

Morphological Analysis of the Flippers in the Franciscana Dolphin, *Pontoporia blainvillei*, Applying X-Ray Technique

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ABSTRACT

Pectoral flippers of cetaceans function to provide stability and maneuverability during locomotion. Directional asymmetry (DA) is a common feature among odontocete cetaceans, as well as sexual dimorphism (SD). For the first time DA, allometry, physical maturity, and SD of the flipper skeleton—by X-ray technique—of *Pontoporia blainvillei* were analyzed. The number of carpals, metacarpals, phalanges, and morphometric characters from the humerus, radius, ulna, and digit two were studied in franciscana dolphins from Buenos Aires, Argentina. The number of visible epiphyses and their degree of fusion at the proximal and distal ends of the humerus, radius, and ulna were also analyzed. The flipper skeleton was symmetrical, showing a negative allometric trend, with similar growth patterns in both sexes with the exception of the width of the radius ($P \leq 0.01$). SD was found on the number of phalanges of digit two ($P \leq 0.01$), ulna and digit two lengths. Females showed a higher relative ulna length and shorter relative digit two length, and the opposite occurred in males ($P \leq 0.01$). Epiphyseal fusion pattern proved to be a tool to determine dolphin's age; franciscana dolphins with a mature flipper were, at least, four years old. This study indicates that the flippers of franciscana dolphins are symmetrical; both sexes show a negative allometric trend; SD is observed in radius, ulna, and digit two; and flipper skeleton allows determine the age class of the dolphins. *Anat Rec*, 00:000–000, 2014. © 2014 Wiley Periodicals, Inc.

Key words: directional asymmetry; allometry; sexual dimorphism; appendicular skeleton

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Cetaceans are mammals that spend all the phases of their lifetime in water. As a result of their full adaptation to inhabit aquatic environments, they present major modifications in bone and muscle structure in comparison to terrestrial mammals. One of these remarkable modifications in the typical mammalian body plan was the evolution of the forelimbs from a quadruped animal into flippers, characterized by a reduced muscle structure, shortening of humerus, radius and ulna, and a higher number of phalanges termed hyperphalangy (Cooper et al., 2007; Reidenberg, 2007; Sanchez and Berta, 2010). These modifications result in flattened flippers which minimize water resistance during swimming (Howell, 1930) and have a fundamental role in locomotion, particularly on stability and maneuverability (Fish, 1996, 2002).

Anatomical and morphometrical studies of the appendicular skeleton and its associated musculature of franciscana dolphin (*Pontoporia blainvillei*) are scarce. Early research focused on descriptive aspects of the flippers (Burmeister, 1868; Pilleri and Gihl, 1976; Strickler, 1978), although, some significant aspects of the anatomy and morphology of the flippers of this species such as directional asymmetry (DA), sexual dimorphism (SD), physical maturity and allometry have not been evaluated yet. The franciscana dolphin occurs from south-eastern Brazil (Siciliano et al., 2002) to northern Patagonia, Argentina and inhabits coastal-marine waters, from the coast to the 30-m isobath (Crespo et al., 2010). Although sometimes described as a “river dolphin,” the franciscana dolphin is not a freshwater species and belongs to the superfamily Iniioidea, being the only extant species of the Pontoporiidae family (May-Collado and Agnarsson, 2006; McGowen et al., 2009). The species show sexual size dimorphism, with females attaining larger sizes and weights (147.33 cm and 36.69 kg) than males (128.96 cm and 27.96 kg) (Kasuya and Brownell, 1979; Pinedo, 1991; Negri, 2010; Panebianco, 2011; Panebianco et al., 2012), a feature opposed from most mammal species (Ralls 1976, 1977). SD has also been reported on skull structures (Pinedo, 1991) but not in the appendicular skeleton. Thus, considering sexual dimorphism in overall size and the fundamental role of the flippers in locomotion, we hypothesize that females may display absolutely larger appendicular skeletal elements than do males.

Asymmetry has been defined as the deviation of an organism, or a part of that organism, from perfect symmetry (Van Valen, 1962) and can be characterized into three different categories. DA occurs when one side of a bilateral trait always develops more than the other side. Fluctuating asymmetry is defined as subtle random deviations from perfect symmetry when the mean of the differences between sides in the studied character do not differ from 0. Antisymmetry occurs when one side of the character is larger than the other, but there is no handed-bias as to which side will be larger. Previous studies have described DA on the flipper skeleton in several species that belong to the superfamily Delphinoidea such as *Phocoena phocoena*, *Lagenorhynchus albirostris*, and *Cephalorhynchus commersonii commersonii* (Galatius and Jespersen, 2005; Galatius, 2006; Gómez-Campos et al., 2010). According to those studies different mechanical loading between left and right flippers might be associated with DA. This feature has not, to the date,

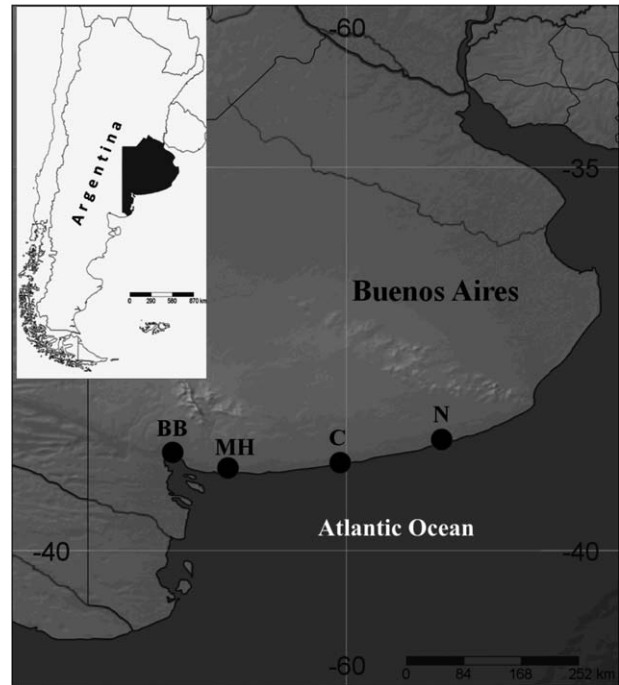


Fig. 1. Sampling localities along the Southern coast of Buenos Aires. Necochea (N), Claromecó (C), Monte Hermoso (MH), and Bahía Blanca (BB).

been evaluated in franciscana dolphins. We hypothesize that the flipper skeleton of the franciscana dolphin will display DA, and that this feature will be more pronounced in older (larger) dolphins.

Morphological and anatomical features of *P. blainvillei* have been poorly studied, in this context the general goal of this article was to contribute with new anatomical and morphometric data studying SD, allometry, physical maturity, and DA on the flipper skeleton of *Pontoporia blainvillei* of southern Buenos Aires (Argentina), using X-ray techniques. The specific objectives were to examine the osteological morphology of the flippers of *P. blainvillei* to determine if they (1) display SD, (2) display DA, and (3) can be used as a tool to determine individual age.

MATERIAL AND METHODS

Sample Preparation and Study Area

Specimens ($N = 37$, 20 males, 17 females) collected during the reproductive season (November–March) between 2003 and 2009 were analyzed. Dolphins were either incidentally caught in artisanal fishing nets or found dead on the beach. The study area embraces southern coastal area of Buenos Aires Province, Argentina, between the localities of Necochea (N, 38°37'S, 58°50'W) and Bahía Blanca (BB, 38°44'S, 62°14'W) (Fig. 1). Fresh flipper pairs (right and left) were cleanly disarticulated at the glenohumeral joint during necropsy, and then frozen until X-ray analysis. X-ray machine operated at 50 mA and 38 kV. Recorded information included gender and total length (according to Norris, 1961).

Age Determination

Teeth were extracted from each specimen and then fixed in a 10% formaldehyde solution for age determination. Teeth were then decalcified in commercial acid mix RDO® and sectioned in a cryostat at -21°C (Pinedo and Hohn, 2000). The sections of $25\ \mu\text{m}$ were stained with Mayer's Hematoxylin and mounted in slides with glycerine. Age was determined by counting growth-layer groups (GLGs). Only central well-contrasted layers were used for GLG readings in both dentine and cement (Negri, 2010; Panebianco et al., 2012). Although no direct validation exists for GLGs, indirect evidence supports that one GLG represents 1 year of age (Kasuya and Brownell, 1979; Pinedo and Hohn, 2000).

Directional Asymmetry

Morphometric characters of the bones were measured by the same observer with a digital caliper ($\pm 0.01\ \text{mm}$) from the X-rays. The morphometric characters recorded were (Fig. 2): humerus length (HL); humerus width (HW); radius length (RL); radius width (RW); ulna length (UL); ulna width (UW); brachium and antebrachium combined length (BL); second digit length (D2L); and flipper skeleton length (FL). Epiphyses were included in all measurements recorded.

DA in each measurement was calculated according to Plochocki (2004):

$$\text{DA} = [2 \times (M_R - M_L / M_R + M_L)] \times 100$$

where M represents the character recorded. R indicates the right bone and L the left one. A positive DA value indicates that the right measurement is larger and a negative one indicates a larger left measurement.

Allometric Trends

Ontogenetic change of each morphometric character (Fig. 2) in relation to the total body length was calculated by the following linear least-squares regression:

$$\text{Log}(Y) = \text{Log}(a) + b(\text{Log}(L))$$

where Y is the length of the measured character (mm), L is the total length (cm) of the specimen, a is a constant determined by the value of Y when L is unity, and b is the allometric growth coefficient. A growth coefficient b significantly less than unity indicates negative allometry, increasing in length at a slower rate than that of TL, b significantly greater than unity indicates positive allometry, increasing in length at a faster rate than that of TL, and b not significantly different from unity indicates isometry, the growth of the character is directly proportional to the growth of TL throughout development.

Physical Maturity

Physical maturity was examined from X-rays. The number of visible epiphyses and their degree of fusion at the proximal and distal ends of the humerus, radius, and ulna were determined in each flipper. The proximal epiphysis of the humerus was not analyzed because it was not easily visualized due to its differential orientation in the

X-ray with respect to the rest of the osseous structures of the flipper. The ossification of the diaphyses was graded according to Ogden et al. (1981): Stage 0, no secondary ossification center present; Stage 1, secondary ossification center present but occupying less than 50% of the width of the adjacent bone; Stage 2, secondary ossification center occupying 50%–100% of the adjacent bone; Stage 3, distance between the bone epiphysis and metaphysis begins to reduce; Stage 4, physis begins to close; Stage 5, physis is completely closed and a radiodense physal line traverses the width of the bone; Stage 6, the physal region is remodeled—the physal line is replaced by mature bone tissue, thus less than 50% of the physal line remains or it has completely disappeared.

Flippers were considered mature when all epiphyses were in stage five or six. The relationship between fusion stage and age was assessed by comparing both parameters (e.g., 3 years old dolphins showed a stage 4 of fusion in the distal epiphysis of the ulna).

Sexual Dimorphism

SD of morphometric characters measured from the flippers' X-rays was evaluated. Immature and mature dolphins were included in this analysis, although this is not a typical approach. We took this approach because the small sample size of adults (two females, five males) precluded a comparison of adults only. We acknowledge that this method will not be directly comparable to other studies of SD that utilize only adult specimens.

Carpals, Metacarpals, and Phalanges

The number of carpals, metacarpals, and phalanges of all digits were recorded in each flipper. The carpals schemes observed in the X-rays were compared with those described by Pilleri and Gihl (1976).

Data Analysis

DA data were analyzed by multiple ANOVAs to detect sexual differences. As sex-related differences were absent, data were pooled and the presence of DA was tested with a T -Student test. Those analyses were performed in both mature and immature dolphins separately to detect if DA arises in larger dolphins. Allometric trends of the flipper bones were evaluated through linear regressions, where sex was set up as a dummy variable. The dummy variable was included to test ontogenetic differences between sexes. The regression coefficients for these dummy variables or their interaction were not statistically significant, due to this, data were pooled together to perform a new regression including both males and females. Finally, morphometric characters were analyzed by principal components analysis (PCA) to explore the data, and evaluate SD. PCA allows revealing patterns in data undetected by analyzing the variables separately. A smaller number of variables (principal components or PCs) that are linear combinations of the original variables were obtained. PCs are orthogonal, and thus independent of each other. PCA was performed with standardized variables to equalize the range and/or data variability. Nonparametric statistical analysis—Kruskal-Wallis or Man-Whitney test—were applied to compare the number of phalanges

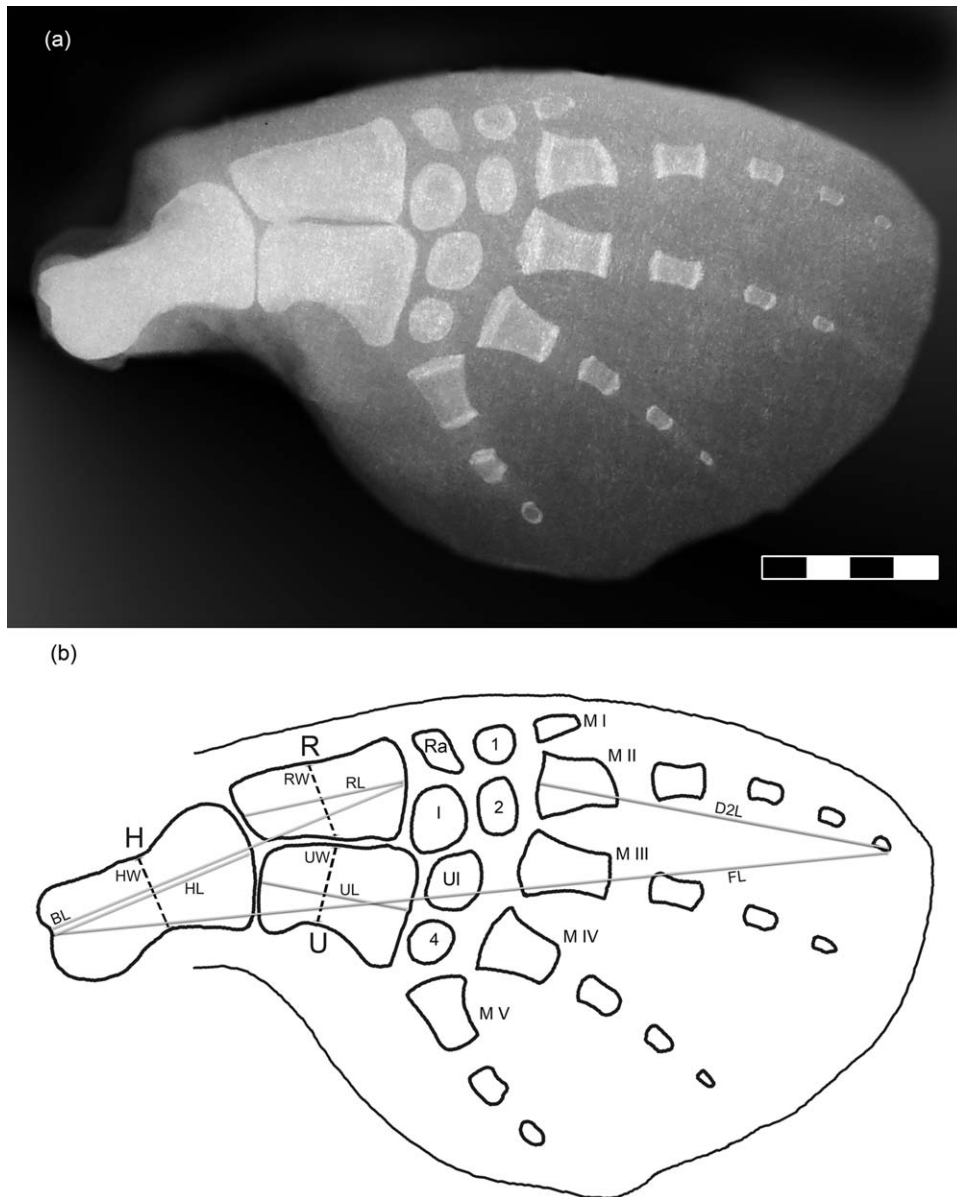


Fig. 2. (a) X-ray of a mature flipper. Note that all epiphyses are fused. (b) Diagram showing bones and morphometric characters analyzed. H: humerus; R: radius; U: ulna; Ra: radiale; I: intermedium; UI: ulnare; 1: carpale one; 2: carpale two; 4: carpale four. M: metacarpals. Dotted lines show the measurements of width and grey lines show the measurements of length (see text). Scale bar: 4cm.

between sexes, when normality and homoscedasticity of the data were not met.

Statistical analyses were performed with the statistical software InfoStat (Di Rienzo et al., 2009). For all tests performed, the level of statistical significance was set at $P \leq 0.05$ and the data presented as mean \pm standard deviation (SD).

RESULTS

Directional Asymmetry and Allometric Trends

Morphometric characters showed no significant DA either in mature or immature dolphins (Table 1) and

also showed a significant negative allometric trend (Table 2), with the exception of UL and UW. The latter morphometric characters did not fit to the allometric model applied. The growth pattern for the morphometric characters HL, HW, FL, D2L, BL, and RL did not significantly differ between sexes. In contrast, RW showed a significantly higher growth coefficient in males than in females ($b_M = 0.54$, $b_H = 0.3$, $N_M = 13$, $N_H = 16$, $F = 12.32$, $P \leq 0.01$). RL showed the lowest growth coefficient ($b = 0.39$), whereas HL showed the highest one ($b = 0.69$). The morphometric characters HW, FL, D2L, and BL showed intermediate b values (Table 2).

TABLE 1. Summary of the directional asymmetries (DA) results for the morphometric characters of *P. blainvillei* flippers analyzed (male and female data pooled)

Morphometric Characters	N	$R > L(\%)$	$R = L(\%)$	$R < L(\%)$	Mean DA \pm SD	T	P value
HL	31	45	26	29	0.59 ± 2.06	1.59	0.12
HW	29	38	10	52	-0.02 ± 3.34	0.03	0.98
RL	35	31	37	31	-0.18 ± 1.18	0.92	0.36
RW	33	52	9	39	0.46 ± 3.07	0.86	0.4
UL	35	48	6	46	-0.17 ± 11.31	0.09	0.93
UW	31	42	13	45	0.12 ± 2.97	0.22	0.83
D2L	34	47	12	41	0.10 ± 5.06	0.11	0.91
FL	29	52	14	34	0.31 ± 2.13	0.78	0.44
BL	30	53	23	23	0.51 ± 1.45	1.94	0.06

TABLE 2. Allometric trends found for the morphometric characters analyzed in the flippers of *P. blainvillei*

Morphometric characters	N	a	$b \pm SE$	P value	R^2
HL	32	0.27	0.69 ± 0.04	<0.01	0.91
HW	31	0.09	0.56 ± 0.07	<0.01	0.66
FL	31	1.10	0.56 ± 0.05	<0.01	0.81
D2L	33	0.67	0.58 ± 0.09	<0.01	0.54
BL	33	0.69	0.59 ± 0.04	<0.01	0.86
RL	34	0.77	0.39 ± 0.05	<0.01	0.62

N, number of specimens; a : constant; b : growth coefficient; P : significance of deviation from isometry. For references of morphometric characters, see text.

TABLE 3. Fusion pattern observed for the epiphyses considered

Epiphysis	Age					
	0	1	2	3	4	≥ 5
Distal humerus	2-3	2-4	4	4	4-5	6
Proximal radius	2	2-3-4	4	4	4-5	6
Distal radius	0	0-1-2	0-2	2-3	4-5	6
Proximal ulna	2-3	3-5	5-6	5-6	5-6	6
Distal ulna	0	0-1-2-3	2-3	4	5	6

Numbers indicate fusion stage graded following Ogden et al. (1981).

Physical Maturity and Sexual Dimorphism

Epiphyses exhibited a proximo-distal gradient of ossification and fusion (Table 3).

Mean values of morphometric characters analyzed were higher in females than in males (Table 4). PCA showed two principal axes (PC) which jointly explained 86% of the data variance. PC1 represented dolphins' size—larger values representing larger dolphins—and all variables showed low correlation between a component and a variable—loading score ~ 0.3 . PC2—orthogonal to the first and therefore independent of the size of the specimens—ordered the dolphins according to sex—females presented higher values on the axis than males (Fig. 3). D2L (loading 0.56) and UL (loading 0.62) showed the highest loadings in this PC where the remaining variables had loadings lower than 0.3. An ANOVA ($N = 31$, $F = 9.03$, $P \leq 0.01$) was performed using

this new variable (PC2) as dependent variable and “sex” as grouping criterion, demonstrating significant differences between sexes in the relative values of UL and D2L.

Most males of franciscana dolphins presented an extra phalanx than females—in digits two, three, four, and five (Table 5). The number of phalanges of digit two in males was significantly higher than in females (Kruskal-Wallis $H = 6.46$, $N_F = 17$, $N_M = 18$, $P \leq 0.01$), whereas the number of phalanges of digit four was similar in both sexes (Kruskal-Wallis $H = 2.16$, $N_F = 17$, $N_M = 18$, $P = 0.09$). Kruskal-Wallis test was not performed on digits three and five because statistical assumption of homoscedasticity was not met.

Carpals, Metacarpals, and Phalanges

Seven different types or schemes of carpal bones in the X-rays of the flippers were observed (Table 6). Types I to IV have been previously described by Pilleri and Gahr (1976), but types V to IX are described in this work for the first time. The flippers showing types III to IX—with less than 6 carpal bones—belong to ≤ 1 -year-old dolphins. The X-rays indicate the presence of five metacarpals (MI–MV), each holding a digit. Metacarpal I was reduced compared with metacarpal II to V, which were highly developed. We observed hyperphalangism in the second and third digits. The phalangeal formula observed in the X-rays is: 0:4-5:2-3-4:2-3:2-3 (metacarpals were not included). Finally, metacarpals and phalanges did not show secondary ossification centers.

DISCUSSION

The results of this study did not support the hypothesis that flipper skeleton of *Pontoporia blainvillei* would display DA. Instead, osteological features within the flippers of both sexes were bilaterally symmetric. This result is consistent with those by Ness (1967), who also reported a nearly symmetrical cranial structure in *P. blainvillei*, which distinguishes this dolphin from most odontocete cetaceans (Ness, 1967; MacLeod et al., 2007). Godfrey and Barnes (2008) have suggested that cranial asymmetry may be the primitive character among Pontoporiidae family based on fossil records, and that symmetrical skull might be a derived character state in the franciscana dolphin. Appendicular skeleton symmetry observed in the species might be associated with this asymmetry reversion. However, further studies are

TABLE 4. Mean values and standard deviation of the morphometric characters, according to sex

Morphometric characters	Matures				Immatures			
	Females		Males		Females		Males	
	N	Mean \pm SD	N	Mean \pm SD	N	Mean \pm SD	N	Mean \pm SD
HL	2	57.82 \pm 2.20	4	49.23 \pm 2.54	15	44.16 \pm 9.50	13	43.08 \pm 8.12
HW	2	21.23 \pm 0.81	4	18.58 \pm 1.62	13	17.08 \pm 1.94	10	15.82 \pm 2.03
RW	2	20.96 \pm 2.43	5	18.42 \pm 0.96	15	17.16 \pm 1.05	11	16.44 \pm 1.62
UW	2	20.54 \pm 0.48	4	18.36 \pm 1.04	14	16.16 \pm 2.01	11	15.43 \pm 2.02
FL	2	213.75 \pm 15.20	4	196.88 \pm 6.68	13	170.85 \pm 15.55	10	164.70 \pm 18.89
D2L	2	89.52 \pm 11.52	5	87.60 \pm 5.83	15	68.33 \pm 5.82	12	69.01 \pm 9.68
BL	2	94.59 \pm 3.89	4	81.88 \pm 3.13	13	78.60 \pm 8.53	12	75.40 \pm 8.12
RL	2	42.49 \pm 2.01	5	38.44 \pm 1.29	15	36.48 \pm 2.72	13	34.35 \pm 2.76
UL	2	38.10 \pm 0.17	5	35.47 \pm 1.61	15	35.59 \pm 2.00	13	34.50 \pm 1.47

Lengths are given in mm.

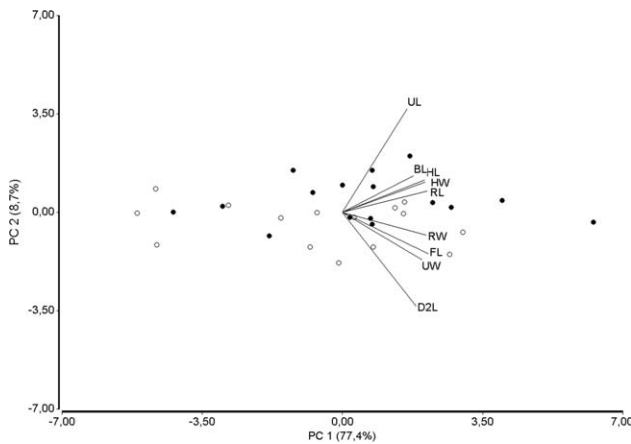


Fig. 3. Biplot of the first two principal component scores for both mature and immature individuals. Black points: females; white points: males.

TABLE 5. Number of phalanges (expressed on percentage) in the flippers X-rays, split by sex

Digit	Sex	Number of phalanges (%)				
		0	2	3	4	5
One	Female	100	—	—	—	—
	Male	100	—	—	—	—
Two	Female	—	—	—	73.5	26.5
	Male	—	—	—	30.6	69.4
Three	Female	—	3	76.5	20.5	—
	Male	—	5.6	30.6	63.8	—
Four	Female	—	44.1	55.9	—	—
	Male	—	19.4	80.6	—	—
Five	Female	—	91.2	8.8	—	—
	Male	—	66.7	33.3	—	—

likely to provide new insights into phylogenetic patterns of skeletal symmetry in Pontoporiidae and in odontocete cetaceans. It is worth pointing out that symmetry found on flipper skeleton is different to the condition of DA found on *P.phocoena*, *L.albirostris* and *C.c.commerstonii* (Galatius and Jespersen, 2005; Galatius, 2006; Gómez-Campos et al., 2010). Galatius and Jespersen (2005), and

Gomez-Campos et al. (2010) suggested that even though flipper DA seems to be an innate feature, it might also be related to differential flipper use, a hypothesis that is in agreement with previous studies where lateralized behavior in cetaceans was observed (Marino and Stowe, 1997; Johnson and Moewe, 1999). The results presented here might indicate that *P. blainvillei* does not exhibit lateralized behavior.

Growth rate was not significantly different between males and females in the variables analyzed, excepting RW, which showed SD on its growth pattern. Additionally, morphometric characters showed negative allometry ($b < 1$), thus suggesting that appendicular skeleton shows a differential growth rate to that of the overall size. These outcomes indicate that males and females follow similar pattern of skeleton development, which is in agreement with the observations made by Galatius (2005) in *P. phocoena*, where flipper skeleton showed negative allometry and no sexual differences. Pinedo (1991) and Negri (2010), suggested that flippers play an important role on early locomotion—based on rapidly growth of external measurements—thus allowing young dolphins to swim efficiently on early stages of life. Nevertheless, Galatius (2005) suggested that the negative allometry of the flipper skeleton combined with positive allometry of the scapula may cause a deficit in maneuverability on small specimens of *P.phocoena*, since they must drive large flippers with a small muscle mass. Considering the opposed hypothesis mentioned here, further morphological studies are necessary to conduct considering other variables (such as scapula and muscle structure development) to elucidate the role of the flippers in early locomotion of the species.

SD hypothesis was not supported by the results obtained. Statistical comparisons of measured characters between mature males and females were not performed due to sample size limitation (two mature females); nevertheless, the evidence showed that both mature and immature females exhibit mean values of all variables (with the exception of D2L on immature dolphins, probably related to the fact that males have an extra phalanx than females) higher than males. UL and D2L showed also an association with sex—according to PCA. Females had a higher relative UL and shorter relative D2L, and the opposite occurred in males. Males also usually showed an extra phalanx than females. These

TABLE 6. Schemes and percentage of carpal bones described for *P. blainvillei* in Pilleri and Gihl (1976) and in this study. Types III to IX in our work only occur in ≤ 1 -year-old dolphins

Type	Pilleri and Gihl 1976				This study						
	I	II	III	IV	I	III	V	VI	VII	VIII	IX
Free carpalia	6	6	5	5	6	5	4	5	5	5	3
Radiale	1	1	–	FCI	1	–	–	1	1	1	–
Intermedium	1	1	1	1	1	1	1	1	1	1	–
Ulnare	1	1	1	1	1	1	1	1	1	1	1
Carpale 1	1	1	1	–	1	1	1	1	1	–	1
Carpale 2	1	1	1	1	1	1	1	1	1	1	1
Carpale 3	–	FMIII	–	–	–	–	–	–	–	–	–
Carpale 4	1	1	1	1	1	1	–	–	FMV	1	–
Percentage	68.0	18.0	5.0	9.0	75.0	10.3	5.9	1.5	2.9	2.9	1.5

FMIII, fused with Metacarpal III; FCI, fused with Carpale I; FMV, fused with metacarpal V.

results are in agreement with those by Calzada and Aguilar (1996) who also found a significant variation on flipper skeleton between sexes in D2L in *Stenella coeruleoalba*, although females presented a larger digit. It is interesting to note that the SD in relative size of D2L, UL and number of phalanges was an unexpected outcome and further studies are likely to provide new insights into the origin and functional implications of this feature.

Distal epiphyses of the humerus and the proximal of the radius were not joined to the diaphysis, although ossified before the first year of life, thus suggesting that fusion process may begin after reaching the first year old, continue until the third, and conclude by the time of the fourth year of life. The ossification of the distal epiphysis of the radius may occur during the first and second year of life; the fusion process may start and conclude at the fourth year of life. The proximal and distal epiphyses of the ulna may ossify before and during the first year of life, respectively. The fusion process of the proximal epiphysis may start and conclude at the first year of life, whereas that of the distal one may start and conclude during the third year. The general trend found in this work—the proximo-distal gradient of ossification of the epiphyses—is similar to that previously described for *S. coeruleoalba*, *P. phocoena*, and *C. c. commersonii* (Calzada and Aguilar, 1996; Dawson, 2003; Gómez-Campos et al., 2010). Results presented in this study show that it is possible to use the fusion pattern of the epiphyses of the flipper skeleton as a tool to determine age of specimens. Franciscana dolphins with mature flippers were, at least, four years old; and dolphins with flippers with no evidence of ossification of the distal epiphyses in radius and ulna were between zero and one year old. X-ray technique may be applied for determine the age of the dolphins instead of the traditional method -counting GLGs in histological slides-. The advantages of the X-ray technique are (1) it is a simpler and noninvasive technique that can be used with live dolphins as well as with preserved specimens in scientific collections and (2) that results can be obtained relatively quickly and economically. However, this technique may provide less accurate results than those of traditional GLGs aging method.

Variability in the number of carpal bones is commonly observed among cetaceans as was reported in *Pseudorca crassidens*, *Stenella coeruleoalba*, (Gihl et al., 1982; Calzada and Aguilar, 1996), and *Pontoporia blainvillei* (Pilleri and Gihl, 1976). Most of the flippers analyzed in this study had six carpals, although this number was

variable—in individuals of one year old or younger—probably due to the lack of ossification, which concludes during the first year of life. However, these observations are in disagreement with those by Calzada and Aguilar (1996) and Gómez-Campos et al., (2010) for *S. coeruleoalba* and *C. c. commersonii*, in which carpal ossification occurs before birth. Metacarpals number (five) showed no variability in franciscana dolphin, although the phalangeal number was variable—within and between individuals. These outcomes are consistent with that observed by Pilleri and Gihl (1976) and are a common feature in cetaceans (Dawson, 2003; Cooper et al., 2007). Cooper et al. (2007) reported that the usual number of digital rays (metacarpals + phalanges) on odontocete cetaceans is five, and that the plesiomorphic odontocete condition is to have one phalanx on digit one, nevertheless, they also reported that the reduction of digit one (i.e., no phalanges) observed on franciscana dolphin has arisen at least four times, reflecting slight degree of variability on this character.

Summarizing, our analyses indicate that franciscana dolphins flippers are symmetrical; both sexes show a negative allometric trend; SD is observed in RW, UL, D2L and number of phalanges of digit two; and flipper skeleton allows determining the age class of the dolphins.

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