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Physical maturity of the vertebral column of *Tursiops truncatus* (Cetacea) from Southern Brazil

Maturidade física da coluna vertebral de *Tursiops truncatus* (Cetacea) do sul do Brasil

Ana Paula Borges Costa[1*]
costa.aninha@gmail.com

Paulo César Simões-Lopes[1]
lamaqsl@ccb.ufsc.br

Abstract

Age, sex and total length are important variables for the analysis of ontogeny, sexual dimorphism and geographic variation among cetaceans. The physical maturity of cetaceans can be determined through analysis of the fusion of vertebral epiphyses. The animal is considered physically mature when all the epiphyses are fused to the vertebral body and the body growth ceases. The focus of the study is to determine physical maturity of the spine of *Tursiops truncatus* and compare with the age of individuals, which was obtained from the counting of Growth Layer Groups (G.L.G.). Twenty-four specimens were analyzed for the degree of epiphyseal fusion. As a result three vertebral maturation patterns were identified: Pattern 1 - vertebral column without fusion of epiphyses or fusion initiating only in cervical epiphyses (from zero to two years old); Pattern 2 - column with four degrees of fusion of epiphyses (from two to eleven/twelve years old); and Pattern 3 - column completely fused (more than eleven/twelve years old). For *T. truncatus* from the coast of Santa Catarina, southern Brazil, the fusion of vertebral epiphyses begins rapidly in the cervical region, followed by the caudal region, with the thoracic and lumbar vertebrae being the last to undergo fusion. Sexual dimorphism may be present during the maturation process of these animals, which became physically mature between eleven and fifteen years old.

Key words: bottlenose dolphin, growth, vertebral epiphyses, age.

Resumo

A idade, o sexo e o comprimento total são variáveis importantes para a análise da ontogenia, dimorfismo sexual e variação geográfica dos cetáceos. A maturidade física dos cetáceos pode ser determinada através da fusão de epífises vertebrais. Quando todas as epífises estiverem fusionadas ao corpo da vértebra, o crescimento corporal cessará e o animal será considerado fisicamente maduro. Tendo *Tursiops truncatus* como foco de estudo, objetivou-se determinar a maturidade física da coluna vertebral comparando-a com as idades obtidas pelas leituras de G.L.G. (em português, Grupo de Linhas de Crescimento, de acordo com Schultz (1996)). Foram analisados 24 exemplares de acordo com o grau de fusão das epífises. Três padrões de amadurecimento vertebral foram diagnosticados: Padrão 1 - coluna vertebral sem fusão de epífises ou estas iniciando apenas nas cervicais (de zero a dois anos); Padrão 2 - coluna com os quatro graus de fusões de epífises (de dois a onze/doze anos); e Padrão 3 - coluna completamente fusionada (mais de onze/doze anos). Para *T. truncatus* do litoral de Santa Catarina, sul do Brasil, a fusão das epífises vertebrais inicia-se rapidamente na região cervical, seguida da região caudal, sendo as vértebras torácicas e lombares as últimas a fusionarem. O dimorfismo sexual pode estar presente durante o processo de amadurecimento desses animais, os quais se tornam fisicamente maduros entre os onze e quinze anos.

Palavras-chave: boto-da-tainha, crescimento, epífises vertebrais, idade.

[1] Laboratório de Mamíferos Aquáticos (LAMAQ), Depto. de Ecologia e Zoologia, Universidade Federal de Santa Catarina, Campus Universitário, Trindade, Cx. Postal 5102, 88040-970, Florianópolis, SC, Brasil.
*Author for correspondence.

Introduction

The relationship between age, sex and total body length can be helpful in studies of ontogenetic, sexual and geographic variation among cetaceans (Ramos *et al*., 2000).

The age at which animals stop growing and become physically mature can clarify many taxonomic variables (Mead and Potter, 1990). In the nineteenth century, zoologists noted the existence of lines in the teeth of marine mammals, but it was only in the following century that these dentinal growth layers were associated with age and defined as Growth Layer Groups (G.L.G.) (Scheffer and Myrick, 1980). Direct evidence of the annual deposit of dentin layers was established for a few species of cetaceans, with most studies being conducted on bottlenose dolphins (Hohn *et al*., 1989; Hohn, 1990; Cockcroft and Ross, 1990).

Although *Tursiops truncatus* is the most studied dolphin species, only a few studies have been conducted on its growth and reproduction, specifically with regard to the physical maturity of its vertebral column (Sergeant *et al*., 1973; Cockcroft and Ross, 1990; Read *et al*., 1993; Fernandez and Hohn, 1998; Pribanic *et al*., 2000).

The physical maturity of cetaceans can be determined by the degree of fusion of the vertebral epiphyses. If all the epiphyses are fused to the vertebral body, the growth of the individual will cease and the animal can be considered physically mature (Mead and Potter, 1990; Rommel, 1990). Sexual maturity, on the other hand, varies with sex and geographic region (Wells and Scott, 1999) and usually occurs before physical maturity (Mead and Potter, 1990; Wells and Scott, 1999; Galatius and Kinze, 2003).

On the east coast of the United States, *Tursiops truncatus* reaches physical maturity at approximately 13 years (Mead and Potter, 1990; Wells and Scott; 1999). Females reach sexual maturity at 7-14 years (Mead and Potter, 1990) and males at 10-13 years (Sergeant *et al*., 1973; Odell, 1975). Females and males in southern Africa became sexually mature at 9-11 years and 10-12 years, respectively, whereas physical maturity is achieved at 12-15 years in this region (Cockcroft and Ross, 1990; Wells and Scott, 1999).

The aim of this study was to determine when physical maturity of the vertebral column is reached in *Tursiops truncatus* of the southern coast of Brazil, by the determination of the degree of fusion of vertebral epiphyses and its correlation with the Growth Layers Groups (G.L.G.).

Materials and methods

We analyzed the vertebral columns of 24 specimens of *Tursiops truncatus* deposited in the collection of mammals of the Departamento de Ecologia e Zoologia, Universidade Federal de Santa Catarina: UFSC 1011, 1072, 1099, 1103, 1105, 1106, 1123, 1126, 1209, 1210, 1225, 1249, 1252, 1281, 1285, 1295, 1299, 1317, 1322, 1334, 1349, 1350, 1361 and 1368. Of these specimens, ten were males, six were females and eight were of unknown sex. The regions of the vertebral column were defined according to the classical system based on Slijper (1979), Rommel (1990) e Crovetto (1991): cervical (C), thoracic (T), lumbar (L) and caudal (Ca). The specimens were separated into patterns of column fusion based on the degree of fusion of the epiphyses to the vertebral body, as well as analysis of the G.L.G.

The degree of epiphyseal fusion of the vertebral column was based on Galatius and Kinze (2003) (Figure 1): (A): no fusion - both epiphyseal plates free or not yet ossified; (B): initial fusion - at least one epiphyseal plate loosely fused to the centra; (C): progressing fusion - both epiphyseal plates fused to the centra, displaying clear sutures; (D): complete fusion - both epiphyseal plates fused to the centra, displaying no sutures.

Specimens in the initial growth phase, i.e., with the entire vertebral column in degree A of epiphyseal fusion or with the beginning of epiphyseal fusion only on the cervical vertebrae, were considered calves in the broad sense (Pattern 1). Specimens in an intermediary or a final phase of growth and with four degrees of epiphyseal fusion through the entire vertebral column included juveniles, sub-adults and sexually mature adults (Pattern 2). Specimens that had the entire column in degree D of epiphyseal fusion were considered adults that had reached physical maturity (Pattern 3).

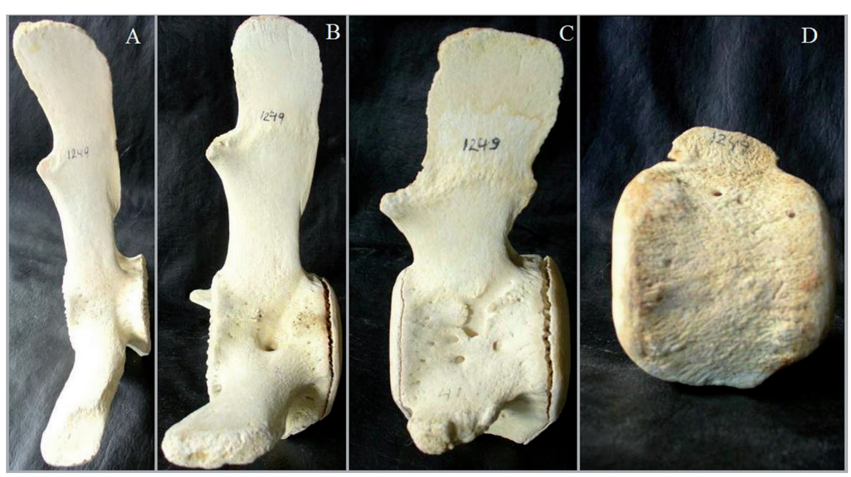

Figure 1. Categories of epiphyseal fusion according to Galatius and Kinze (2003) (see text for details about the degrees of fusion). Degrees A, B, C and D.

The preparation of teeth for G.L.G. counting was performed according to the method of Pierce and Kajimura (1980) with adaptations by Schultz (1996). G.L.G. of specimens UFSC 1072, 1099, 1103, 1105, 1106, 1123, 1126 and 1209 was analyzed previously by Schultz (1996).

Growth Layer Groups (G.L.G.) are concentric layers of dentine presented in the odontocete teeth and it can be used as a method of age determination (Scheffer, 1950). According to Sergeant (1959) the deposition of G.L.G. in *Tursiops truncatus* teeth is annually, i.e., a rate of one layer is set down per year.

For each individual, a longilineal tooth from the maxilla or mandible was chosen and embedded with transparent acrylic resin. The tooth was then abraded to its sagittal plate with an electric grinder. After moistening of the tooth with a solution of soapy water, it was sequentially polished with sandpaper of varying weight (300, 400 and 600), decalcified with formic acid (25%) for 1.5 hours and then washed in tap water. The specimen age was determined using a stereomicroscope (Zeiss, model GSC).

The specimens were separated by sex, whenever possible during the collecting activity, based on the morphology of the genital region of its carcass.

Results

Of the twenty-four specimens analyzed, nine did not have any sign fusion of epiphyses (degree A) or had fusions that were just starting in the cervical vertebrae (degrees B and C) (Table 1). These specimens were included in Pattern 1 of ontogenetic development of the vertebral column, corresponding to fetuses, newborn animals and animals less than two years old (2 G.L.G.), i.e., calves in the broad sense. The male calf UFSC 1368, 3-years-old, seems to be an exception in this group.

Specimens with various degrees of epiphyseal fusion (degrees A, B, C and D) belong to Pattern 2 of ontogenetic development of the vertebral column. These animals include a wide range of age categories corresponding to juvenile, sub-adult and sexually mature adults that have not yet reached physical maturity. Pattern 2 is found in individuals older than two years and is characterized by accelerated maturation of the caudal region (Table 1).

At this stage, the cervical region is already at an advanced degree of maturation, with 88% of the vertebrae of all specimens at degree C or D of epiphyseal fusion (Figure 2). The thoracic and lumbar regions were almost completely engaged in the early stages of development (degree A) (Figures 3 and 4). In the caudal region, the accel-

Table 1. Relationship between age and degree of epiphyseal fusion in each vertebral column sector (cervical, thoracic, lumbar and caudal) of each individual analyzed (n=24). The values indicated only by letters represent 100% of the epiphyses fused to the vertebral body. Degree A, B, C and D. F = Female, M = Male and I = Indeterminate Sex.

	UFSC	Sex	Age	C (%)	T (%)	L (%)	Ca (%)
Pattern 1	1210	I	Fetus	A	A	A	A
	1361	M	0.0*	A	A	A	A
	1072	M	<0.5	A	A	A	A
	1123	M	0.5	A	A	A	A
	1334	F	1.0	A	A	A	A
	1225	M	<0.5	57 A; 43 B	A	A	A
	1105	M	<0.5	29 A; 29 B; 42 C	A	A	A
	1103	M	<0.5	57 B; 43 C	A	A	A
	1368	M	3.0	14 A; 29 B; 57 C	A	A	A
Pattern 2	1126	M	4.5	29 A; 14 B; 57 C	A	A	76 A; 24 B
	1106	I	2.0	14 B; 86 C	A	A	74 A; 26 C
	1295	M	2.5	14 B; 86 C	A	A	64 A; 32 C; 2 D
	1285	F	3.5	14 A; 86 C	A	A	52 A; 16 B; 28 C; 4 D
	1281	F	4.0	14 A; 86 C	A	A	63 A; 3 B; 21 C; 13 D
	1099	F	5.5	14 B; 14 C; 71 D	A	A	62 A; 4 B; 34 D
	1349	F	7.0	D	92 A; 8 C	A	48 A; 4 B; 8 C; 40 D
	1249	I	15+	D	92 A; 8 B	A	24 A; 24 B; 8 C; 44 D
	1299	I	12.0	D	53 C; 47 D	C	38 C; 62 D
Pattern 3	1350	M	11.0	D	D	D	D
	1317	I	12+	D	D	D	D
	1322	I	12+	D	D	D	D
	1252	I	13.0	D	D	D	D
	1011	I	14+	D	D	D	D
	1209	F	17+	D	D	D	D

Note: *Fetal folds present (see McBride and Kritzler, 1951).

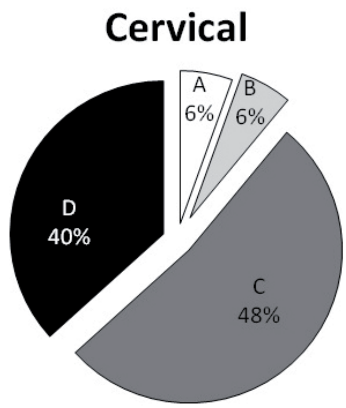

Figure 2. Degree of epiphyseal fusion in the cervical region (Pattern 2). Degrees A, B, C and D.

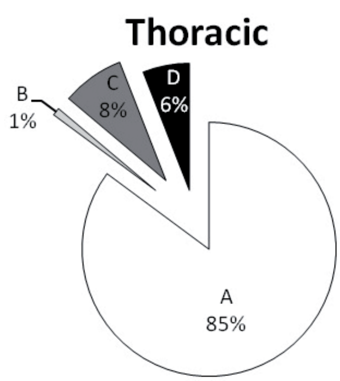

Figure 3. Degree of epiphyseal fusion in the thoracic region (Pattern 2). Degrees A, B, C and D.

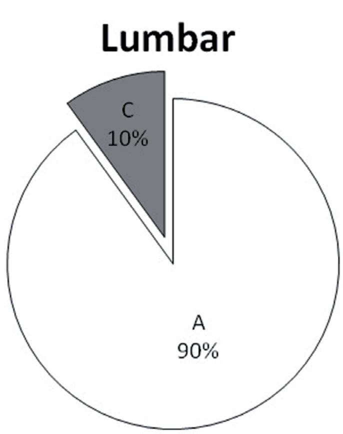

Figure 4. Degree of epiphyseal fusion in the lumbar region (Pattern 2). Degrees A and C.

erated maturation resulted in at least 40% of vertebrae in degree C or D of epiphyseal fusion, but half of the vertebrae in this sector had not yet shown any degree of fusion (Figure 5).

In individuals with Pattern 2, we observed initial fusion of posterior epiphyses to the vertebral body of the last caudal vertebrae, which are the first vertebrae in the entire column, to reach degree D. The thoracic region is the third to initiate the epiphyseal fusion process, with T1 being the first vertebra in this sector to fuse to the vertebral body through the anterior epiphysis. The lumbar region is the last region to show the various stages of ontogenetic development. Final fusion of the vertebral column seems to occur in the anterior lumbar vertebrae. Passage through degree B (initial fusion) is very fast, as only 6% of the cervical, 1% of the thoracic and 9% of the caudal vertebrae were found in this condition.

The upper age limit for Pattern 2 and the onset of physical maturity (Pattern 3) encompasses great individual variability. Specimen UFSC 1350 was the youngest to reach physical maturity, at 11 years. The remaining specimens were physically mature at age 12. Specimen UFSC 1249 took longest to reach physical maturity, displaying varying degrees of fusion (Pattern 2) at age 15 (Table 1).

Sexual dimorphism in vertebral column maturity could not be fully addressed, due to the small number of individuals with a determined sex in each pattern. However, in Pattern 1, the male calves less than one year old (UFSC 1103, 1105 and 1225) had already initiated epiphyseal fusion to the vertebral body, with specimen UFSC 1103 displaying all cervical vertebrae with epiphyseal fusion (degree B and C), while the only female (UFSC 1334) in this pattern, it was one year old, had the entire column in degree A. It was not possible to establish sex correlations for the next pattern, due to the small number of males in this group (n=2). It is noteworthy that when the only two male specimens were compared, we found that specimen UFSC 1295, which was two and a half years old, presented the cervical and caudal region in a more advanced stage of ontogenetic development than UFSC 1126, which was four and half years old. The male UFSC 1295 specimen presented also the cervical region in a stage more advanced than the female UFSC 1285 (three and half years). However, we did not observe significant differences in the ontogenetic development of the caudal vertebrae among these specimens (Table 1). Whereas, when the males UFSC 1368 (Pattern 1) and UFSC 1126 (Pattern 2) and the females UFSC 1281 (two years old) and 1285 (both Pattern 2) were compared, we noticed that the both sex presented the cervical vertebrae with epiphyseal fusion, but only the females had initial fusion in the posterior caudal.

Discussion

The dentin layers deposited annually in the teeth revealed that *Tursiops truncatus* can live more than 40 years (Hohn *et al*., 1989; Cockcroft and Ross, 1990; Wells and Scott, 1999). Although males are larger than females, the latter reach their maximum length earlier (Mead and Potter, 1990; Wells and Scott, 1999).

These sex differences in growth rate were observed in the first phase of column maturation (Pattern 1), suggesting that male calves initiate the process of vertebral column maturation before females. During the Pattern 2, we noticed unclear differences between the sexes, however, the females UFSC 1281 and 1285 showed a more advanced stage of ontogenetic development than the two males UFSC 1126 and 1368 of almost the same age. Some authors suggest that in the second phase (Pattern 2) the trend of the Pattern 1 reverses itself and females subsequently begin to grow faster (Read *et al*., 1993; Galatius and Kinze, 2003). According to

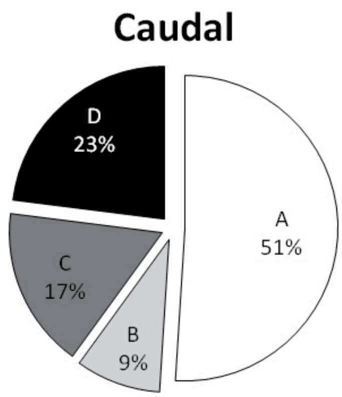

Figure 5. Degree of epiphyseal fusion in the caudal region (Pattern 2). Degrees A, B, C and D.

Read *et al*. (1993), male and female *Tursiops truncatus* from Sarasota (U.S.A.) showed distinct patterns of growth and achieved different final body lengths. Females grew faster than males during the first decade of life, whereas males continued growing for several more years after the growth of the females had ceased. These authors did not, however, measure young dolphins still together with their mothers. Sexual dimorphism can be explained as the result of a diversion of energy from growth to reproduction in females at an earlier age than in males (Wells and Scott, 1999). This trend can also be explained by the interference of environmental factors such as temperature and nutritional and metabolic status, among others. However, to further clarify the size differences in physically mature animals, it is necessary to increase the sample size of individuals with an identified sex.

According to Kato (1988), Lockyer *et al*. (1988) and Galatius and Kinze (2003), the fusion of epiphyses begins at the anterior cervical region and subsequently at the posterior caudal region and then proceeds from both ends toward the middle region of the vertebral column. A similar pattern was observed for *Tursiops truncatus* in southern Brazil, where the fusion of vertebral epiphyses begins in the cervical region with the anterior epiphyses. This process starts from the atlas-axis in the first two years of life and is followed by fusion of the last caudal vertebrae, which begin their fusion by posterior epiphyses starting from the 2 years of age. The last caudal vertebrae are the first ones to have both epiphyses completely fused to the vertebral body (degree D), whereas the first lumbar vertebrae are the last ones to undergo fusion.

The thoracic and lumbar regions are the last to initiate the process of epiphyseal fusion, but it is unclear whether there is a relationship between this process and column flexibility and swimming movements. Buchholtz and Schur (2004) emphasize that the flexibility in the posterior torso is localized at the synclinal point and that displacement of the fluke is due primarily to dorsoventral movement of vertebrae posterior to that point. The synclinal point occurred at approximately caudal vertebra 11 (Ca11) in the sample from southern Brazil (Costa, 2009). The vertebrae posterior to this point (last caudal) are the first to reach degree D, giving early support to the tail movements during swimming.

The youngest individual to show complete fusion of all epiphyses to the vertebral body was specimen UFSC 1350, an 11-year-old male. The oldest specimen still physically immature was UFSC 1249 (unknown gender), aged 15. Physically immature animals of advanced age have been previously reported by other authors (Ito and Miyasaki, 1990; Mead and Potter, 1990; Galatius and Kinze, 2003).

We conclude that the physical maturity of at least some individuals is initiated between 11 and 12 years. This finding is similar to that seen for North American (13 years) and South African (12-15 years) *T. truncatus* (Cockcroft and Ross, 1990; Mead and Potter, 1990; Wells and Scott, 1999). However, it was not possible to compare physical and sexual maturity in the samples analyzed, given the scarcity of data regarding this species in Brazil. The few studies on sexual maturity refer to *Pontoporia blanvillei* and *Sotalia guianensis* (Ramos, 1997; Rosas, 2000).

Since the Slijper's (1946) finding, minimal attention has been give to the postcranial skeleton of cetaceans, in especial to the vertebral ontogeny. This study is one of few works which demonstrate the cetacean's growth compared with the age, being a very important methodology to future studies in age determination and physical maturity of the vertebral column of cetaceans.

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