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**OSTEODENSITOMETRIC DIFFERENCES OF THE FLIPPER AS INDICATORS OF MUSCLES ACTIVITY IN BOTTLENOSE DOLPHIN (*Tursiops truncatus*) AND STRIPED DOLPHIN (*Stenella coeruleoalba*) FROM THE ADRIATIC SEA**

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**Abstract**

The aim of this study is to examine the differences of the flipper osteodensitometric characteristics in two dolphin species which are morphologically very similar, but live in different environments. The pectoral flippers of 24 cetacean specimens representing two odontocete genera were obtained, 12 bottlenose dolphins (*Tursiops truncatus*) and 12 striped dolphins (*Stenella coeruleoalba*). External body measurements were taken and osteodensitometric properties (BMC, bone mineral content, and BMD, bone mineral density) were measured by DEXA method. Osteodensitometric parameters in the examined area were positively correlated to the flipper morphometric properties, especially to the cranial flipper length. Correlation of the greatest flipper width and osteodensitometric parameters is different in examined dolphin species. According to the bigger body size and stronger skeleton, bottlenose dolphin has higher osteodensitometric values than striped dolphin. Intensive body activity increase muscle mass and bone mineral density, and suggest higher forelimb muscle activity in bottlenose dolphin than in striped dolphin.

**Keywords:** bottlenose dolphin, striped dolphin, DEXA, flipper muscle activity

## Introduction

Cetaceans are exceptional mammals that have adapted to life in a marine environment. The two recent suborders, Odontoceti (toothed whales) and Mysticeti (baleen whales), display a variety of adaptations that aid in an aquatic lifestyle, such as hind limb and hair loss, streamlined and fusiform body contours, and blowholes on the top on their heads. Cetacean pectoral flippers are composed of the bony elements plesiomorphic to those of tetrapods (humerus, radius, ulna, carpals, metacarpals and digits), all encased in dense connective tissues, creating a stiffened flipper (COOPER et al, 2008). Most active muscles in flipper movement are triceps and deltoid muscles (COOPER et al., 2007a) attached at the humerus and antebrachial bones.

Bone mineral density (BMD) is a clinical test for the diagnosis of the bone pathology, and in animals it is mostly used in research of the osteoporosis in laboratory animal models. Several investigations were done with the aim to determine changes in bone mineral density in dolphin during its lifetime, and to develop alternative method for age determination (GUGLIELMINI et al., 2002; LUCIĆ, 2006; BUTTI et al., 2007). Stronger muscles activity increases the bone mineral density during the growth of humans and animals (RYAN and ELAHI, 1998; MITCHELL et al, 2001).

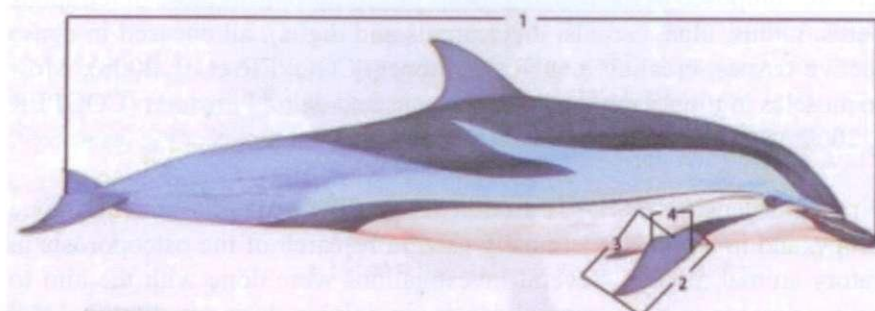
The aim of this study is investigation of differences of the flipper osteodensitometric characteristics in two dolphin species which are morphologically very similar, but live in different environments. First one is bottlenose dolphin, resident species in Adriatic Sea, and striped dolphin, which is not resident but it is a most common guest cetacean species in Adriatic Sea (GOMERČIĆ et al, 1998). Croatian part of Adriatic Sea is complex habitat because of its coastal geographic variety and bottlenose dolphin as a mesopelagic species; probably have better turning performances for manoeuvring in it. In the contrast, striped dolphin inhabit pelagic environment whereas faster but wide manoeuvres are dominant in turning movements.

## Material and methods

The pectoral flippers of 24 cetacean specimens representing two odontocete genera were obtained from dead stranded animals found at the Croatian coast of the Adriatic Sea during the period from 1997 to 2002. Animals were transported to the Faculty of Veterinary Medicine, University of Zagreb for

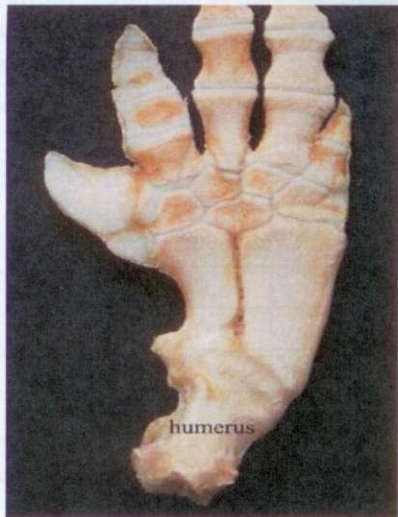


necropsy. Species included 12 bottlenose dolphins (*Tursiops truncatus*) and 12 striped dolphins (*Stenella coeruleoalba*). Before necropsy, body measurements were taken according to PERRIN (1975): total body length, cranial flipper length, caudal flipper length, and greatest width of flipper as shown in Fig. 1. After necropsy, right flipper of all examined dolphins was fully defleshed and stored. Dolphin age was determined by counting annual layers of tooth dentin (MOLINA and OPORTO 1993).



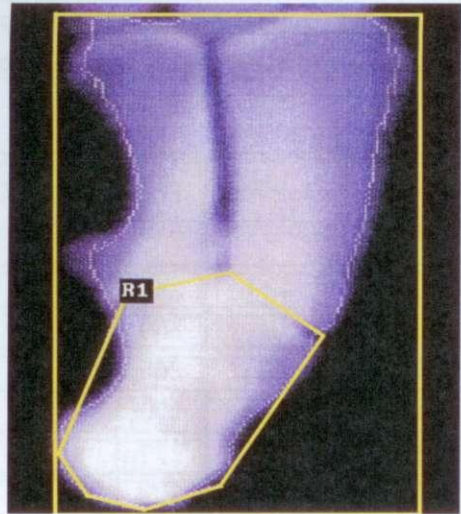
**Fig. 1** External body measurements: 1- total body length; 2 – cranial flipper length; 3 – caudal flipper length 4 – greatest flipper width.

Bone mineral density of the right flipper was measured by Hologic QDR-4000 osteodensitometer (S/N 55428; Hologic Inc., Waltham, MA, USA). Bone preparation was placed at lexan-plate in dorsopalmar position before measuring of bone mineral density. Soft tissues of flippers were simulated by using specially designed plate, which is called lexan-plate (Acrylic Scan Platform, Hologic Inc., Waltham, MA, USA) and was made of pure acrylic with the same absorption intensity of X-rays as soft tissues. Area of measurement was marked at the osteodensitometric image of a flipper, as global region of interest (Groi). Bone mineral density (BMD) was measured in selected area.



**Figure 2**

**Fig. 2** Bones of the right flipper with marked humerus of the bottlenose dolphin (D072, male, 10 years old).



**Figure 3**

**Fig. 3** Osteodensitometric image of the humerus and antebrachial bones in the dorsopalmar projection of the right flipper of the bottlenose dolphin (D072, male, 10 years old) (R1, humerus; yellow rectangle, global region of interest, Groi).

## Results

External body measurements were taken from 24 dolphins in total, which were divided in two groups according to the dolphin species, 12 bottlenose dolphins, and 12 striped dolphins.

Osteodensitometric data were obtained by measuring the bone mineral content (BMC) and bone mineral density (BMD) of the humerus and antebrachial bones (Groi, global region of interest).

Collected data are shown in Table 1 and Table 2 according to the dolphin species.

**Table 1** External body measurements (total body length, TBL; cranial flipper length, CrFL; caudal flipper length, CaFL; greatest flipper width, GFW) and BMC and BMD of the humerus and antebrachial bones (Groi) of the bottlenose dolphins

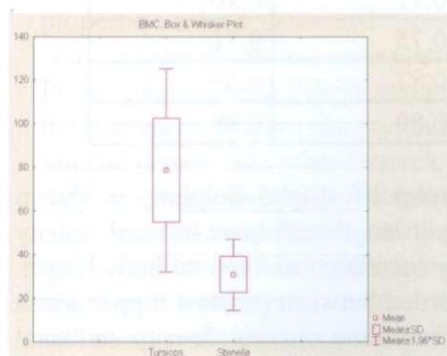
Dolphin number	Dolphin age /year	TBL /cm	CrFL /cm	CaFL /cm	GFW /cm	BMC (Groi) /g	BMD (Groi) /g/cm <sup>2</sup>
D088	11	249	34	20	12	58,06	0,787
D036	10	260	30	21	11	82,01	0,939
D126	19	266	41	29	16	79,28	0,866
D100	12	270	41	31	16	88,21	0,879
D104	20	277	44	33	14	121,95	1,109
D063	2	200	28	20	10,5	35,91	0,677
D127	7	231	36	22,5	16	52,16	0,774
D035	14	258	40	26,5	14	64,38	0,843
D041	12	261	42	30	16	79,09	0,878
D102	20	262	42	29	18	80,25	0,883
D017	13	274	44	30	16	102,87	1,08
D025	12	278	48	34	17,5	99,88	1,016



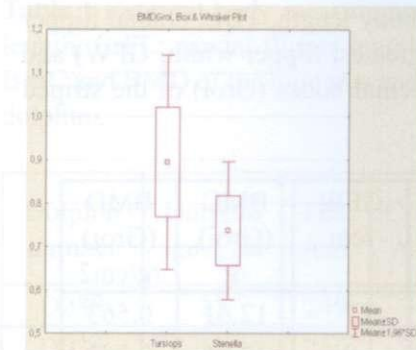
**Table 2** External body measurements (total body length, TBL; cranial flipper length, CrFL; caudal flipper length, CaFL; greatest flipper width, GFW) and BMC and BMD of the humerus and antebrachial bones (Groi) of the striped dolphins

Dolphin number	Dolphin age /year	TBL /cm	CrFL /cm	CaFL /cm	GFW /cm	BMC (Groi) /g	BMD (Groi) /g/cm <sup>2</sup>
D034	3	132	23,5	16,5	7	12,61	0,563
D081	12	188	28	19	8	32,05	0,759
D027	11	198	28	20	9,5	35,61	0,784
D071	13	208	26	19	9,5	37,64	0,829
D089	23	209	30	20	10,5	40,57	0,827
D029	5	171	19	15	8	17,3	0,599
D115	22	197	28	20	9	31,29	0,744
D079	22	198	25	17,5	8,5	34,78	0,779
D074	15	199	28	20,5	10	32,04	0,76
D078	15	202	25,5	17	8,5	26,54	0,709
D121	12	203	27	20	10	36,51	0,744
D073	17	207	28,5	21,5	10	33,63	0,754

Results of descriptive statistical analyses are shown below. Mean values as well as range of BMC<sub>Groi</sub> and BMD<sub>Groi</sub> were higher in bottlenose dolphins than in striped individuals (Fig. 4, Fig. 5).



**Fig. 4.** Mean values, standard deviation and standard error of the bone mineral content (BMC<sub>Groi</sub>) in bottlenose dolphin (Tursiops) and striped dolphins (Stenella)



**Fig. 5.** Mean values, standard deviation and standard error of the bone mineral density (BMDGroi) in bottlenose dolphin (Tursiops) and striped dolphins (Stenella)

Results of correlative statistical analyses are shown below. Correlation coefficients, those are higher than 0.50 are statistically significant. Cranial flipper length, caudal flipper length and bone mineral density of the bottlenose dolphins were positively correlated to the total body length. Furthermore, low correlations were recorded between greatest flipper width and bone mineral density, same as between bone mineral density and total body length and greatest flipper width and total body length (Table 3).

**Table 3.** Correlation coefficients between osteodensitometric parameters (BMCGroi, bone mineral content of global region of interest; BMDGroi, bone mineral density of global region of interest) and external body measurements (CrFL, cranial flipper length; CaFL, caudal flipper length; GFW, greatest flipper width; TBL, total body length) in both investigated dolphin species.

	Bottlenose dolphin		Striped dolphin	
	BMCGroi	BMDGroi	BMCGroi	BMDGroi
<b>CrFL</b>	0.75	0.69	0.77	0.76
<b>CaFL</b>	0.82	0.72	0.75	0.71
<b>GFW</b>	<b>0.43</b>	<b>0.36</b>	<b>0.82</b>	<b>0.75</b>
<b>TBL</b>	0.89	0.84	0.89	0.88

Correlative statistical analysis in the group of striped dolphins is shown below. Cranial flipper length, caudal flipper length and bone mineral density of the bottlenose dolphins were positively correlated to the total body length. Furthermore, good correlations were recorded between greatest flipper width and bone mineral density, same as between bone mineral density and total body length and greatest flipper width and total body length.



## Discussion

Dolphin flippers control the body position and have a great influence on stability and precise manoeuvrability in aquatic locomotion. Flipper mobility is lower in cetacean species which swim faster than in the species which swim slower with lot of manoeuvres. Mobility and placement of the body in aquatic environment appear to be associated with prey type and habitat. Flexibility and slow, precise manoeuvring are found in cetaceans that inhabit more complex habitats, whereas high-speed manoeuvres are used by cetaceans in the pelagic environment (FISH, 2002). According to that, forelimb muscles are better developed in species which live in waters of lower density and depth. Example of that was described in Amazon River dolphin that has very mobile flippers, even with a mobile radius (KLIMA et al., 1980).

Research on bone osteodensitometric parameters includes bone mineral content and bone mineral density of the major and strongest flipper bones, humerus and antebrachial bones. Flipper length in most cetaceans has a constant relation to the body length (FISH and ROHR, 1999). According to that, bone mineral density of examined flipper bones has a positive correlation to the total body length in both dolphin species. Increase of osteodensitometric parameters was described in earlier investigations in striped dolphin (GUGLIELMINI et al., 2002) and bottlenose dolphin (LUCIĆ, 2006). Osteodensitometric parameters in examined area were positively correlated to the flipper morphometric properties, especially to the cranial flipper length. That length is based mostly on the cranial surfaces of humerus and radius that were placed in the osteodensitometric area, and strongest shoulder muscles are attached at those bones, especially humerus. Caudal flipper length is based on carpo-metacarpal and phalangeal bones mostly, and that length had a lower correlation to the osteodensitometric properties in both examined species. Correlation of the greatest flipper width and osteodensitometric parameters is different in examined dolphin species. Positive correlation of these variables was record in the striped dolphins, but in the bottlenose dolphin the correlation was low and insignificant ( $r < 0.5$ ). Bone mineral density and related muscle activity influence the flipper width in striped dolphins, but in bottlenose dolphins it was affected by some other properties. That properties can be the small phalangeal bones which are connected with fibrous tissue and also wider interdigital spaces between the first and second digits in the bottlenose dolphin flipper which can be related to the external influences such as the resistance to water media when flipper movements and short manoeuvres are frequent. The forelimb of cetaceans has been radically



modified during the limb-to-flipper transition. Recent cetaceans have a soft tissue flipper encasing the manus and acting as a hydrofoil to generate lift (COOPER et al., 2007b), more or less depend of species and its habitat characteristic (WOODWARD et al., 2006). Strong muscle activity increases the bone mineral density, and it is important for increasing of total bony mass, but intensity of mineral depositioning in bone depends from type and category of muscle activities (RYAN and ELAHI, 1998).

According to the bigger body size and stronger skeleton, bottlenose dolphin has higher osteodensitometric values than striped dolphin. Bone mineral content is higher in osteodensitometric area of the bottlenose dolphin, but that area is bigger in bottlenose dolphin because of bigger humerus and antebrachial bones than in the striped dolphin. On the other hand, bone mineral density is defined by the unit surface in the measuring area (1 cm<sup>2</sup>) and that values are higher in bottlenose dolphin. Intensive body activity increases muscle mass and bone mineral density (RYAN and ELAHI, 1998) and suggests higher forelimb muscle activity in bottlenose dolphin than in striped dolphin.

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