

Ontogenetic Development and Sexual Dimorphism of Franciscana Dolphin Skull: A 3D Geometric Morphometric Approach

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ABSTRACT The aim of this work was to study the postnatal ontogenetic development of *Pontoporia blainvillei* skull, identifying major changes on shape, and relating them to relevant factors in the life history of the species. We analyzed a complete ontogenetic series (73♂, 83♀) with three-dimensional geometric morphometric techniques. Immature dolphins showed a very well-developed braincase and a poorly developed rostrum, and the principal postnatal changes affected the rostrum and the temporal fossa, both structures implied functionally to the feeding apparatus, thus suggesting a specialized mode for catch fast prey in *P. blainvillei*. Osseous elements associated with sound production were already well developed on immature dolphins, suggesting the importance of this apparatus since the beginning of postnatal life. Sexual dimorphism was detected on both shape and size variables. Females were bigger than males, in accordance with previous studies. Shape differences between sexes were found on the posterior part of premaxillaries and external bony nares ($P < 0.01$), suggesting that this sexual dimorphism is related to differences on vocalization capabilities. *J. Morphol.* 000:000–000, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: geometric morphometrics; ontogeny; *Pontoporia blainvillei*; sexual dimorphism; skull

INTRODUCTION

Cetacean skull morphology differs radically from other mammals, as they are fully adapted to aquatic life, and they can provide valuable information about cetacean life history (e.g., Reidenberg, 2007; Kurihara and Oda, 2009; Frandsen and Galatius, 2013). One of the main skull rearrangements is the telescoping, that is, the disposition of cranial bones in cetaceans, including the back expansion of the maxillary bones, elongated rostrum and dorsal position of the nares and narial passages (Miller, 1923). Telescoping forms a frontal concavity occupied by structures involved in sound generation mechanisms (Ketten, 1992).

Odontocete cetaceans follow the common pattern of mammals, that is, sexual dimorphism with increasing body size (Ralls, 1977; Tolley et al., 1995). However, a few odontocete species show a less common pattern of inverted sexual dimorphism in size, and females usually reach larger sizes than males. This feature has been recorded for *Pontoporia blainvillei*, *Phocoena phocoena*, *Platanista gangetica*, *Berardius bairdii*, and species of the genus *Cephalorhynchus* (Kasuya and Brownell, 1979; Lockyer et al., 1988; Ralls and Mesnick, 2002; Galatius, 2005). Several studies in cetaceans address the issues of cranial ontogeny and sexual dimorphism (e.g., Van Waerebeek, 1993; Turner and Worthy, 2003), although only a few approach these issues through geometric morphometrics (see Galatius et al., 2011; Frandsen and Galatius, 2013). Even though traditional morphometry

Additional Supporting Information may be found in the online version of this article.

Contract grant sponsor: ANPCyT; Contract grant numbers: PICT 2008-1798 and PICT 2012-1588.

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Author contributions: Study design was done by del Castillo, Flores, Cappozzo. Acquisition of data was done by del Castillo. Analysis and interpretation of data was done by del Castillo, Flores, Cappozzo. Manuscript preparation was done by del Castillo, Flores, Cappozzo. Statistical analysis was made by del Castillo.

Received 9 April 2014; Revised 2 June 2014; Accepted 5 July 2014.

Published online 00 Month 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jmor.20309

provides important information regarding changes in morphology, it exhibits some limitations such as the difficulty to separate the components of shape and size (Rohlf and Marcus, 1993). Cranial studies of the franciscana dolphin, *P. blainvillei*, has been addressed from qualitative and morphometric (both linear and geometric) approaches (e.g., Flower, 1867; Pinedo, 1991; Mazzetta, 1992; Higa et al., 2002; Ramos et al., 2002; Trimble and Praderi, 2008; Negri, 2010). Higa et al. (2002) published the only study applying a geometric morphometric approach for this species, although focused on the exploration of sexual dimorphism and differences between populations in mature specimens.

P. blainvillei is the only extant species of the family Pontoporiidae (May-Collado and Agnarsson, 2006; McGowen et al., 2009), and is one of the smallest cetaceans, maximum body length reported is 171 and 152 cm for females and males, respectively (Kasuya and Brownell, 1979; Botta et al., 2010). *P. blainvillei*, together with *Inia geoffrensis*, form the sister group to the Delphinoidea, which includes monodontids, porpoises, and ocean dolphins (Yan et al., 2005). The species occurs from south-eastern Brazil (Siciliano, 1994) to northern Patagonia, Argentina, and inhabits coastal-marine waters, from the coast to the 30-m isobath (Crespo et al., 2010). Sexual maturity is reached between 2 and 4 years (Brownell, 1989; Danilewicz et al., 2000; Panebianco et al., 2012) and life span is about 20 years (Pinedo and Hohn, 2000).

We aim to identify major changes in shape during postnatal ontogeny, as well as differences in the skull shape and size associated with sex, relating these changes to relevant factors in the life history of this species, and comparing them with previously reported data on Delphinoidea. Our goal is to contribute to the understanding of development of the skull of *P. blainvillei*, and provide new information of a family in which the knowledge is still limited. This analytical approach will expand the current knowledge about skull development of odontocete cetaceans.

MATERIALS AND METHODS

Sample Size and Cranial Landmarks

We analyzed a sample of 156 sexed skulls of a complete ontogenetic series (73♂, 83♀) of *Pontoporia blainvillei* (Gervais and d'Orbigny, 1844), deposited on the mammal collection of Museo Nacional de Historia Natural (see Supporting Information), from Uruguay. Following Kasuya and Brownell (1979), we considered mature all males with total length of body (TL) equal or greater than 131 cm, and females with TL equal or greater than 140 cm. In our sample, 51 males and 59 females were considered as immature. In five dolphins, we could not assign maturity stage, because TL data were not available. We digitized 57 landmarks on each skull (Fig. 1) with a 3D-digitizer Microscribe®, which were chosen following Sydney et al. (2012) with some modifications. Each skull was digitized at least twice and an average of both configurations was taken.

Geometric morphometrics allow us to study separately the two components of the form (shape and size), providing information that is not possible to obtain through traditional morphometry (e.g., Rohlf and Marcus, 1993; Adams et al., 2004). Several authors (e.g., Zelditch et al., 2004; von Cramon-Taubadel et al., 2007; Viscosi and Cardini, 2011; Gillick, 2012; Cardini, 2013), reported a limitation associated with Procrustes-based geometric morphometric analyses, known as “Pinocchio effect.” The positions of the landmarks represent shape differences as a whole but the method cannot necessarily convey the amount of shape variation occurring at individual landmarks between individuals (Viscosi and Cardini, 2011). When significant variation between landmark configurations is limited to only one or a few landmarks within the configuration, then the variation between these landmarks may be spread across all of the landmarks used (Zelditch et al., 2004; von Cramon-Taubadel et al., 2007). This may misleadingly decrease the variation occurring at different landmarks and, possibly, generate inconsistent estimators of mean form and shape (Lele, 1993). In this sense, we consider such artifact regarding to the rostral landmarks of *Pontoporia* skull (see below).

Superimposition of landmark configurations were performed by a generalized procrustes analysis (Goodall, 1991; Rohlf, 1999) and size was measured as the centroid size. Collected data was analyzed with MorphoJ (Klingenberg, 2011) and InfoStat (Di Rienzo et al., 2008).

Ontogenetic Changes and Sexual Dimorphism

To analyze the main changes in shape during ontogeny, we worked with the covariance matrix of the symmetrical component of the shape (Klingenberg et al., 2002). We conducted a principal component analysis (PCA) to identify major components of variation of the entire sample. The rostrum is clearly the most prominent structure in *P. blainvillei* skull, and presumably would show the major shape differences between immature and mature dolphins. Taking this into account, we performed the analyses excluding the landmarks corresponding to the rostrum (i.e., landmarks 1, 22, 38, and 39, Fig. 1), thus avoiding the inconsistent mean shape estimators associated to the “Pinocchio effect” (see above). Finally, we performed a size-corrected PCA (i.e., PCA of the residuals of the regression of shape into size), to test if shape differences between sexes were size-related.

We performed multivariate regressions of the Procrustes coordinates against the centroid size to detect how shape variation is associated with size. For comparison between males and females, the angles between regression vectors of both sexes were compared. Angles were computed as the arccosines of the signed inner products between the regression vectors (Drake and Klingenberg, 2008; Klingenberg and Marugán-Lobón, 2013). As ontogenetic vectors were not statically different between sexes (the angle between the two regression vectors was 8.22; $P < 0.001$, under the null hypothesis that the vectors have random directions in the shape tangent space), data were pooled together and shape variation during ontogeny was analyzed for the entire sample. The analysis was performed using MorphoJ (Klingenberg, 2011).

To analyze shape differences between sexes, we worked with the covariance matrix of the symmetrical component of the shape, also discarding the landmarks corresponding to the rostrum (Fig. 1). First, we run these analyses with the complete ontogenetic series, and then on immature and mature individuals as separate subsets to detect if sexual dimorphism on shape or size was only expressed in adults periods, or dimorphism is already expressed during juvenile stages of growth. We conducted a PCA for each subset, and when some difference on the shape space was detected, we proceeded to explore sex differences with a discriminant analysis (DA). DA was performed to detect the landmarks showing most variation between sexes, because DA tends to over-estimate groups differences when the sample size is small relative to the number of landmarks

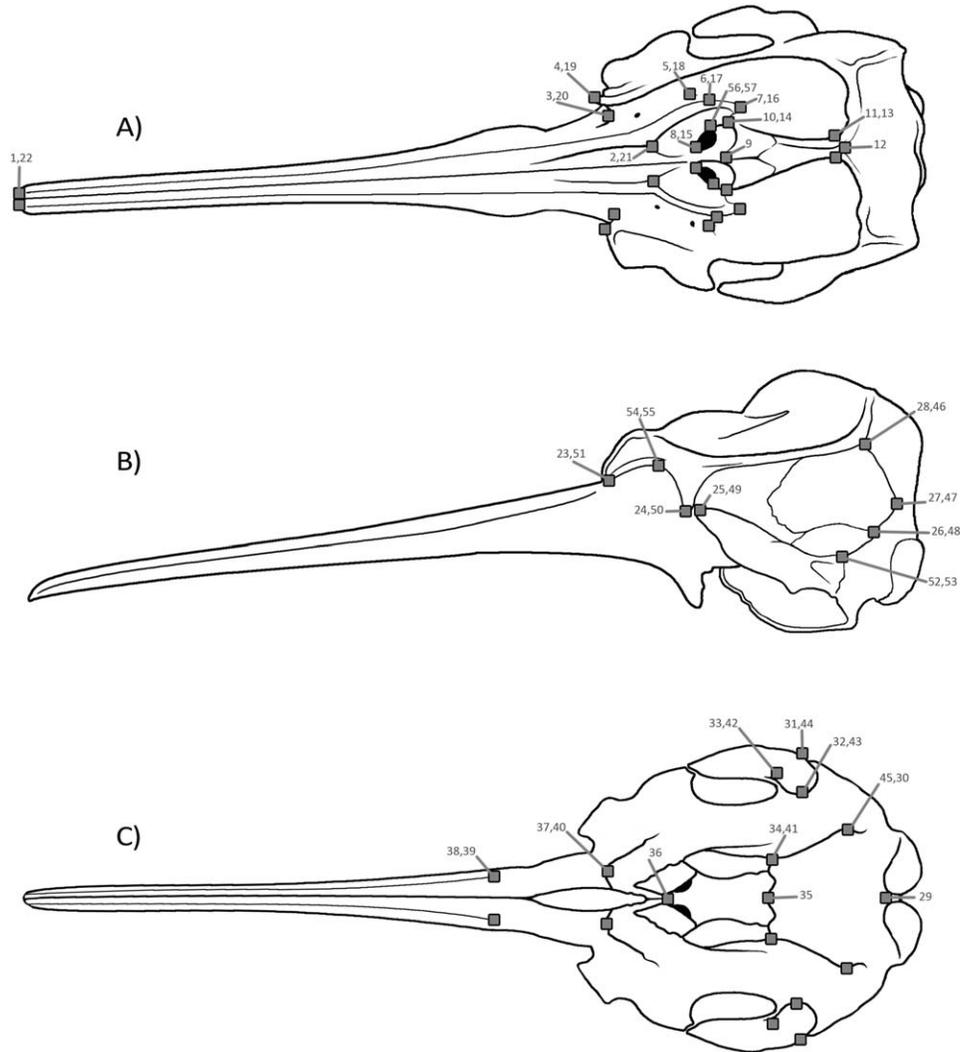


Fig. 1. *Pontoporia blainvillei* landmarks in dorsal (A), lateral (B), and ventral (C) views of the skull.

(which is our case). We select only a few landmarks recognized as the most variables between sexes on the DA (see Results) and conducted a PCA for each subset (mature and immature samples) with such selected landmarks. When difference on the shape space was detected, we also proceeded to explore sex differences with a DA. Finally, we performed ANOVA analyses using centroid size as dependent variable, to analyze size differences between males and females. Here, we also carried out these analyses with the complete ontogenetic series, as well as on immature and mature subsets separately. We tested the normality of the sample with a Shapiro–Wilks test in both our complete sample and cutoff subsets (complete sample: $W = 0.99$, $P = 0.86$; immature subset: $W = 0.97$, $P = 0.07$; mature subset: $W = 0.95$, $P = 0.34$).

RESULTS

Ontogenetic Changes

Rostrum was the structure that showed most variation in our sample as evidenced the PCA that takes into account all cranial landmarks (Fig. 2; first PC explained 67.15% of the variance and was highly related to the rostrum length). Most mature

specimens of both sexes were placed on the positive (right) side of the first component, although few immature specimens (male and female) were also placed on the right side of this component. Mature (and some immature specimens as well) female dolphins occupied the right extreme on the first component, denoting the most elongated rostral morphology, and immature and mature morphology was more clearly distinct in males (Fig. 2) than in females.

Cranial shape variation excluding the rostrum is described in Figure 3. The First PC explained 16.95% of the variance, mature female dolphins were mostly placed on the right side of this component, and male dolphins were more dispersed on this component. Cranium shape was similar according to sex (except the extreme position of mature female dolphins), and some differences between mature and immature specimens were detected (although with some overlapping), thus

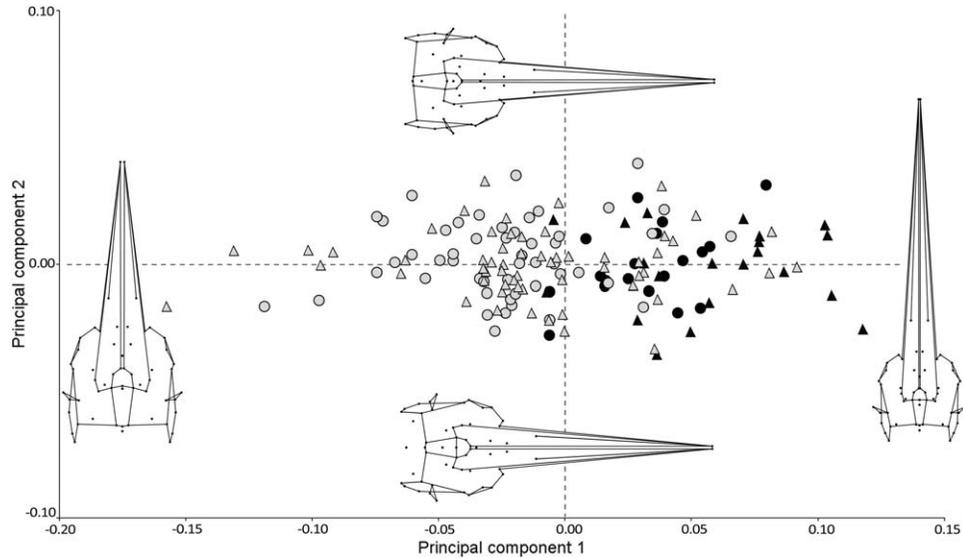


Fig. 2. *Pontoporia blainvillei* PCA of the 3-dimensional shape variables including all landmarks. Circles: male dolphins; triangles: female dolphins. Light gray: immature dolphins; dark gray: mature dolphins.

denoting a continuous pattern of variation. Sorting according to sexual maturity on the first PC was more obvious in the PCA analysis that included rostral landmarks (Figs. 2 and 3).

As mentioned above, PCA analysis showed that females have a longer allometric trajectory than males (Fig. 2), suggesting that overall shape differences between sexes could be associated with size. This fact was confirmed using the size corrected PCA which did not show spatial differences on the shape-space between sexes, thus indicating that differences on skull shape associated with sex are caused by allometric variation (Supporting Information Fig. S1).

The regression of Procrustes coordinates versus centroid size including all the 57 landmarks was highly significant ($P < 0.01$) and the size explained 54.1% of the shape changes. However, the same analysis excluding rostrum landmarks (Fig. 4) was also significant ($P < 0.01$) but size explained only 10.9% of the shape changes. Ontogenetic trajectory describes shape variation from skulls showing a rounded general shape, a not developed nuchal crest, orbits pointing to the front, rounded and thin temporal fossa, thin rostrum base pointing down, wide posterior part of premaxillary bone, mandibular (glenoid) fossa close to midline, and posterior part of the skull more developed, toward skulls with a more triangular global shape, a well-developed nuchal crest, orbits pointing more laterally, oval and wide temporal fossa, wide rostrum base aligned with the skull, thin posterior part of premaxillary bone, mandibular fossa placed further away from the midline and anterior part of the skull more developed (Fig. 4).

Sexual Dimorphism

Sexual dimorphism was not evident using PCA including both mature and immature dolphins. Therefore, we divided the dataset in subsets of individuals, mature and immature, and explored sexual dimorphism in both groups. The PCA corresponding to immature dolphins did not show any special discrimination corresponding to sexual dimorphism, in comparison with that corresponding to mature dolphins that did show a spatial pattern according to sex. The DA performed to explore those sexual differences showed that main differences between sexes were similar to those observed between the extremes of the regression analysis (see Fig. 4; i.e., the skull shape of female

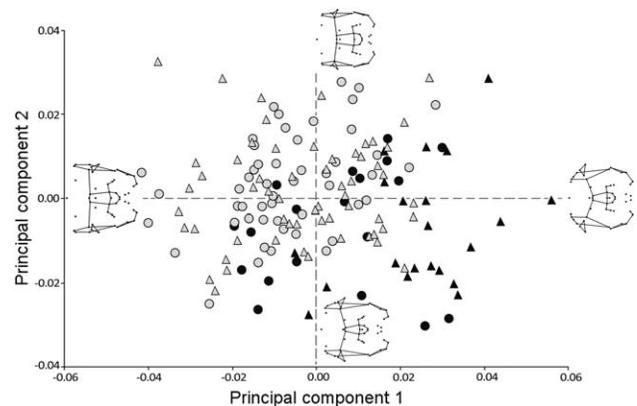


Fig. 3. *Pontoporia blainvillei* PCA of the 3-dimensional shape variables excluding landmarks 1–22, 38–39. Circles: male dolphins; triangles: female dolphins. Light gray: immature dolphins; dark gray: mature dolphins.

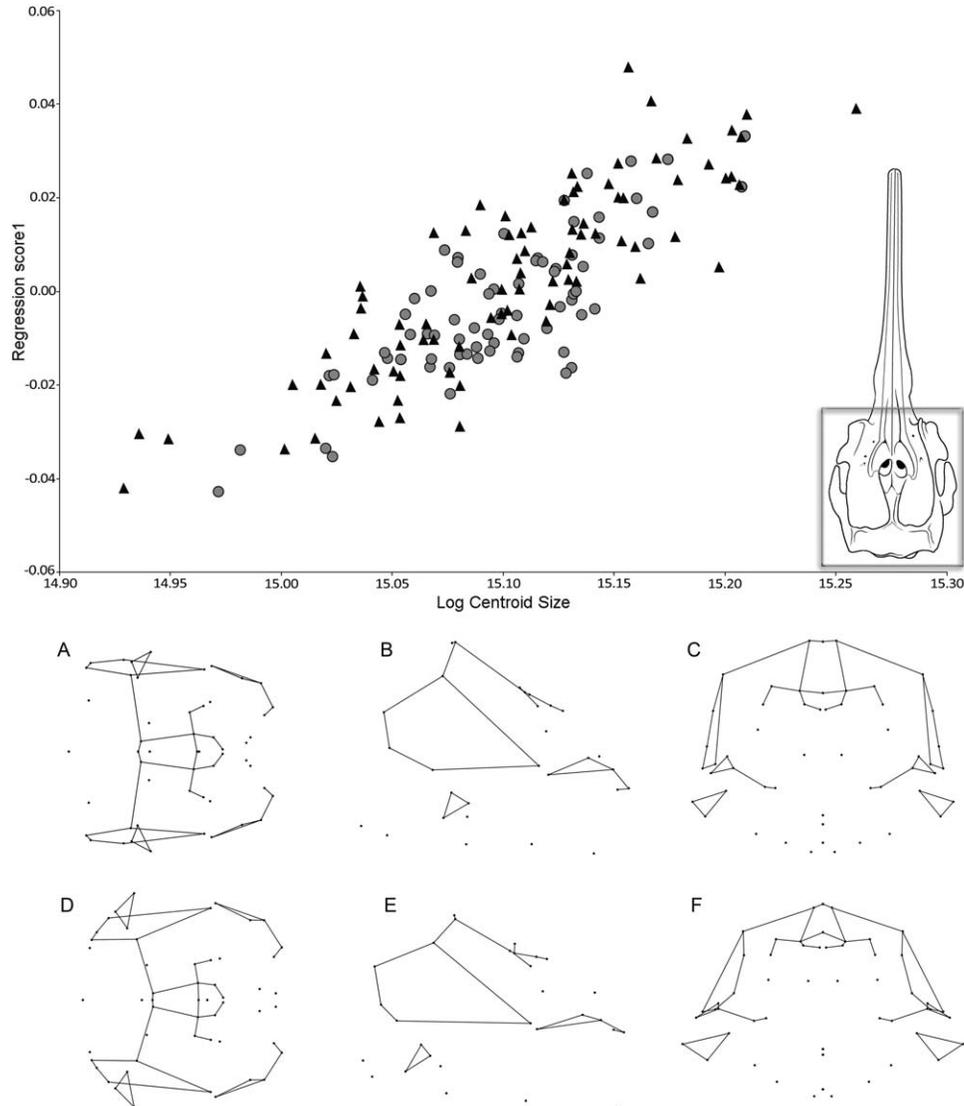


Fig. 4. *Pontoporia blainvillei* procrustes distance from average shape plotted against CS. Triangles: females, Circles: males. (A, D) Dorsal; (B, E) lateral; (C, F) frontal views of lower and upper size extremes, respectively (magnification $\times 2$).

dolphins is alike to the shape of the mature dolphins, whereas the skull shape of male dolphins is alike to the shape of the immature dolphins). Moreover, additional differences on the posterior part of the premaxillaries and external bony nares were also detected in the DA ($P < 0.01$). The latter region showed clear differences according to sex, evidenced on Figure 5 (PCA performed taking into account only the landmarks implied in such area; i.e., landmarks 6–17, 7–16, 8–15, 9, 10–14, 56–57), and on Table 1, that shows a high rate of correct classification with cross validation method.

Sexual differences in centroid size were tested by ANOVA excluding rostrum landmarks and no significant differences were found (ANOVA $n = 156$, $F = 0.48$, $P = 0.49$). A similar outcome was found for immature dolphins (ANOVA, $n = 110$,

$F = 0.37$, $P = 0.54$). However, centroid size showed sexual dimorphism for mature dolphins favoring females (ANOVA, $n = 41$, $F = 9.29$, $P < 0.01$). Sexual differences in centroid size revolved the same pattern when the complete landmark configuration was analyzed (ANOVA complete data set: $n = 156$, $F = 2.52$, $P = 0.11$; immature dolphins: $n = 110$, $F = 0.48$, $P = 0.49$; mature dolphins: $n = 41$, $F = 16.63$, $P < 0.01$).

DISCUSSION

Ontogenetic Changes

The rostrum was the feature that showed the most drastic growth during postnatal ontogeny. This growth pattern was also observed in other dolphins such as *Tursiops* spp. and *Sotalia*

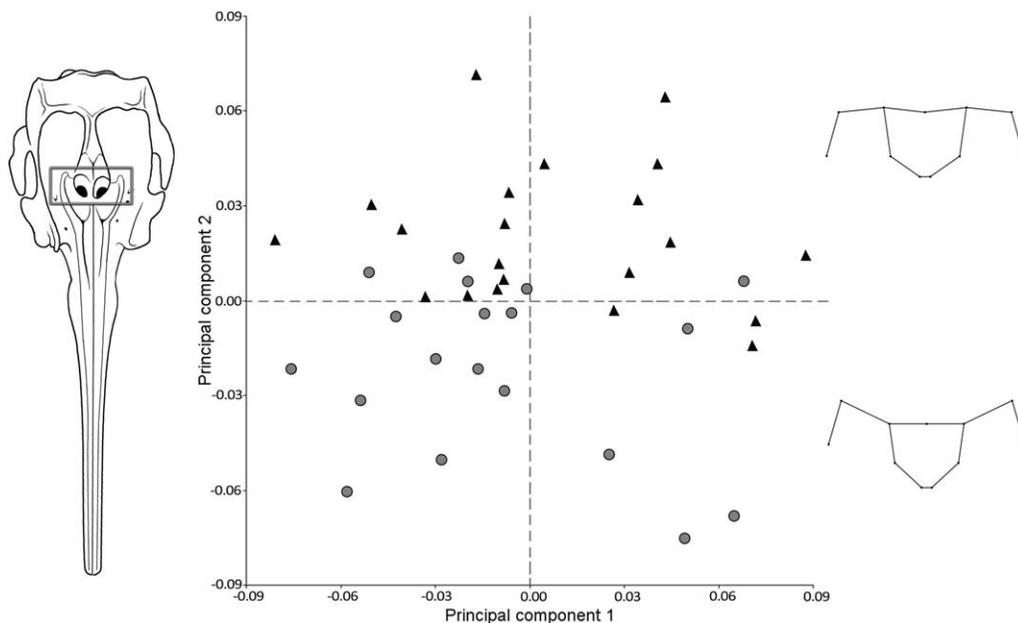


Fig. 5. *Pontoporia blainvillei* PCA of the 3-dimensional shape variables of the narial region. Only mature dolphins are plotted. Circles: male dolphins; triangles: female dolphins.

guianensis (e.g., Kurihara and Oda, 2009; Sydney et al., 2012), although in some pedomorphic species such as porpoises and *Cephalorhynchus commersonii* the rostrum was not particularly elongated during ontogeny (Galatius et al., 2011). According to Werth (2006), mandibular bluntness on odontocete cetaceans is related to feeding strategy, in which species with blunt heads and wide jaws have more circular mouth opening, improving water flow for suction feeding. Taking these into account, the drastic growth of the rostrum in adults of *P. blainvillei* (Fig. 2) is indicative of specific feeding strategies, where suction feeding is likely less important than a catching mode of predation. Young and adults franciscana dolphins exhibit differences in their feeding habits (Rodríguez et al., 2002), which is also reflected in a notably longer rostrum in adults, probably associated with a better performance of catching bigger and faster preys. The small teeth disposed in a proportionally long tooth row work as a highly efficient tool for catching preys. The shape change pointed to enlarging the temporal fossa (Fig. 4) is probably associated with this performance; a longer rostrum acting through well-developed temporal muscles may allow dolphins to catch fast preys efficiently. In addition, the lateral displacement of the squamosal in adults (Fig. 4) derives in an amount of the available space for the accommodation of the temporalis. This character seems to be common in mammals (and perhaps a plesiomorphic condition), as was detected in the ontogeny of several groups of marine and terrestrial mammals

(e.g., Segura and Prevosti, 2012; Flores et al., 2013; Segura et al., 2013; Tarnawski et al., 2014a, 2014b). Although the longer rostrum suggests a decreasing of the mechanical advantages at the tip (Preuschoft and Witzel, 2002; Segura and Prevosti, 2012; Segura et al., 2013), its length, associated with a well-developed temporal musculature, also implies a gain in mouth-closing speed at the tip, making prey catch function in adults more efficient. These observations are in agreement with those by Loy et al. (2011), who suggested that the elongation of the rostrum might be related to differences in feeding habits for different populations on *Stenella coeruleoalba*.

Mature franciscana dolphins showed a rostrum more aligned with the skull -and with the column- than immature dolphins. This fact has been also observed by Sydney et al. (2012) on *Sotalia guianensis*, who suggested that this feature is associated with a shift toward a more pelagic feeding.

Sound production in odontocete cetaceans is driven by pressurized air within a complex nasal

TABLE 1. *Pontoporia blainvillei* classification summary shape variables using cross-validation

Sex	Frequency of female classification (<i>n</i>)	Frequency of male classification (<i>n</i>)
Female	17/21	4/21
Male	2/19	17/19
Total	19/40	21/40

DA performed with the landmarks: 6–17, 7–16, 8–15, 9, 10–14, 56–57.

system (Madsen et al., 2012), where sound vibrations propagate through the melon and emerge into the environment (McKenna et al., 2012). The echolocation system allows dolphins to forage, avoid obstacles, and orient under water (Thomas et al., 2004), and involves sound production as well as sound receiving. According to Cranford et al. (1996), premaxillary bones are linked to the shape and position of soft tissues responsible for sound production and probably influence the sonar beam formation process. In this sense, the early development of these bones in *P. blainvillei* may be associated with the precocious development of the sound production apparatus. Previous studies performed on odontocetes (e.g., Hendry, 2004; Li et al., 2007; Favaro et al., 2013) indicate that sound emissions in some species (*Tursiops truncatus* and *Neophocaena phocaenoides asiaeorientalis*), starts at 22 days of age or earlier. Although there is no data about the age at which echolocation actually begins in *P. blainvillei*, the early development of such capacity is probably a characteristic widespread in odontocete cetaceans (e.g., Yurick and Gaskin, 1988); the proportionally wider premaxillary bones observed in immature franciscana dolphins (Fig. 4) may be associated with that early development. In this context, Racicot and Berta (2013) recently found that pterygoid sinuses on paedomorphic porpoises are already present on neonates, suggesting the importance of bidirectional hearing in earlier stages of the ontogeny. Our results reinforced all previous evidence pointed to the early acquisition of echolocation system in odontocete cetaceans.

The dorsal displacement of the midpoint of the nuchal crest (Fig. 4) in adults may increase the surface for insertion of the muscles inserted on the occipital plate, such as the m. *semispinalis* (Strickler, 1980), thus increasing the stability during swimming. The nuchal crest is anteriorly displaced during ontogeny, in agreement with Galatius et al. (2010), who suggested that this displacement may be associated to the supraoccipital telescoping over the parietal and frontal bones during ontogeny.

The general direction of the shape change during postnatal ontogeny observed on *P. blainvillei* skull was expected according to the common pattern observed for mammals and particularly for odontocetes, that is, an enlargement of the rostral part of the skull, and a compression of the neurocranium, which is proportionally larger in earlier stages of postnatal development (Moore, 1966; Galatius et al., 2011; Sydney et al., 2012; Flores et al., 2013; Segura et al., 2013). Specific features, such as the early development of the premaxillaries and external nares, are probably restricted to odontocetes, because the skull function in this group is not only pointed to trophic and sensitive functions, but also to sound production mechanisms. Echolocation is also believed to influence skull size

because neural processing needs to be associated with either echolocation per se or its elaboration into a complex perceptual system in odontocete cetaceans, leading to an increase in encephalization at the origin of the clade (Marino et al., 2004).

Sexual Dimorphism

Our results showed inverted sexual dimorphism of size, consistent with previous studies in *Pontoporia* and other dolphins (e.g., Lahille, 1899; Kasuya and Brownell, 1979; Mazzetta, 1992; Pinedo, 1991; Higa et al., 2002). Although the inverted dimorphism of *Pontoporia* is not a novelty, we discuss the specific shape differences between sexes.

The main shape differences between sexes were associated with size differences, as noted in Figures 2 and Supporting Information S1. The skull shape of female dolphins is alike to the one of mature dolphins, whereas the shape of the skull of male dolphins is alike to the one of the immature dolphins. This fact probably occurs because females grow for a longer period of time than males, which is in agreement with Barreto and Rosas (2006) and Negri (2010), whom suggested that dimorphism is reached by growth extension of the trajectories of females (i.e., hypermorphosis, *sensu* Leigh, 1992). Moreover, an alternative explanation is that female dolphins start its postnatal growth with a larger size than males. However, our regression analysis of Procrustes distances on CS (Fig. 4) seems to indicate that females are not larger than males on the earlier stages of growth. Indeed, the youngest female of our sample exhibit lesser CS than the youngest males analyzed. These results suggest that sexual dimorphism in skull shape is size-related, as has been reported in other mammals (e.g., Cardini and Elton, 2008; Flores and Casinos, 2011; Tarnawski et al., 2014a, 2014b), although, as a general trend, favoring males.

Different hypotheses have been proposed to explain inverted sexual dimorphism in size observed on small cetaceans. Ralls (1976) proposed that larger body size on females exhibits better chances in competition for resources, but an alternative (not exclusionary) explanation pointed to the physiological needs of a minimum newborn size to maintain body temperature. Our results of size and size-related shape sexual dimorphism observed on mature dolphins are in accordance with both hypotheses.

The structure of the premaxillary bones and the nasal opening, condition the morphology of soft tissues responsible for vocalization capabilities (Cranford et al., 1996). In this sense, sexual differences on this area detected in our DA may be related to possible differences on the vocalizations between sexes. Previously, Sayigh et al. (1995) have suggested that on matrilineal cetaceans, vocalizations capabilities may be under greater

selection pressures on females, as they would need to be capable to produce whistles different from their mothers to be recognized. Fripp et al. (2005) suggested that males and females *Tursiops* may use signature whistles differently as adults, based on whistle recordings of wild dolphins. Recent studies have suggested that matrilineal units would be the social unit on franciscana dolphins (Valsecchi and Zanelatto, 2003; Costa-Urrutia et al., 2012), for which females and males franciscana dolphins may have a distinct selection pressure for vocalization capabilities, reflected on the soft tissues responsible for this, as well as in the osseous elements supporting them. Even though whistles have not been recorded in franciscana dolphins (Melcón et al., 2012; Morisaka, 2012), previous studies proposed that the species that do not whistle may compensate this loss by using other types of sound or clicks for communication (Morisaka, 2012). Taking this into account, it is clear that further research is needed to elucidate the nature and possible consequences of sexual dimorphism observed in this area. Even though the report of Higa et al. (2002) detected absence of sexual dimorphism on skull shape of *P. blainvillei*, our results indicate some shape differences. However, we consider that Higa's finding is not in complete disagreement with our results, mainly due to that the former authors considered only one landmark in the area where we detected sexual dimorphism in shape (i.e., the nasal opening).

Summarizing, our results indicate that the main shape changes on the postnatal ontogeny of *P. blainvillei* are associated to the changes in feeding habits, as it affects principally to the trophic apparatus and its capabilities. Moreover, the early development of the bony elements associated with sound production, suggests the major importance of this apparatus since the beginning of postnatal life. Finally, sexual dimorphism found on the dorsal part of the premaxillaries and the external bony nares may be associated to differences on vocalization capabilities according to sex, a topic that should be also addressed in a behavioral context in this species. The detection of additional sexual differences and its expression during the ontogeny in other delphinid species is also highly interesting and necessary to detect patterns of dimorphism and ontogenetic shape changes in phylogenetic and functional grounds, considering the strongly specialized life history in cetaceans, and the different habitats (i.e., coastal and off-shore species) where they inhabit.

ACKNOWLEDGMENTS

The authors thank Enrique Gonzalez for permitting access to collection specimens and Sabrina Riverón who was very hospitable during sampling. The authors also thank Marcelo del Castillo who

kindly helped with the figures of this manuscript, and María Victoria Panebianco for the English corrections. This research project is a part of DdC's PhD thesis for which the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) from Argentina granted her a postgraduate fellowship. Finally, thanks to the two anonymous reviewers for their valuable corrections and suggestions which greatly improved this manuscript.

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