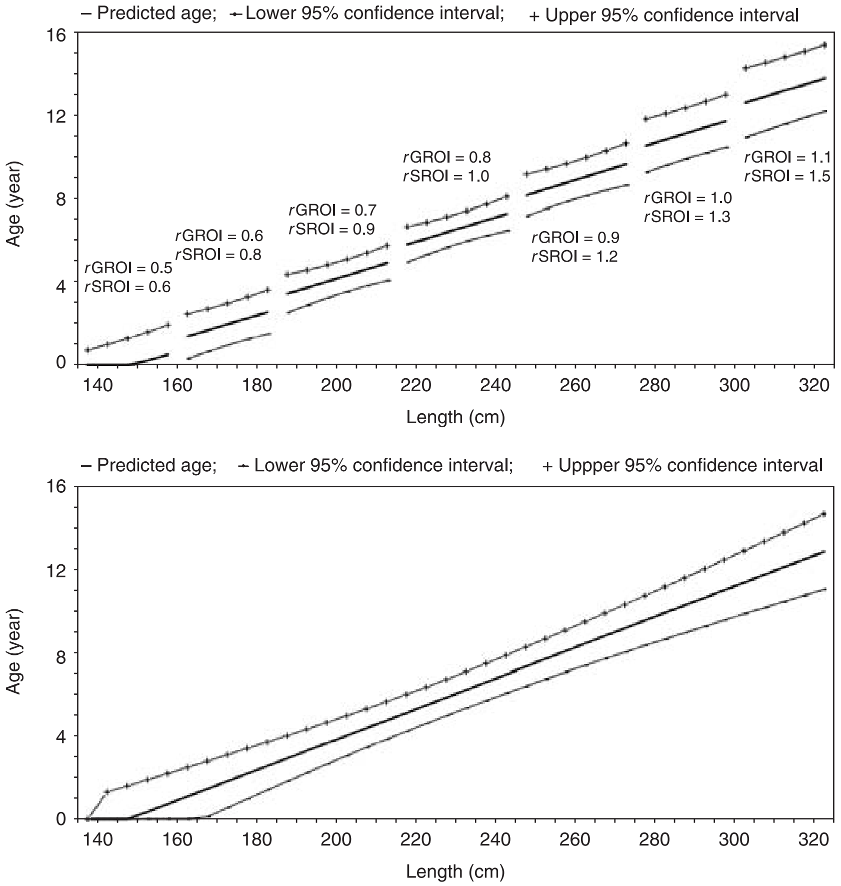

**Fig. 2** Graphical representation of our proposed age prediction model (2A) and graphical representation of a simpler age prediction model, without considering *r*GROI and *r*SROI (2B).

Mean values of BMD measured by means of DXA techniques in our sample of dolphins are lower if compared with the range of BMD values measured with DXA in many healthy terrestrial mammals. However, the relationship between bone density and weight is not yet fully understood: the bone density of equine limb bones is approximately double that of corresponding human structures, although the weight of the average horse is fivefold the human's. The lower density of the bottlenose dolphin arm and forearm bones fits well with the findings of De Buffrenil & Schoevaert (1988) about the cancellous texture and the reduction or absence of compact cortices in cetacean flipper bones. The BMD values that we obtained in the bottlenose dolphin (see Table 1) are slightly higher than those reported in the striped dolphin (Guglielmini et al. 2002). These BMD feature findings are better understood if we consider the cetacean flipper as a structure not bearing weight. The cetacean flipper is in fact subject to transverse loading by water resistance and does not have to download the weight onto the ground, or otherwise support a heavy workload (Berta & Sumich, 1999). Furthermore, the lack of any statistical difference between the flippers of the two sides indicate that there is no muscular factor related to laterality that modifies bone mineral content, contrary to what is reported in normal humans (Taaffe et al. 1994) and in athletes (Tsuji et al. 1995). Our results show no sex difference in the BMD. Similar results have been obtained in the striped dolphin (Guglielmini et al. 2002).

According to our data, to estimate the age of an individual *Tursiops truncatus* one should use the linear model reported in Table 6 (age = –14.8 + Length × 0.1 + *r*GROI × 48.3 – SROI × 29.4). Use of the model is graphically represented in Fig. 2A, where age is the dependent variable predicted in function of body length, *r*GROI and *r*SROI. Note that only a few combinations of *r*GROI and *r*SROI are considered, to allow a two-dimensional graph. In fact, the linear model proposed here for age prediction is four-dimensional. When considering body length as the unique predictor for age, the result is a simpler but less reliable model which is represented in Fig. 2B. The relatively poor accuracy of length as age predictor has already been discussed (Zweifel & Perrin, 1980; for review see Perrin & Myrick, 1980). The use of the BMD information increases significantly predictive ability in comparison to simply considering the body length. The advantage can be quantified by the increase of the $R^2$ regression coefficient from 86.0% (linear model with only length) to 92.4% (our proposed model with *r*GROI and *r*SROI as well). The $R^2$ increase of 7.4% is proved to be significant (at the significance level $\alpha$ equal to 0.05) by using the Chow test (Davidson & MacKinnon, 1993) which provides a *P*-value of 0.026.

The removal of a flipper is an easy procedure to perform on stranded cetaceans, even in cases in which body conditions are poor. So the use of DXA techniques in age determination may yield precious information on a number of toothed whales. Methods other than X-rays are invasive (GLGs count) or require fresh organs (gonads). BMD values are more accurate age predictors than X-rays alone because they consider bone deposition and possibly reabsorption through life rather than only epiphysial plate fusion. So BMD values can be used to assess the age of animals properly maintained in museum collections, without risk of compromising the integrity of the specimen. Further studies may also apply this technique to those odontocetes (including Ziphiidae) where GLGs count is scarcely feasible due to reduction of the teeth and sometimes because stranded individuals belong to a rare species whose skeletal remains are precious. DXA devices are very common in hospitals all over the world, and BMD values can obviously also be assessed in live dolphins, although the animals must be anesthetized to obtain their complete stillness during the procedure. This possibility could be considered when a dolphin strands alive and rehabilitation is attempted. Since DXA converts a three-dimensional structure into a two-dimensional image, BMD is measured in an area rather than a volume (Genant et al. 1991). Thus, correct positioning of the bone to be scanned is mandatory to obtain accurate and precise measurements (Markel et al. 1994; Rozenberg et al. 1995; Muir & Markel, 1996).

To the best of our knowledge, the phenomenon of postmenopausal osteoporosis, described in aged women and in non-human primates (Champ et al. 1996), has never been described in marine mammals; however, the study of BMD may be applied also to those conditions in which bone demineralization or pathology is suspected in cetacean (Sweeny et al. 2005), including in suspected cases of decompression sickness in cetaceans (Moore & Early, 2004).

In conclusion, our study on a sample of 24 *Tursiops truncatus* from the Adriatic Sea indicates that BMD values of the thoracic limb are reliable indicators of age, thus confirming also in this species what has already been reported for the striped dolphin. This population can be considered representative of bottlenose dolphins from the Mediterranean Sea (Natoli et al. 2004; Reeves & Notarbartolo di Sciara, 2006), although a further subdivision into an East Mediterranean subpopulation has been proposed based on microsatellite analysis (Natoli et al. 2005). We are well aware that oceanic populations of *Tursiops truncatus* may show differences in bone density patterns, as already demonstrated in various human ethnic groups (Bhudhikanok et al. 1996). Further studies are required to deal with population diversities in bone density and their eventual relationship to the dolphin habitats.

The method that we present here may likely constitute a future standard for age determination in toothed whales, alone or combined with former standardized techniques including GLGs count.

## Acknowledgements

The authors thank the Mediterranean Marine Mammal Tissue Bank at the Department of Experimental Veterinary Science of the University of Padova (Italy) and the *Fondazione Cetacea Onlus*, Riccione (Italy) for providing cetacean tissues. We acknowledge the helpful advice of Dr Christina Lockyer on the age determination protocol used for GLGs count and of Dr Giovanni Bearzi on the population structure of the Mediterranean bottlenose dolphin. We wish to thank also Mr Giovanni Caporale, Mr Emanuele Zanetti and Ms Maristella Giurisato for their expert technical assistance.

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