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Source: Journal of Mammalogy, 96(3):541-551.
Published By: American Society of Mammalogists
URL: http://www.bioone.org/doi/full/10.1093/jmammal/gyv059

Journal of Mammalogy, 96(3):541–551, 2015
DOI:10.1093/jmammal/gyv059

Thoracic limb bone development in *Sotalia guianensis* (Van Beneden 1864) along the coastline of Espírito Santo, Brazil

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The skeleton is often the only remaining structure of the Guiana dolphin, *Sotalia guianensis*, after decomposition of carcasses. This study investigates the bone development of Guiana dolphins beached on the coastline of the state of Espírito Santo, Brazil. External measurements of 43 thoracic limbs were obtained. Internal structures (radius, ulna, and humerus) were also measured. Dual-energy X-ray absorptiometry (DXA) was used to evaluate bone mass. The variables concerning the thoracic limb were tested using the Akaike information criterion to scale the best growth model when correlated with age and by the allometric model when they were correlated with total body length. The efficacy of DXA was also tested. The Brody growth model (best fit) showed that the thoracic limb stopped growing around the age of 2, while total body length ceased to grow at the age of 5.5. The thoracic limb presented early growth (negative allometry) compared with total body length. The methodology used to measure bone mass was efficient when considering ash weight. No difference in bone density was observed between the right and the left forelimb ($P > 0.05$), male and female ($P > 0.05$), or between dolphins found in the 3 sites we monitored. The deposition of bone mass was high in the early stages of life, and stabilization occurred at around the age of 13.

O esqueleto muitas vezes é a estrutura que resta da espécie *Sotalia guianensis*, boto-cinza, devido ao processo de decomposição em que as carcaças são encontradas. O objetivo do presente estudo é entender o desenvolvimento ósseo dos animais encontrados encalhados no litoral do Espírito Santo. Para isso, 43 pares de nadadeiras peitorais foram submetidos às mensurações externas e de estruturas ósseas internas (rádio, ulna e úmero) e à técnica de absorciometria de raio-x de dupla energia (DEXA) para massa óssea. As variáveis das nadadeiras foram testadas pelo critério de Akaike para escalonamento do melhor modelo de crescimento quando correlacionadas com a idade, e para o modelo alométrico quando correlacionadas com o comprimento total (CT). Foi testada a eficácia da DEXA. através do modelo de crescimento de Brody (melhor ajuste) e pode-se observar que as nadadeiras param de crescer por volta dos 2 anos de idade e o comprimento total do corpo aos 5.5 anos, apresentando essas estruturas crescimento precoce (alometria negativa) em relação ao CT. A metodologia aplicada para mensuração da massa óssea mostrou-se eficaz quando correlacionada com peso das cinzas. Não houve diferença de densidade óssea entre nadadeira direita e esquerda ($P > 0.05$), macho e fêmea ($P > 0.05$) e nem entre os animais dos três diferentes locais de ocorrência, pois todos estavam dentro do mesmo intervalo de confiança. A deposição da massa óssea é acentuada no início da vida com posterior estabilização por volta dos13 anos de idade.

Key words: bone development, Guiana dolphin, *Sotalia guianensis*, thoracic limb

Sotalia guianensis (Van Beneden 1864), commonly known as the Guiana dolphin, is distributed from Honduras, in Central America, to the state of Santa Catarina, Brazil (Flores and Silva 2009). Mangroves, bays, and estuaries are some of the coastal environments preferred by this species (Borobia et al. 1991).

In Brazil, Guiana dolphins are found beached on Brazilian shores throughout the year (Zerbini 1999), rousing interest in the investigation of biological parameters and cause of death (Guglielmini et al. 2002). Since bones resist the decay process, these structures are the best study material in postmortem investigations. In spite of that, oestological descriptions and studies on the maturity of thoracic limb in sea mammals are scarce (Calzada and Aguilar 1996), even for Guiana dolphins (Menezes and Simões-Lopes 1996; Carvalho 2011).

Like skeleton maturity, bone mineral density (BMD) increases with development (Katzman et al. 1991). In humans, BMD may be influenced by several factors, such as genetic predisposition, age, body mass, gender, ethnicity, hormone deficiency, intensity of physical exertion, vitamin intake, and use of medical drugs (Brandão and Vieira 1999; Nascimento et al. 2009). In aquatic mammals, BMD has been linked with behaviors such as dive depth, aggressiveness, feeding habits (suction feeding and capture—Cozzi et al. 2010), and gender attributes, as well as other factors like environmental pollution (Sonne et al. 2004), age, and size (Guglielmini et al. 2002; Butti et al. 2007; Lucic et al. 2010).

In the past, the quantification of BMD in cetaceans was based on the Archimedes principle (Felts 1966; Wall 1983), in which density is measured as the volume of water shifted by a whole bone and expressed as g/cm^3 (Stein 1989; Cozzi et al. 2009). Currently, new methods are used, such as computed tomography. However, dual-energy X-ray absorptiometry (DXA) is a noninvasive, fast, accurate technique that is widely used in humans and small and large domestic mammals alike, such as cats, rabbits, dogs, pigs, sheep, horses, ferrets, iguanas, and primates (Grier et al. 1996; Turner 2001; Borges et al. 2008).

Although DXA is used in investigations of cetaceans and polar bears (*Ursus maritimus*—Guglielmini et al. 2002; Sonne et al. 2004; Butti et al. 2007; Cozzi et al. 2009, 2010; Lucic et al. 2009, 2010; Zotti et al. 2009), no study has discussed the precision and accuracy of the technique.

Ideally, precision and accuracy of a method should be tested before the actual experiment, so as to evaluate error for individual species (Rozenberg et al. 1995; Borges et al. 2008). The precision of the method, which is the capacity to obtain the same result in several repeated measurements, is assessed based on the coefficient of variation. Accuracy is evaluated comparing results obtained with different methods (Grier et al. 1996). Studies have tested accuracy of the use of DXA in laboratory mice, comparing 2 systems—Norland XR-26 (Norland Corporation, Fort Atkinson, Wisconsin) and HologicQDC 1000/W (Hologic Inc., Waltham, Massachusetts) (Hagiwara et al. 1993)—or comparing the results with ash weight or calcium content (Cazes et al. 1994; Rozenberg et al. 1995).

The present study investigated bone maturity and density of the Guiana dolphin based on the correlation of measurements and DXA of thoracic limb with biological parameters like total body length (TBL) and age estimates and to evaluate the efficiency of the technique.

Materials and Methods

Forty-three *S. guianensis* carcasses found beached or accidentally captured in fishing nets on the coastline stretching between the municipalities of Conceição da Barra and Presidente Kennedy, in the state of Espírito Santo, Brazil, were used (Fig. 1). The dolphins were salvaged by the ORCA Institute (environmental awareness organization, Guarapari, Espírito Santo, Brazil) between March 2009 and February 2013.

Individuals were submitted to a postmortem examination. When the degree of decay allowed, gender was established and external measurements were taken, including TBL, which was measured between the rostral tip of the upper jaw to the median notch between tail flukes and width, cranial length, and caudal length of thoracic limb (W_{tl}, CaL_{tl}, and CrL_{tl}). After measurements, right and left thoracic limbs were removed at the glenohumeral joint and macerated in water upon detachment of soft tissues. Width and length were also measured in bone, radius, ulna, and humerus (W_{hum}, L_{hum}, W_{uln}, L_{uln}, W_{rad}, and L_{rad}) using digital calipers. Length was measured between the proximal to distal ends of bone, while width was measured in the middiaphyseal region.

The age of 37 dolphins was estimated based on the counts of growth layer groups in dentin, as described by Perrin and Myrick (1980) using the technique developed by Hohn et al. (1989). Each layer group, formed by a stained and a nonstained region, was considered as 1 year of life. Dolphins that presented only the neonatal line (a clearly visible line formed soon after birth) on teeth were given the age of 0.5. In turn, those that did not present the neonatal line were considered newborns with age of 0 (zero). Teeth were obtained macerating crania in water.

The bones of the thoracic limb were placed together on an acrylic tray that had the same absorption intensity as that of soft tissues. Then, bone density of the right and left thoracic limb was measured using a densitometer (Lunar Prodigy Advance DXA-GE Healthcare, Diegem, Belgium) on the dorsal–ventral projection position. After readings, BMD was measured in regions of interest corresponding to the humerus, radius, and ulna using the software Encore (GE Healthcare 2010), with the protocol used to analyze the human forearm. All measurements were carried out by the same observer in order to minimize experimental error.

The efficacy of the DXA to analyze the thoracic limb of Guiana dolphin was evaluated using the bone ash weigh of the humerus, used after maceration and reading of DXA, of 20 animals. The humeri of 20 thoracic limbs were dried in a stove at 55°C for 72 h and then triturated into a homogenized fine power in a ball mill. Afterward, 2 g of the milled material was weighed in a china crucible and incinerated at 550°C for 4 h. The ash obtained was used to calculate the total ash of the whole humerus.

The statistical analyses first tested the normality of the distribution of all variables that included age, TBL, thoracic limb

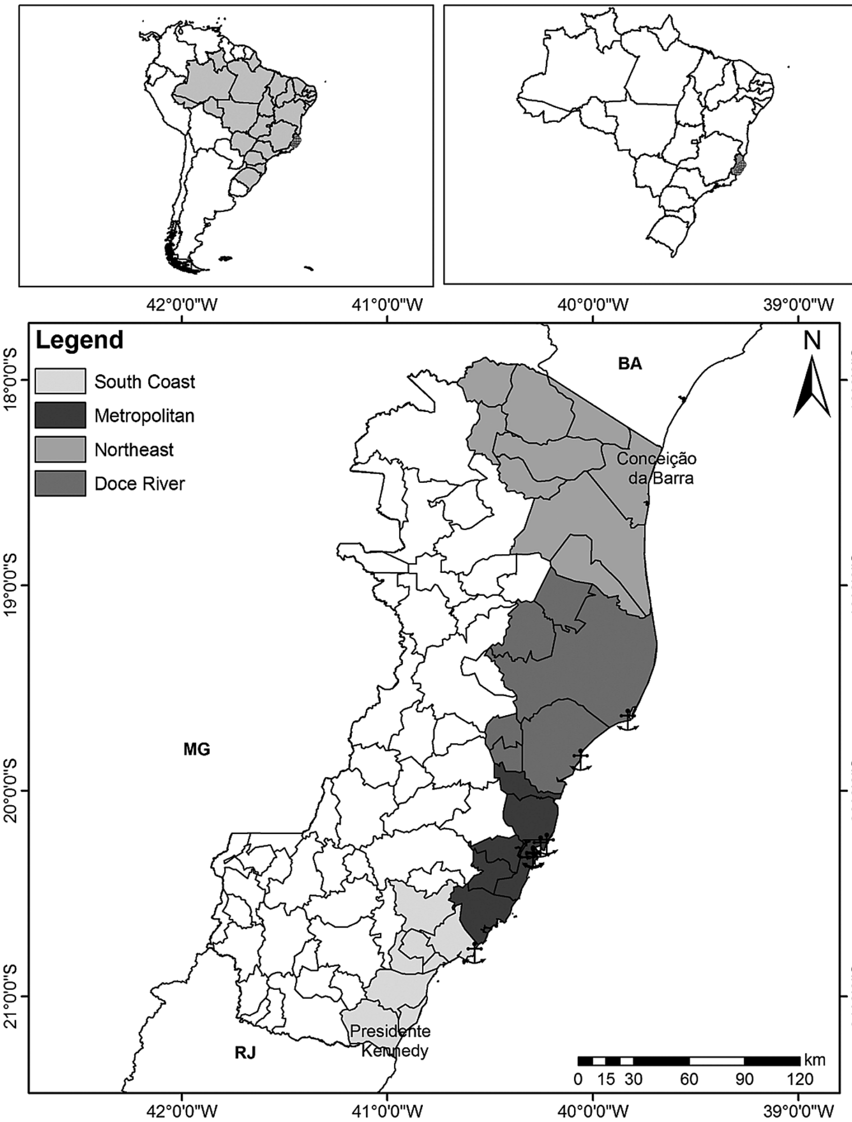

Fig. 1.—Map showing the geographical state limits where this study was conducted (Espírito Santo), southeastern Brazil, and the sites where harbors are located (anchors).

external measurements (W_{tl}, CaL_{tl}, and CrL_{tl}), thoracic limb bone measurements (W_{hum}, L_{hum}, W_{uln}, L_{uln}, W_{rad}, and L_{rad}), and BMD of radius, ulna, and humerus using the Shapiro–Wilk (W) test. The Student's t-test parametric was used to evaluate differences between right and left thoracic limb and between thoracic limb from males and females for variables with a normal distribution. For the other variables, the nonparametric Mann–Whitney test (U) was used. The correlation of the BMD values of radius, ulna, and humerus with age was adjusted to the model of nonlinear correlation (Spearman's r).

Descriptive statistics was used to calculate mean, maximum and minimum values, and *SD*.

Nonlinear regression models of growth were tested using the Akaike information criterion (AIC—Akaike 1974) to assess the best growth profile for all variables correlated with age. The corrected AIC (AIC_c) was calculated using the sum of squared errors (SSE), number of parameters estimated, including residual variance (θ), and sample size (n). The differences between AIC_c values (Δ), likelihood probability (w), and evidence ratio (ER) were calculated using the equations of Brody, Gompertz, and Richards described by Vieira et al. (2012).

The final inflection time (t_f) for the model with the best prediction power was calculated to estimate exact age development ceases, using the equation:

$$t_f = \frac{-1}{k}\ln\left(\frac{y_f - 0.95y_f}{y_f - y_0}\right)$$

The allometric model developed by Huxley and Teissier (1936) was used to scale the variables of external and internal measurements of thoracic limb in relation to TBL, with the equation:

$$Y = \alpha . X^{\beta}$$

Allometry.—Where X is the TBL, Y is the measurement of one variable required, α is the integration constant that defines the intercept with the y-axis, and β is the measurement of the proportion of relative growth rates or allometry coefficient. If $\beta = 1$, there is isometry (isometric growth), if $\beta < 1$, allometry is negative (early growth), and if $\beta > 1$, allometry is positive (late growth).

The AIC (Akaike 1974) was also used to evaluate the occurrence of scaling in variables, comparing the parameters associated with allometric equations with the parameters of the means of variables investigated.

The accuracy of the DXA technique was evaluated using the correlation between weight (g) of ash and BMD of the humerus using linear regression, in the equation $Y = a + bx$ (where y is the ash weight, a and b are constants, and x is the BMD of the humerus). Linear models with and without intercepts were also tested using the Akaike criterion to apply the model that best fits the data. Precision was obtained using the coefficient of variation (%).

Dolphins were sorted based on the location they were found to test the differences in BMD. These locations were 4 geographical microregions in the state of Espírito Santo, Brazil. Data were organized separately, using the growth curve model that best fits the respective confidence intervals, in the regions that include the Northeast (n = 3) and Rio Doce (n = 5), the Greater Vitória region (n = 11), and South Coast (n = 18). The animals from the Northeast and Rio Doce regions were grouped, since they numbered fewer than the other animals.

All analyses were carried out using the SAS software (SAS Institute 2002) version 9.0.

Results

Of the 43 dolphins analyzed, 24 were males, 11 were females, and 8 did not have their gender determined. Age varied between 0 and 35 years (n = 37) and TBL ranged from 122 to 206 cm (n = 42). Means, maximum and minimum values, *SD*, and *d.f.* of all variables are shown in Table 1. Except for the variable BMD ($P > 0.05$), no other variable followed the normal distribution ($P < 0.05$). Side-of-body asymmetry and sexual dimorphism were not detected ($P > 0.05$). Therefore, side and gender did not require separate calculations.

A significantly positive strong correlation was observed between the BMD of humerus and radius and age ($r > 0.7$). A moderate correlation was observed between the BMD of ulna and age ($0.4 < r < 0.7$; $r = 0.78$, $r = 0.73$, $r = 0.64$; $d.f. = 36$, $P < 0.05$).

Table 2 shows the estimates obtained for the parameters of the 3 models in the adjustment of growth curves of all variables of the Guiana dolphin using the AIC. All nonlinear models studied presented appropriate fit. The Gompertz and Brody model exhibited the best results for all variables (ER closer to 1). However, since the beginning of the growth curve of the Guiana dolphin (Fig. 2) exhibited an exponential pattern, the Brody curve is more suitable in this study.

The respective parameters of the growth curve equation are given in Table 3, together with the t_f values, in which the exact age when growth stabilizes is observed. Anterior and posterior lengths and width of thoracic limb measured externally reached asymptotic growth at 25.7, 19.9, and 10.7 cm, respectively, at the age of 2. Length of humerus and ulna stabilized at 5.6 and 6 cm at the ages of 2.7 and 2.3 years, respectively, while radius length reached 6.9 cm a little later: 3.6 years of age. Width of humerus and ulna reached 2.2 and 1.66 cm (respectively, both at the age of 3.4), while width of radius reached 3 cm (at the age of 5.4).

The data show that BMD increased exponentially with age, but only in the beginning of the development process. After a peak (Fig. 2), a stabilization stage started at the ages of 13, 14, and 16 years for humerus, ulna, and radius, respectively (Table 3).

Negative allometry ($\beta < 1$; early growth) was seen for measurements of the thoracic limb (Table 4). The parameters of the AIC, likelihood probability (w = 0.9999), and evidence ratio (ER = 1) demonstrate the scaling of variables in the allometric model.

The linear regression with intercept showed the correlation between BMD of radius and ash mass, as revealed by the parameters of the AIC (w = 0.999, RE = 1), as opposed to the regression without intercept ($w = 9.68 \times 10^{-6}$, RE = 10.4197.36) and R^2 = 0.9181 (Fig. 3). The coefficient of variation for the precision analysis was 7.8%.

Table 1.—Mean, maximum and minimum values, *SD*, and *d.f.* of characteristics measured for *Sotalia guianensis* specimens recovered from the coast of Espírito Santo, Brazil, between 2009 and 2013. Variable definitions as defined in "Materials and Methods."

Variables	$\bar{X}$	Maximum	Minimum	*SD*	*d.f.*
Age (years)	10.28	35	0	±9.44	37
TBL (cm)	176.20	206	122	±19.40	37
BMD humerus (g/cm^2)	1.03	1.46	0.62	±0.19	37
BMD ulna (g/cm^2)	0.49	0.66	0.30	±0.09	37
BMD radius (g/cm^2)	0.63	0.95	0.40	±0.11	37
CaL_{lt} (cm)	26.74	47	18	±4.41	36
CrL_{lt} (cm)	20.08	28	7.5	±3.91	36
W_{lt} (cm)	11.08	13.5	7	±1.46	36
L_{rad} (cm)	6.74	7.47	5.31	±0.47	32
W_{rad} (cm)	2.92	3.32	1.98	±0.29	32
L_{uln} (cm)	5.91	6.43	4.61	±0.39	32
W_{uln} (cm)	1.60	1.98	1.09	±0.19	32
L_{hum} (cm)	5.53	6.31	3.82	±0.44	32
W_{hum} (cm)	2.20	2.49	1.66	±0.16	32

Table 2.—Values of the Akaike information criterion to test 3 growth curve models for the morphometric and bone density variables measured for *Sotalia guianensis* specimens recovered from the coast of Espírito Santo, Brazil, between 2009 and 2013. Variable definitions as defined in "Materials and Methods." *SSE* = sum of squared errors and AIC_c = corrected Akaike information criterion.

Variable	Model	*SSE*	AIC_c	ΔAIC	*w*	*RE*	Θ	*n*
TBL (cm)	Brody	2717.1	148.98	0	0.459	1	3	37
	Gompertz	2733.3	149.17	0.19	0.417	1.09	3	37
	Richards	2717.1	151.61	2.624	0.123	3.71	4	37
BMD humerus (g/cm^2)	Brody	0.4755	−127.83	0	0.47	1	3	37
	Gompertz	0.4802	−127.52	0.314	0.402	1.17	3	37
	Richards	0.4755	−125.21	2.624	0.126	3.71	4	37
BMD ulna (g/cm^2)	Brody	0.1729	−160.2	0.018	0.423	1	3	37
	Gompertz	0.1728	−160.22	0	0.427	1	3	37
	Richards	0.17	−158.12	2.101	0.149	2.86	4	37
BMD radius (g/cm^2)	Brody	0.254	−147.89	0	0.445	1	3	37
	Gompertz	0.2544	−147.84	0.05	0.434	1.02	3	37
	Richards	0.254	−145.27	2.624	0.119	3.71	4	37
CaL_{tl} (cm)	Brody	1921.4	137.89	0.005	0.436	1	3	36
	Gompertz	1921.1	137.89	0	0.437	1	3	36
	Richards	1913.2	140.38	2.492	0.125	3.47	4	36
CrL_{tl} (cm)	Brody	1116.6	120.53	0.083	0.415	1.04	3	36
	Gompertz	1113.7	120.44	0	0.432	1	3	36
	Richards	1095.4	122.54	2.094	0.151	2.85	4	36
W_{tl} (cm)	Brody	276	75.8	0.035	0.428	1.02	3	36
	Gompertz	275.7	75.77	0	0.435	1	3	36
	Richards	273.1	78.09	2.321	0.136	3.19	4	36
L_{rad} (cm)	Brody	2.4323	−75.6	0.069	0.368	1.03	3	32
	Gompertz	2.427	−75.67	0	0.381	1	3	32
	Richards	2.2955	−74.83	0.841	0.25	1.52	4	32
W_{rad} (cm)	Brody	0.7472	−113.37	0	0.471	1	3	32
	Gompertz	0.7548	−113.04	0.324	0.401	1.17	3	32
	Richards	0.7472	−110.74	2.624	0.127	3.71	4	32
L_{uln} (cm)	Brody	1.8551	−84.27	0.164	0.376	1.08	3	32
	Gompertz	1.8456	−84.43	0	0.408	1	3	32
	Richards	1.7698	−83.15	1.282	0.215	1.89	4	32
W_{uln} (cm)	Brody	0.3349	−139.05	0.115	0.424	1.06	3	32
	Gompertz	0.3337	−139.16	0	0.449	1	3	32
	Richards	0.333	−136.6	2.557	0.125	3.59	4	32
L_{hum} (cm)	Brody	1.7772	−85.64	0.387	0.357	1.21	3	32
	Gompertz	1.7558	−86.03	0	0.434	1	3	32
	Richards	1.6937	−84.56	1.472	0.208	2.08	4	32
W_{hum} (cm)	Brody	0.4166	−132.06	0	0.458	1	3	32
	Gompertz	0.419	−131.88	0.184	0.418	1.09	3	32
	Richards	0.4166	−129.44	2.624	0.123	3.71	4	32

The curves obtained for the 3 microregions did not differ, according to the amplitude of confidence intervals (Fig. 4).

Discussion

Growth model.—As expected, no sexual dimorphism was observed in the Guiana dolphins examined in the present study. According to Di Beneditto and Ramos (2004), the species does not present sexual dimorphism, as observed in cranial morphometry studies (Borobia 1989) and odontometry investigations (Ramos et al. 2000b).

Growth curves are an appropriate parameter whenever the variable age encompasses all growth stages (Fekedulegn et al. 1999). Although growth varies across individuals, it follows a well-defined pattern in all animal populations, in terms of age (Brody 1945; Arango and Vleck 2002). The main nonlinear models used to describe growth rates are Brody, Von Bertalanffy, Gompertz, logistic, and Richards (Mendes 2007).

The AIC was the diagnosis tool that best fits in the variables studied. According to Rocha (2013), this method may be used to indicate, with greater accuracy, the best growth profile, as opposed to the determination coefficient (R^2) and the residual *SD*, used in most studies.

The Gompertz model is conventionally used in studies of the morphology of dolphins (Fernandez and Hohn 1998; Ramos et al. 2000a, 2010, 2002; Di Beneditto and Ramos 2004). However, in the study by Ramos et al. (2010), it did not show good fit with the variables TBL and age of Guiana dolphins in Espírito Santo, Brazil, when the asymptote was reached at the age of 10.

In the present study, based on the results obtained, although all models presented appropriate fit for the variables studied, the Brody model offered the best fit, due to the exponential behavior of the beginning of the curve. Moreover, the curve does not evolve following a sigmoidal pattern and does not present an inflection point.

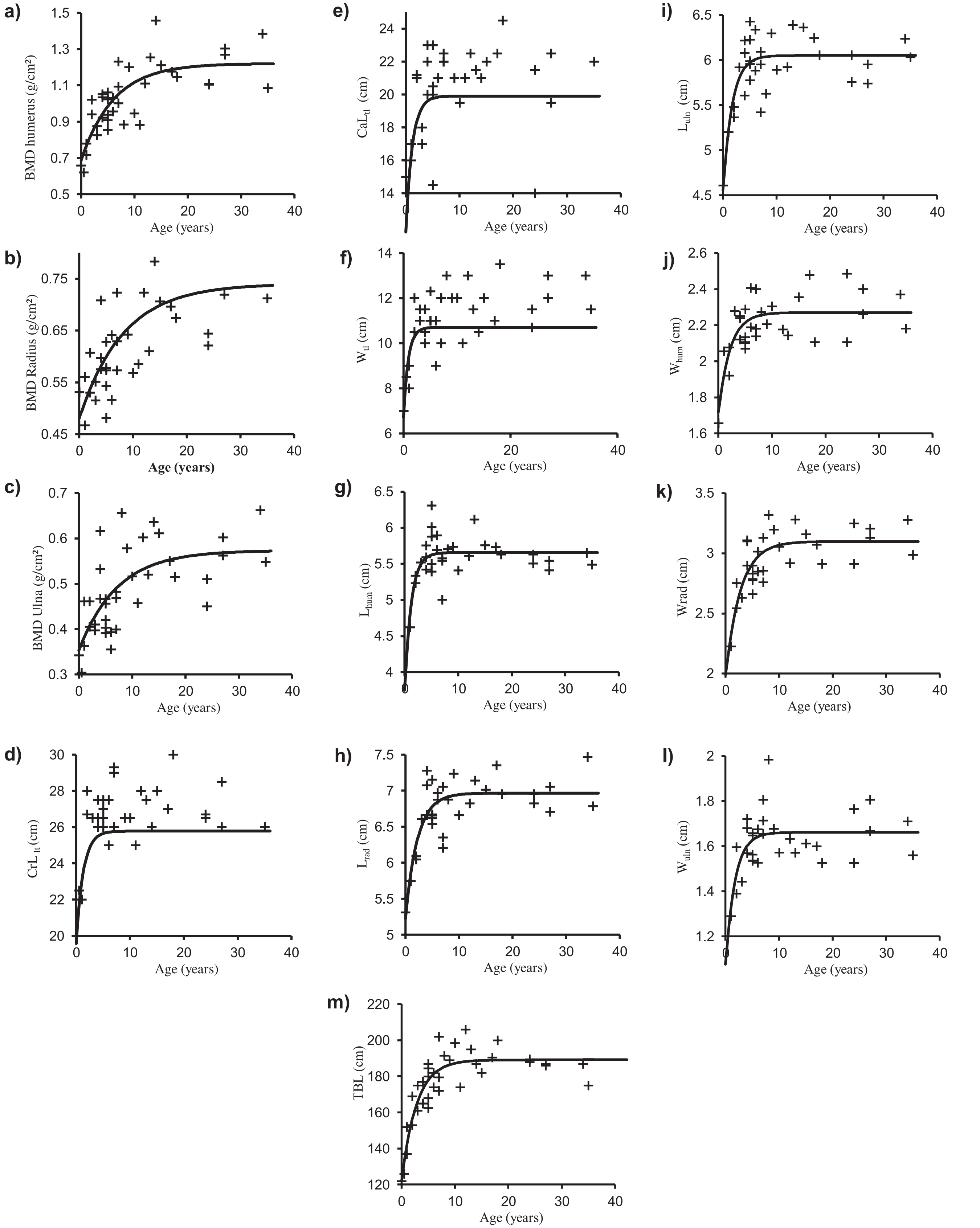

Fig. 2.—Growth curves according to the Brody model for the variables: a) bone mineral density (BMD) of humerus, b) BMD of radius, c) BMD of ulna, d) CrL_{tl}, e) CaL_{tl}, f) W_{tl}, g) L_{hum}, h) L_{rad}, i) L_{uln}, j) W_{hum}, k) W_{rad}, l) W_{uln}, and m) TBL of *Sotalia guianensis* on the coast of the state of Espírito Santo, Brazil.

Table 3.—Parameters of the growth curve according to the Brody model for the morphometric and bone density variables measured for *Sotalia guianensis* specimens recovered from the coast of Espírito Santo, Brazil, between 2009 and 2013. t_f indicates the exact age when growth stabilizes. Other variable definitions are as defined in "Materials and Methods."

Brody variables	Parameters			
	y_0	y_f	k	t_f
TBL (cm)	121.70	189.1	0.3584	5.48
BDM humerus (g/cm^2)	0.69	1.2208	0.1633	13.28
BMD ulna (g/cm^2)	0.35	0.5735	0.1386	14.71
BMD radius (g/cm^2)	0.48	0.7403	0.1215	16.03
CaL_{tl} (cm)	19.57	25.7828	0.7618	2.06
CrL_{tl} (cm)	11.65	19.9045	0.8131	2.60
W_{tl} (cm)	6.71	10.7075	1.1194	1.80
L_{rad} (cm)	5.23	6.966	0.4434	3.63
W_{rad} (cm)	1.99	3.0987	0.3583	5.49
L_{uln} (cm)	4.56	6.0501	0.5873	2.72
W_{uln} (cm)	1.08	1.6614	0.5622	3.47
L_{hum} (cm)	3.75	5.6572	0.8111	2.35
W_{hum} (cm)	1.72	2.2703	0.4581	3.46

Table 4.—Parameters and respective confidence intervals of the allometric equation for the morphometric and bone density variables measured for *Sotalia guianensis* specimens recovered from the coast of Espírito Santo, Brazil, between 2009 and 2013. Variable definitions as defined in "Materials and Methods."

Variables (cm)	Estimated parameters					
	α	95% *CI*		β	95% *CI*	
		Minimum	Maximum		Minimum	Maximum
CaL_{tl}	3.6805	13.6437	21.0047	0.372	0.538	1.282
CrL_{tl}	1.1650	−4.5930	6.9230	0.542	−0.413	1.496
W_{tl}	0.8228	−2.8301	4.4757	0.492	−0.366	1.349
L_{rad}	0.4525	0.0152	0.8899	0.521	0.890	0.707
W_{rad}	0.0441	−0.0163	0.1044	0.808	0.545	1.072
L_{uln}	0.5270	0.0212	1.0327	0.466	0.281	0.651
W_{uln}	0.0551	−0.0321	0.1423	0.650	0.345	0.954
L_{hum}	0.2864	−0.0395	0.6124	0.571	0.352	0.790
W_{hum}	0.1496	−0.0202	0.3194	0.518	0.300	0.737

Bone maturity.—The TBL of the Guiana dolphin in Espírito Santo, Brazil, asymptotic growth was reached at 189 cm and 5.5 years of age, according to the Brody model. Differences are observed in animals from the states of São Paulo and Paraná in Brazil, where the asymptotic growth was reached at 182.6 cm (Schmiegelow 1990) and 179.8 cm (Santos et al. 2003), and in animals from northern Rio de Janeiro which reached the asymptote at 191 cm (Di Beneditto and Ramos 2004). For Ramos et al. (2000a), the difference in the value at which total length reaches the asymptote may be a consequence of the different models used, of the absence of age groups used in the analyses, or even of differences between populations.

The development and structure of bones are some of the most interesting anatomical aspects in cetaceans. These parameters reveal a great deal of information about adaptation to the environment, and analysis can indicate how flippers evolved (Felts 1966). Studies on physical maturity mainly focus on TBL and when other morphometric variables correlated with age are used, the focus is on the differentiation of populations, as in the study by Ramos et al. (2010) and Lima (2012). Additionally, studies that consider stages of epiphyses ossification in the thoracic limb, such as in Guiana dolphins, use age and TBL to assess bone maturity (Ogden et al. 1981; Calzada and Aguilar 1996; Di Giancamillo et al. 1998; Carvalho 2011). However, few studies have investigated the development and growth stages of different parts of the skeleton, like the flipper bones (Calzada and Aguilar 1996).

The lengths of the radius and ulna of the dolphins examined in the study were shorter than that of the humerus, as reported for *Stenella coeruleoalba* (Calzada and Aguilar 1996). However, the Guiana dolphins examined reached bone maturation earlier in life, that is, between 2 and 3 years of age, compared with *S. coeruleoalba*, which reached between 5 and 6 years.

The age (between 2 and 3 years) when maximum forelimb length is reached coincides with the age when total tooth length reaches its asymptote and Guiana dolphin teeth cease to grow (Azevedo 2012). This may reflect the need to be prepared, at this age, to capture prey, since mothers breastfeed their offspring until they are 9 months old (Rosas et al. 2010). In Fish (2002), the thoracic limb controls the position of the body and consistently influences the precision of stability and maneuvers during movements in the water. These behaviors are directly associated with the type of prey and habitat. Arnould et al. (2003) confirm this notion, stating that the offspring has to optimize growth and skills before the end of maternal dependence, in order to meet the requirements of an independent life.

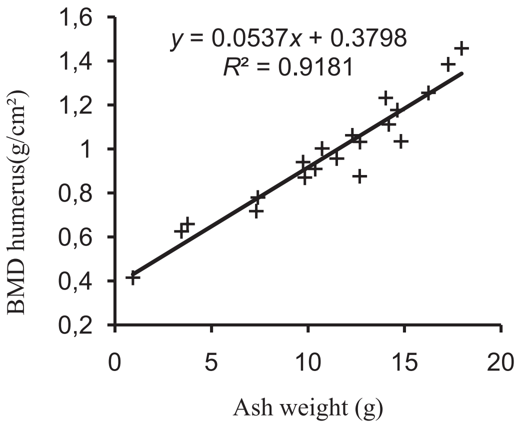

Fig. 3.—Correlation between ash weight and bone mineral density (BMD) of the humerus of the Guiana dolphin, *Sotalia guianensis*, salvaged from the coast of the state of Espírito Santo, Brazil, using linear regression with intercept and the respective equation and determination coefficient (R^2).

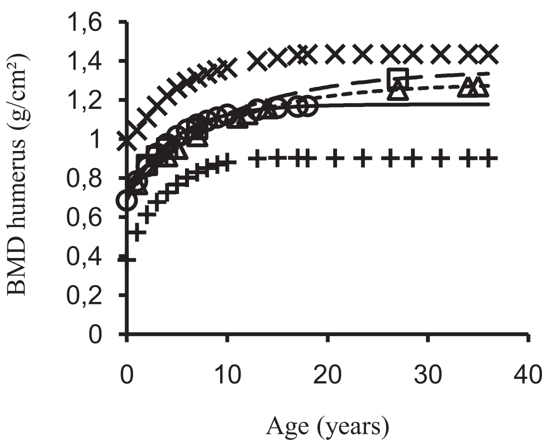

Fig. 4.—Growth curve according to the Brody model with confidence intervals to compare bone mineral density (BMD) of the humerus of the Guiana dolphins, *Sotalia guianensis*, salvaged from the coast of the state of Espírito Santo, Brazil: □ (Northeast and Rio Doce); Δ (Greater Vitória Region); ○ (South Coast).

The negative allometric growth observed for all variables studied in the thoracic limb of the Guiana dolphins examined indicates that these limbs grow to a smaller proportion, when compared to TBL. In other words, thoracic limbs develop fast and early in life before the animal reaches its full adult size. The same behavior was observed in measurements of thoracic limb of *Phocoenoides dalli* (Amano and Miyazaki 1993), when thoracic limbs were shown to grow rapidly between birth and weaning, and of *Delphinus delphis* (Murphy and Rogan 2006), when the same allometry was observed.

Bone mass.—Protocols to use DXA in humans have been applied in small and large animals (Grier et al. 1996). Nevertheless, though it has been used in cetaceans, its precision and accuracy have never been tested, as with mice (Hagiwara et al. 1993; Cazes et al. 1994; Rozenberg et al. 1995). For Rozenberg et al. (1995), precision depends on several factors, including the position of the animal, the definition of the region to be analyzed, and the intrinsic precision of the equipment, and may be between 1% and 8%, depending on the study site. In this sense, the results of the present study demonstrate the precision of the DXA protocol used. In addition, BMD of the humerus presented high linear correlation with ash weight, which supports the accuracy of the technique (Fig. 3).

During the evolution of aquatic mammals, the bone mass of terrestrial ancestors underwent the required adaptations to life in aquatic environments (Taylor 2000). Paleontological records show the increase in bone density at the early adaptation stage for aquatic life (Felts 1966), which lent the skeleton the role of a "bone ballastin," to overcome floatability (Houssayer 2009).

These characteristics were observed, for a short time, in cetaceans and pinnipeds, though they remained present in sirenians (de Buffrénil and Rage 1993). The development of the ability to collapse lungs, which is essential to overcome floatability during deep dives, led to a secondary reduction of density in the limbs of cetaceans and of pinnipeds (Wall 1983). The decrease in bone density documented is characterized by the replacement of the cortical bone (the compact bone tissue) and of its medullary cavities by cancellous bone tissue (Mass 2009) and was followed by the development of a dense conjunctive tissue that helped maintain the strength of these limbs (Berta et al. 2006).

The bone ballastin reduces the ability to speed up and to carry out maneuvers in the water (Taylor 2000), which may lead to an increase in energy consumption in sea mammals that need to swim fast and remain submerged for longer periods, for feeding, for instance (Taylor 1994).

Therefore, the differences in bone mass across families or even species may be due to distinct behaviors, like feeding habits (suction and capture), dive depths, and aggressive behavior (Cozzi et al. 2010).

The results of the measurements show that mean BMD of the humerus (1.030 g/cm², *d.f.* = 37) of the Guiana dolphin was higher than that observed in the works by Gugliemini et al. (2002), Butti et al. (2007), and Lucic et al. (2010), which showed that the mean obtained with raw data was 0.99998 g/cm² for *S. coeruleoalba*, 0.837435 g/cm² for *Tursiops truncatus*, and 0.737583 and 0.89425 g/cm² for *S. coeruleoalba* and *T. truncatus*, respectively.

Gray et al. (2007) confirmed these findings and stated that high bone density allows the animal to control floatability in shallow waters. For Lucic et al. (2009), high bone density of the thoracic limb is associated with slow and precise maneuvers, which are carried out with greater mobility. In this sense, the higher bone mass of the humerus of the thoracic limb observed in the present study may be the result of the adaptation of the Guiana dolphin to the shallow waters in the coastal environment this species lives in (Borobia et al. 1991), when compared to *T. truncatus* and *S. coeruleoalba*, which inhabit mesopelagic and pelagic zones, respectively (Lucic et al. 2010).

It should be stressed that other adaptations, such as the size of lungs and thickness of the fat layer also affect floatability, though BMD may be considered a key feature in the adaptation of aquatic mammals (Taylor 2000).

Age is one of the factors that affects bone mass of mammals. A high correlation between age and BMD of the humerus of odontocetes has been reported (Guglielmini et al. 2002; Butti et al. 2007; Lucic et al. 2010). However, in spite of the strong correlation between BMD and age, the data clearly show that BMD increases exponentially with age only in the beginning of development and that, after a peak, stabilization of the humerus, ulna, and radius start at the ages of 13, 14, and 16 years, respectively (t_f values; Table 3). Sonne et al. (2004) also reported an increase in BMD with age in polar bears, but only in subadult individuals. In adults, the authors observed stability. This pattern is a result of the cyclic bone production and resorption process. Bone production is higher than resorption in young individuals, and there is a balance in the adult phase (Pessoa et al. 1997).

Due to these characteristics of bone mass, age of Guiana dolphins could only be estimated based on densitometry results of the forelimb up to the age the peak is reached, different from what was observed by Guglielmini et al. (2002), Butti et al. (2007), and Lucic et al. (2010), who reported that BMD of thoracic limb of odontocetes may be used as a new standard to estimate age. This difference is possibly explained by the fact that the animals used in these studies did not cover all age groups, since they were essentially in the growing stage, which explains why data fit linear regression models.

Therefore, if age is considered an important parameter to know the likely cause of death and the general biology of animals in nature (Butti et al. 2007), and if the limitations of the technique described by Hohn et al. (1989) are taken into account, which include the experience of observers, time in laboratory, and the need to dissect carcasses (Guglielmini et al. 2002), it is possible to state that the measurement of BMD of the forelimb may be used as a complementary and/or alternative technique to the method described by Hohn et al. (1989), for animals up to the age of approximately 13.

The increase in BMD occurs in parallel with skeleton maturity (Katzman et al. 1991). If we consider skeleton maturity of Guiana dolphin to occur around 6 years of age and 189 cm in length, the increase in bone mass in this species goes beyond the physical maturity stage. However, it should be stressed that Fettuccia (2001) estimated physical maturity of the Guiana dolphin at 12 years based on the fusion of vertebral body epiphyses and that Chivers (2002) believes that physical maturity is reached after fusion of vertebral epiphyses.

Influence of pollutants.—Pollutants negatively affect estrogen levels, which are an important regulation factor in the speed of reorganization and renovation of bones (Swenson 1984). BMD of *Halichoerus grypus* (grey seal—Lind et al. 2003) and the *U. maritimus* (polar bear—Sonne et al. 2004) varied significantly considering periods of different pollution degrees. The greater Vitória region is where most harbors and freight terminals are located in the state of Espírito Santo. Domestic sewage and harbors are responsible for the highest concentrations of heavy metals in the region (Jesus et al. 2004). In spite of the likely difference in environmental pollution between different regions, the growth curves of animals collected in the 3 microregions surveyed in the present study did not differ significantly (Fig. 3). However, more detailed studies are required, since low BMD is associated with bone diseases, alveolar loss, and tooth decay (Lind et al. 2003). If, according to Van Bressem et al. (2007), malformation may lead to fractures and therefore facilitate capture in fishing nets, low BMD values could be associated with these factors.

More studies have to be carried out to shed more light on the effect of pollution on bone development of sea mammals in their habitats. In this sense, knowledge about bone maturation and deposition patterns is important, since it affords to detect any abnormality.

Acknowledgments

We thank Organização Consciência Ambiental for the material used, Laboratório de Morfologia e Patologia Animal of Universidade Estadual Norte Fluminense, Laboratório de Mamíferos Aquáticos e Bioindicadores of Universidade Estadual do Rio de Janeiro, and Departamento de Saúde of Universidade Federal de Viçosa.

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Submitted 9 June 2014. Accepted 20 November 2014.

Associate Editor was Jeanette A. Thomas.