

Reproductive Parameters of Female Long-finned Pilot Whales (*Globicephala melas edwardii*) from the Southwestern Atlantic

Soto Florencia Anabella^{1,*}, Grandi María Florencia², García Néstor Aníbal², Crespo Enrique Alberto^{2,3}, and Dans Silvana Laura^{2,3}

¹Universidad Nacional del Comahue, Centro Regional Universitario Bariloche, Río Negro, Argentina

²Laboratorio de Mamíferos Marinos, Centro para el Estudio de Sistemas Marinos (CESIMAR). CENPAT-CONICET. E-mail: grandi@cenpat-conicet.gob.ar; garcia@cenpat.edu.ar

³Universidad Nacional de la Patagonia San Juan Bosco, Sede Puerto Madryn, Chubut, Argentina. Kike.Crespo@uv.es; dans@cenpat-conicet.gob.ar

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Soto Florencia Anabella, Grandi María Florencia, García Néstor Aníbal, Crespo Enrique Alberto, and Dans Silvana Laura (2017) Mass strandings represent a valuable source of information, especially when it comes to difficult-to-access pelagic species, such as *Globicephala melas edwardii*. We collected reproductive organs from 27 female pilot whales stranded in Caleta Malaspina, Chubut, Argentina. Our main objective was to analyze reproductive parameters of female long-finned pilot whales in the southern hemisphere. To achieve this, we studied age and reproductive status in order to approximate age and body length at sexual maturity, breeding interval, and fertility of this subspecies. The maturity state was determined by recording different ovulation bodies obtained from histological sections and by assessing the presence of milk in the mammary glands and a fetus, and we concluded that 5 of the females were immature and 22 were mature females. Finding mature females in different reproductive states (resting, in lactation, pregnant, or pregnant and in lactation) along with the great variety of fetus sizes would indicate that copulation and birth are not governed by seasonality. Age at sexual maturity was 8 years old and length at sexual maturity would reach 365.50 cm. The annual pregnancy rate was constant with age for mature females at 0.41 ovul / year, with a breeding interval of 2.4 years. The average fertility varied between 0.19 and 0.21. All the parameters are within the estimated range for females of the North Atlantic subpopulation, except for the breeding interval, which was significantly lower than estimates for the northern subspecies. This study could be limited because all individuals come from a single mass stranding. While this study allowed us to reach some conclusions about the reproductive biology of female *G. melas edwardii* in the Southwestern Atlantic Ocean, further studies are needed to fully comprehend the behavior and biology of this species.

Key words: Age at sexual maturity, Breeding cycle, Fertility, Length at sexual maturity, Ovarian activity.

BACKGROUND

Cetaceans (Chordata, Cetacea) include 87 species of whales, dolphins and porpoises (Hoyt 2001). This diverse group ranges in adult sizes from less than 1.5m to 33m in length, inhabits all major ocean basins and adjoining seas as well as certain lakes and river systems, and exhibits a

diverse array of life history strategies (Perrin 1991; Rojas-Bracho et al. 2006). Among the strategies, the overall pattern of their social structure suggests that herds or groups are structured by sex and age depending on the degree of sexual dimorphism and mating system of the species; the more dimorphic and polygynous they are, the greater the separation by groups of different ages and

*Correspondence: Fax: +54 0280 488-3184. E-mail: sotoflorenciaa@gmail.com

sexes is (Gaskin 1980; Norris and Dohl 1980). The most dimorphic and polygynous cetaceans are the sperm whale (*Physeter macrocephalus*), killer whale (*Orcinus orca*), false killer whale (*Pseudorca crassidens*) and pilot whale (*Globicephala* sp.); and all present a complex social structure. In these species, groups are in stable partnerships over the time and consist mostly of females and immature individuals of both sexes with a few or even a single adult male temporally associated with the herd (Norris and Dohl 1980).

Long-finned pilot whales, *G. melas*, inhabit cold temperate waters of both the North and South Atlantic Oceans. These whales are isolated in the two hemispheres and recognized as subspecies: *G. melas* in the northern and *G. melas edwardii* in the southern hemisphere. Pilot whales are polygynous; huge aggregations of pilot whales are occasionally reported and it is believed that males move between family groups to mate during these temporary aggregations. This type of social structure in which adult males live with related females and copulate elsewhere is common among pilot whales (Oslo and Stephen 2002). The average herd size is 80 individuals and ranges from 10 to hundreds of whales. All ages and sex classes are represented in each group, with a high ratio of females to males (Sergeant 1962; Bloch et al. 1993a).

There is little known information on the reproductive parameters of the southern species, and knowledge of *Globicephala* reproductive biology comes from populations of the northern subspecies: *G. melas*. The breeding season occurs during spring and summer and shows a marked synchronization cycle (Sergeant 1962; Martin and Rothery 1993), a feature of polygynous systems. Sexual maturity is delayed with age and differs between sexes: males mature at 10–12 years while females mature at 6–7 years (Desportes et al. 1993; Martin and Rothery 1993). The body length at sexual maturity (LSM) ranges from 430 to 490 cm in males and 366 to 378 cm in females (Lockyer 1993). The gestation period was estimated to be 15 to 16 months and lactation to be 22 months, so a full reproductive cycle lasts about 40 months. According to the longevity of the species, a female can give an average of 9 pups in its life, thus the breeding interval would be 3.3 years on average. Age-specific fertility (m_x) ranges from 0.18 to 0.24 (Martin and Rothery 1993). Females reach menopause at age 40 when they become senescent and, after giving birth to her last calf, this process can last more than 15

years (Sergeant 1962; Martin and Rothery 1993). The adaptive significance of senescence is still unknown but it probably contributes to increasing reproductive success.

The above reproductive parameters come from studies of the northern subspecies, but there is a lack of information on these parameters for the southern species. Our main objective was to fill this knowledge gap by analyzing the reproductive parameters of female long-finned pilot whales in southern hemisphere. We expected to find differences among reproductive parameters in both subspecies due to their life histories. To achieve this, we studied age and reproductive status of this subspecies in order to approximate its age and length at sexual maturity, the breeding interval, and fertility.

MATERIALS AND METHODS

Collection of specimens

A total of 50 long-finned pilot whales were found in a massive stranding that occurred in 2009 at Caleta Malaspina (45°10'S, 66°30'W). This cove is a vast and shallow sea entrance to the continent located at the south of Bahía Bustamante, Chubut, Argentina. There were 31 females and 19 males in this sample. Female standard body length was measured as a straight line from the tip of the rostrum to the fluke notch (Norris and Prescott 1961). Some teeth were taken for age estimation. From each necropsy, reproductive tracts were dissected out, including intact pairs of ovaries, uteri ($n = 27$) and, in some cases, fetuses. These samples were fixed in 10% formaldehyde and then preserved in 70% alcohol solutions for further analysis.

Age estimation

Age determination of each individual was performed by counting growth layer groups (GLG) in the dentine (Fig. 1a) and cementum (Fig. 1b) of tooth sections. Teeth preparation was done using Crespo et al.'s (1994) technique. Tooth sections obtained were examined under a stereomicroscope (30X) with transmitted light in order to count GLGs, considering that one GLG represent a one-year period for this species (Kasuya 1988; Lockyer 1993) and half GLG a 0.5 year-old calf (Sergeant 1962). Each section was read independently at least twice by three experienced

observers to estimate each individual age. If readings differed by no more than one year, the mean was used; if they differed by more than one year, sections were re-examined or re-prepared.

Reproductive analysis and definitions

Ovaries were weighed and measured. For macroscopic inspection, slides were made by hand-sectioning at 2mm intervals parallel to the attachment of the ovarian ligament, recording amount and size of ovarian corpora. Four types of corpora were identified:

- Corpora lutea (CL): sponge-like with highly vascularized pigmented formation and endocrine function. Once Graafian follicle is ruptured, its wall collapses into a folded structure, the active CL. Regressing CL results from a pregnancy in the preceding cycle.

- Corpora albicantia (CAI): dense, connective tissue scar resulting from the regression of a corpora lutea from earlier pregnancies or ovulations.

- Corpora atresia (CAt): scar resulting from the degeneration of a follicle before ovulation. Irregularly shaped and smaller than a CAI, it may have yellow or brown pigmentation.

- Graafian follicles (GF): hollow tissue mass containing the oocyte in a liquid medium.

When possible, the diameter of the uterine horn was measured and then opened to check for the presence of an embryo, a fetus, or signs of pregnancy (placental ligaments or dark scars). Mammary glands were also examined in the field in order to record the presence or absence of milk.

The reproductive status for each female was defined according to the first ovulation (DeMaster 1984), classifying them according to the terminology of Perrin and Donovan (1984) as immature (absence of CAI and CL) or mature (presence of at least one CAI or CL). They were then characterized as: resting (neither pregnant nor with milk in the mammary glands), in lactation (with milk in the mammary glands), pregnant (presence of fetus), or pregnant and in lactation (with a fetus and milk in the mammary glands).

To describe the follicular cycle, Graaf follicles greater than 1mm were counted and measured and the average follicular diameter (AFD) was calculated using the 5 largest follicles.

Considering that ovulations in *G. macrorhynchus* occur more frequently in the left ovary at any age (Kasuya and Marsh 1984), a student *t*-test was performed to analyze whether there are significant differences in the size and ovarian activity (measured as CAI + CL) of the right and left ovaries in mature females (*n* = 22). We first used logarithms to test for normality in

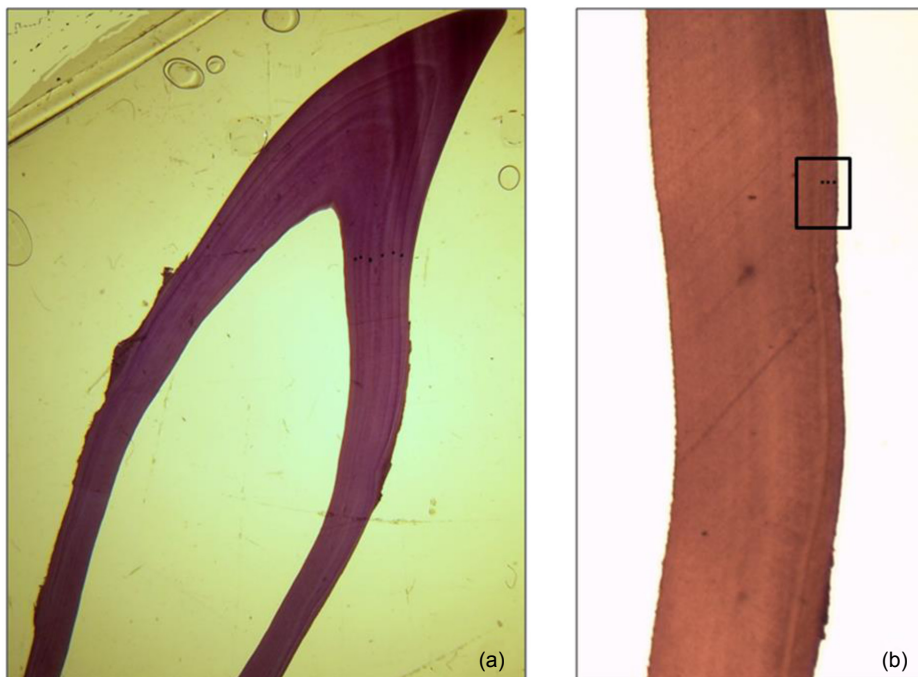


Fig. 1. Deposition pattern on dentine (a). Deposition pattern on cement (b). In both cases, each point represents a GLG.

the data; if there was a lack of normality then we worked with non-parametric. Additionally, we evaluated if there is a relationship between the size of fetus and corpus luteum, fitting the data to a simple linear regression ($n = 7$).

Age at sexual maturity (ASM) was defined, according to the criteria established by DeMaster (1984), as the age at first ovulation. The average age at sexual maturation (ASM) was defined as the age at which 50% of females are mature (DeMaster 1984). ASM was then estimated by fitting a logistic model to the proportion of mature females by age class (p_x). This p_x was estimated using two methods:

$$a - p_x = \frac{CL_x + CAI_{x+1}}{n_x + n_{x+1}}$$

where CL_x is the number of females in the age class x with one corpora lutea, CAI_{x+1} is the number of females in the age class $x + 1$ with one recent corpora albicantia, n_x is the number of females in the class age x , and n_{x+1} is the number of females in the age class $x+1$ (Smith 1973).

$$b - p_x = \frac{\sum (\text{N}^\circ \text{ of first ovulated females})}{\text{total females}}$$

Length at sexual maturity (LSM) was estimated by relating ASM to the average body length obtained from body growth curves. Two models were adjusted, von Bertalanffy and Gompertz, and defined by the following equations:

$$L_t = L_\infty(1 - e^{-k(t-t_0)}) \quad \text{And} \quad L_t = L_\infty e^{-c_0 e^{-kt}}$$

where L_t is the length (cm) at age t , t is the unit of time in years, t_0 is the hypothetical age at which the body length is zero, L_∞ is the asymptotic length (e.g. the average length achieved if the species grow indefinitely), k is the growth rate, c_0 is a constant of proportionality, and e is the natural logarithm base.

Models were adjusted with Excel spreadsheets while parameter estimation was performed using maximum likelihood methods by minimization algorithms (Microsoft Excel Solver). The selection of models was made based on the lowest Corrected Akaike Information Criterion (AICc) (Johnson 2004).

Fertility (m_x), defined as the average number of female offspring generated by every pregnant female (Caughley and Gunn 1996), was estimated as $\frac{1}{2}$ the annual pregnancy rate (APR) and the proportion of mature females that were pregnant at

the time of stranding in each age class (Martin and Rothery 1993).

The breeding interval (BI), defined as the time between two pregnancies of the same female, was estimated from 21 mature females and calculated as $BI = 1/APR$.

RESULTS

Age, length and reproductive status

Observed inside out, the pattern of dentine growth layers was a thin translucent band constituting the neonatal line and then a succession of (light and dark) alternating bands that, until 10-12 years, had a width that decreased in size. After that time, a bonded set of much finer bands surrounding the pulp cavity was observed. Age ranged from 0.5 to 35 years.

Body length ranged from 221 to 479 cm (Fig. 2), with an average length of 409 cm. The largest immature female measured 353.5cm and the smallest mature one measured 377 cm.

Regarding the reproductive status from the whole sample, 5 were immature and 23 were mature; among matures: 8 were pregnant, 6 in lactation and 9 at rest. No females were found simultaneously pregnant and in lactation.

Age and length at sexual maturity

The ASM was estimated as 7.81 ± 0.29 years (\pm SD) according to the method proposed by Smith and 8.00 ± 0.12 years (\pm SD) according to the DeMaster criteria (Fig. 3). Given that the age of the DeMaster method was best adjusted it had the lowest confidence interval subsequent analysis used this method for ASM estimation.

The data were supported by both Gompertz and von Bertalanffy models. Asymptotic length was 441.28 cm and 439.15 cm for von Bertalanffy and Gompertz curves, respectively (Fig. 4). Akaike Information Criterion corrected (AICc) showed differences of less than 2 units between models, so model selection could not be performed following this criterion. However, we worked with von Bertalanffy model because it had the least estimated parameters and was the most parsimonious. We used the von Bertalanffy model to estimate that the LSM was 365.50 ± 24.03 cm (\pm SD) when females became sexually mature at 8 years old.

Ovarian activity

Ovaries measurements and weight of mature and immature females are summarized in table 1. The youngest immature female (0.5 years) had more than 10 Graafian follicles less than 1 mm wide while the AFD in pregnant females was 2.2 mm and 3.2 mm in resting females. The oldest immature female (13 years) had a Graafian follicle of 4.5 mm while the largest Graafian follicle of a resting female measured 5.4 mm, which suggests that the youngest immature female was close to its ovulation time.

Even though ovulation appeared to take place from any point on the surface of either ovary, more ovulations occurred in the left ovary (Fisher LSD = 4.4233, *d.f.* = 21, *p* = 0.0006), as Marsh and Kasuya (1991) described. However, in this study there was no evidence of differential growth between the ovaries' volume (*t*-student = 0.2729, *d.f.* = 21) and weight (*t*-student = 0.8003, *d.f.* = 21) (Fig. 5).

When we evaluated the relationship between the size of fetus and corpus luteum, the linear

regression was not significant ($F = 0.88, R^2 = 0.15, p = 0.3897$). However, we noted that the size of the CLs varied from about 36 to 46 cm (Fig. 6), regardless of fetus size, showing a tendency to stabilize its growth close to this range. This can be clearly demonstrated because the smallest fetus measured 23 cm and its corresponding CL measured 39.8 mm, while the CL of a female whose fetus was near term (160cm) measured 40.51 mm.

Fertility and breeding cycle

The estimated fertility from the APR (Table 2) shows an average of 36.66 % pregnant females; this value implies an annual pregnancy rate of 0.37 ovum/year. If we consider a sex ratio at birth of 1:1 (Sergeant 1962), then half have females and fertility would be equal to $m = 0.19$.

The annual pregnancy rate (slope value) indicates that females of this stranding generate 0.41 ovules per year (Fig. 7), thereby resulting in a breeding interval of 2.4 years. The rate is linear and therefore constant for all age classes.

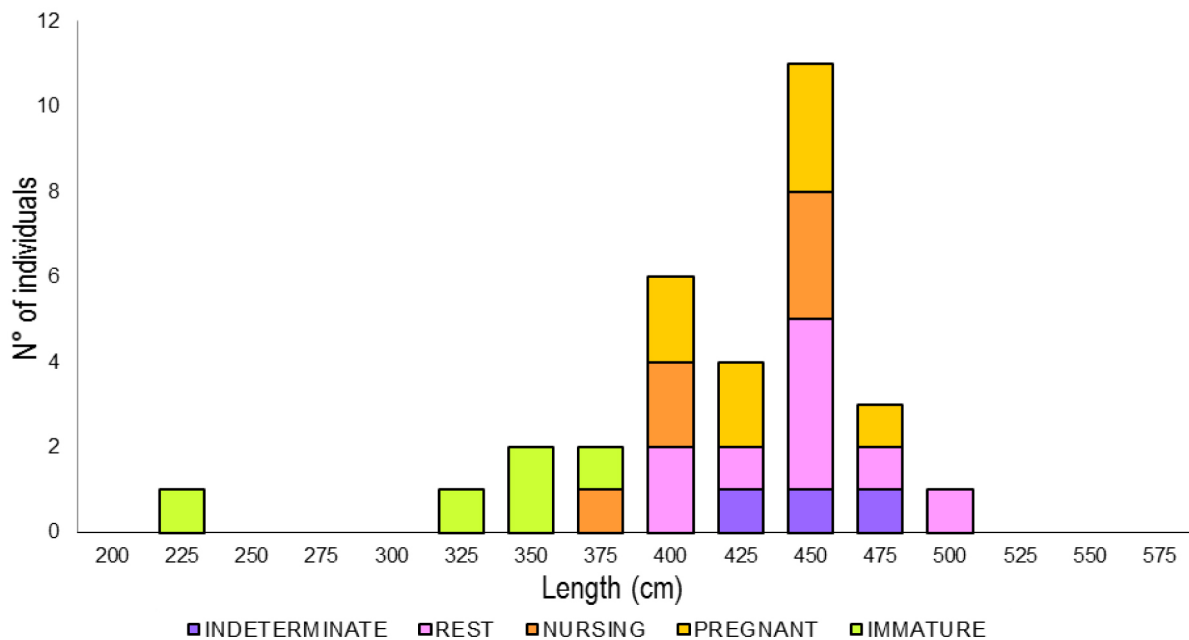


Fig. 2. Sexual maturity: number of females (*G. melas edwardii*) by reproductive status and size.

Table 1. Average measurements and weights from both ovaries

	Weight (g)	Length (mm)	Width (mm)	Thickness (mm)
Mature	29.51	56.91	33.44	22.48
Immature	5.01	37.58	19.94	12.34

Linear regression among ovarian corpora number and age was significant ($F = 15.44$, $R^2 = 0.45$, $p = 0.0009$), showing that there is a relationship between the number of ovarian corpora and mature female age.

DISCUSSION

This study analyzed reproductive data of female pilot whales (*G. melas edwardii*) for the first time and contributes to the knowledge of

reproductive parameters for this subspecies. Those parameters are estimate from size and age data.

The maximum age recorded for this stranding was lower than that in Newfoundland and the Faroe Islands subpopulations, where longevity reached 50 years (Sergeant 1982; Bloch et al. 1993b). The failure to find females greater than 40 years in this work made impossible to study the senescence state described for the species (Marsh and Kasuya 1991; Norris and Pryor 1991; Chivers 2002).

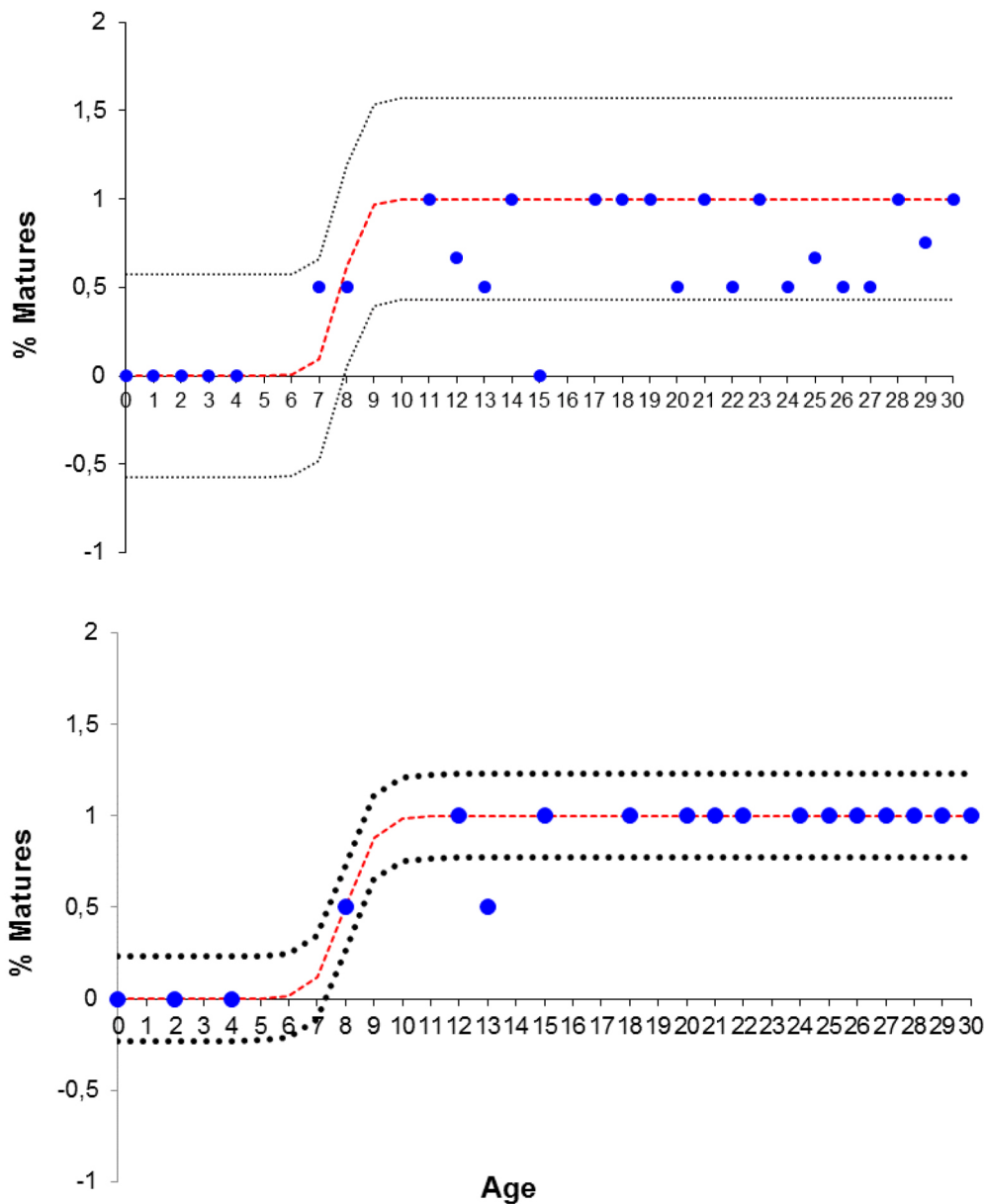


Fig. 3. Percent of mature females (*G. melas edwardii*) by age class (px : •) and expected values for the adjustment (-) and its 95% CI (-). Above px according to Smith method (1973) and below px by DeMaster method (1984).

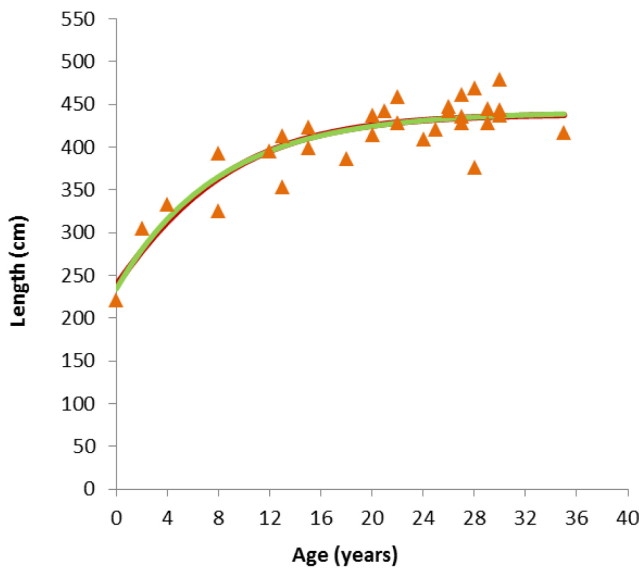


Fig. 4. Body growth models by age. Green line designates data from von Bertalanffy model. Orange line designates data from Gompertz model. Symbols designate observed data.

Although the individuals of this stranding were younger, the growth range was within the range measured in northern subspecies, 163-512 cm (Lockyer 1993), and within the documented range for the southern subspecies, 160-530 cm (Garcia 2007), and this would be expected for other herds of the same species.

Both ASM and the LSM were within the estimated range for females of the North Atlantic subpopulation, summarized in table 3 (Bloch et al. 1993c; Lockyer 1993; Evans 2001). Besides, fertility for stranded females (*G. melas edwardii*) was within the range estimated for the north subpopulation (Martin and Rothery 1993). However, the breeding interval found here was significantly lower than that estimated for the northern subspecies (Evans 2001). In this particular case, and bearing in mind that in the northern hemisphere whale capture was persistent during the last three centuries, it was expected that the reproductive parameters are different between

Table 2. Annual pregnancy rate by age class

Age (years)	Matures females	Pregnant females	APR
8	1		0
12	1	1	100
13	1	1	100
15	1		0
18	1		0
20	1		0
21	1	1	100
22	2	1	50
24	1		0
25	1		0
26	2		0
27	2		0
28	2	2	100
29	2		0
30	3	1	33.33
35	1	1	100

Table 3. Reproductive parameters of female pilot whales from North Atlantic and Argentina

	North Atlantic	Argentina
ASM (average and range)	8.7 [5-15] ¹ 8.5 [5.5-15]	8.0 [8-13]
LSM (cm)	366-378	341-389
Fertility range (m)	0.18-0.24	0.19-0.21
Breeding interval (years)	3.5	2.4

¹Age at sexual maturity estimated by Bloch et al. 1993c and Martin and Rothery 1993 in differents strandings in North Atlantic.

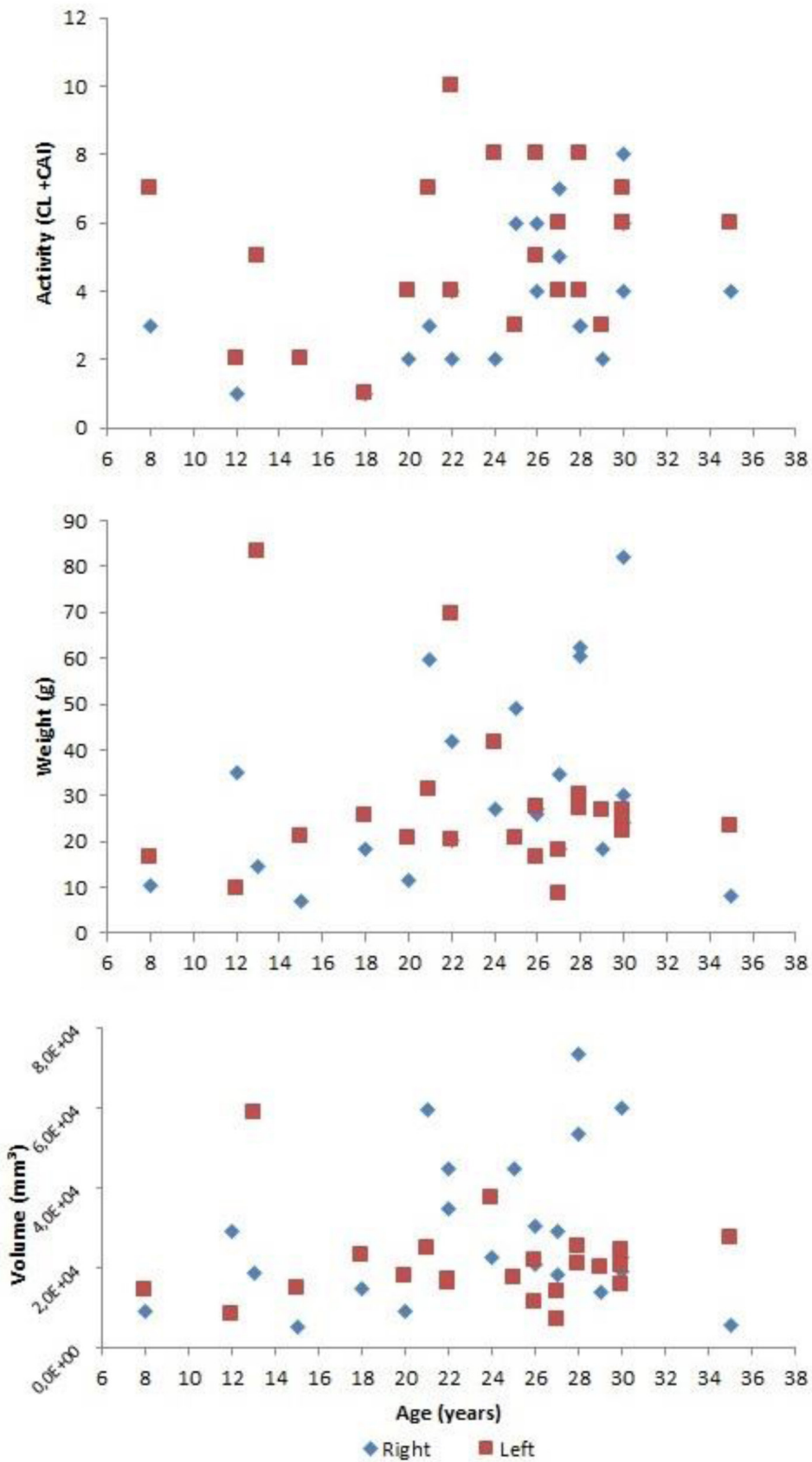


Fig. 5. Size and ovarian activity by age class from right and left ovaries.

the North and South Atlantic populations (Garcia 2007). Notwithstanding, it would be expected that females from populations that were subject to capture reach faster sexual maturity, having lower ASM and LSM than those that were not capture subject even reaching higher fertility, but this was not observed. For example, the highest record breeding interval for the northern subpopulation was (3.5 years) probably because, being females that were subject to exploitation, they must increase the time of pregnancy, lactation and especially rest. Something similar happens with another dolphin species, *Delphinapterus leucas*, in which gestation periods ranges from 12 to 14½ months (Moore 2008), but information from captive females recorded a longer time with an average of

475 days (15.8 months) (Robeck et al. 2005).

The macroscopic and microscopic gonad analysis showed that in over 80% corpora lutea of pregnant females are larger on average than those that were not pregnant. In any case, the presence of corpora lutea in both ovaries was recorded, suggesting an alternate ovulation between reproductive cycles. Although the ASM was estimated to be 8 years old, the youngest pregnant female of this stranding was 12 years; this is likely because the breeding interval was 2.4 years and mature females (not pregnant) between 8-12 years were in a resting period when beaching, so may have been pregnant in previous cycles. It was unusual for this study to find a 13-year-old female. However, something similar happened in a study

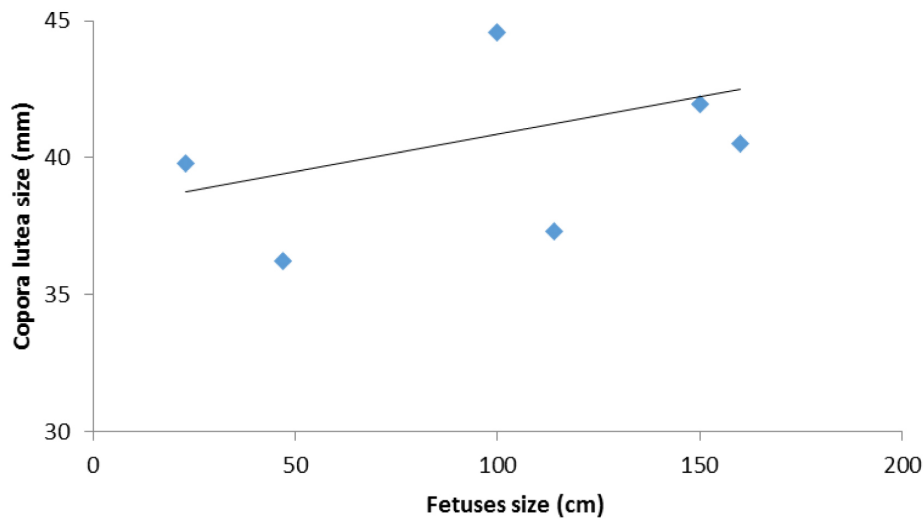


Fig. 6. Relationship between corpora lutea size and fetal size from female pilot whales (*G. melas edwardii*). The dark line corresponds to the linear regression ($R^2 = 0.1506$)

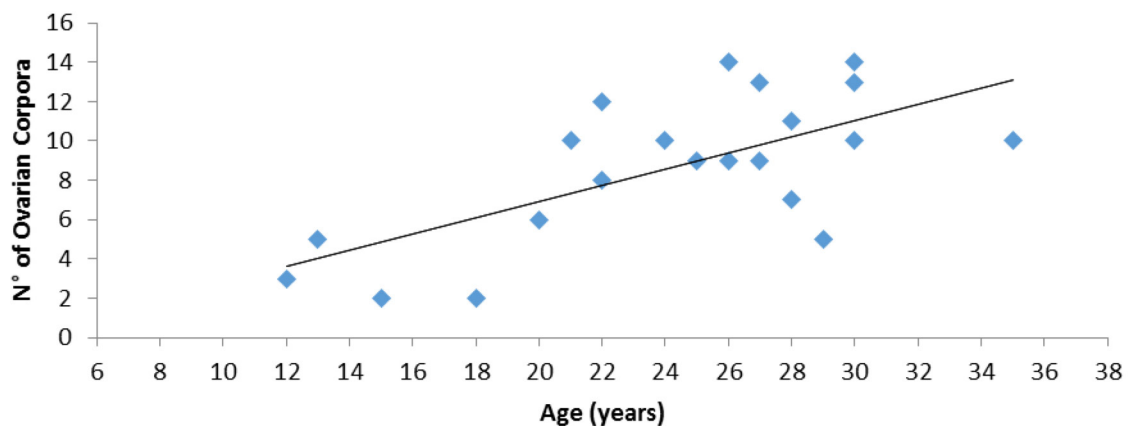


Fig. 7. Relationship between the number of ovarian corpora and age from female pilot whales (*G. melas edwardii*). The dark line corresponds to the linear regression ($R^2 = 0.4483$).

on the north subpopulation in which a 15-year-old female, still immature, was found (Martin and Rothery 1993; Bloch et al. 1993a). This could be a case of infertility caused by hormone deficiencies, although one of the Graafian follicles measured 4.5mm, reaching the AFD of a resting female, and so it was presumed that this female was close to ovulation time.

No simultaneously pregnant and lactating females were recorded, showing that females do not ovulate when they are in lactation, this being a usual behavior in female pilot whales (*G. melas edwardii*). In fact, it has been shown that females stop ovulating at 40 years old even though they continue to lactate for more than a decade (Marsh and Kasuya 1991). Thus, they have the potential to not only extend the long-term care of their offspring, but also to influence the fate of their progeny. Bilateral differences in ovarian activity, which showed a tendency to produce more ovulation in the left ovary, were described for both species (*G. melas* and *G. macrorhynchus*). However, the prevalence of activity in the left ovary is less dramatic in *Globicephala* spp. than in other toothed whales (Marsh and Kasuya 1991). In fact, in many odontocetes, the left ovary appears to be active early in life, while the right ovary becomes active in late stages (Boyd et al. 1999). On the other hand, ovarian measurements found in this study are within the range described in the northern subspecies by Marsh and Kasuya (1991).

As we did not find a linear relationship between the fetal size and corpora lutea, they cannot be used to estimate the size of the fetus. In fact, they reach a certain size and stop growing regardless of the fetus, as cited for the species (Marsh and Kasuya 1991). Besides, the low regression suggests that, while the number of ovarian corpora vary with age, it is not the only factor explaining the relationship. Perhaps the corpus luteum stops growing at some point because the size could be enough to secrete the progesterone needed to hold pregnancy.

Furthermore, one of the fetuses was 160cm in length and possibly near gestation term. This size was almost within the range of fetus size at birth in the northern subspecies: 165-190 cm (Lockyer 1993). Fetus sizes let us infer that conception and birth can occur at any time of the year, suggesting there is no marked seasonality for reproduction, but birth numbers may peak in summer and autumn, as recorded by Martin and Rothery (1993). There was a great variation in reproductive history among individuals in this species. Some mature

early in life, others much later. Some die young, others live for more than 50 years. Some ovulate relatively frequently, others slowly (Martin and Rothery 1993).

As mentioned at the beginning of this paper, mass strandings represent valuable sources of information for the study of pelagic species that are difficult to access, as is the case of *G. melas edwardii*. Previous studies on reproductive biology of cetaceans are mostly based on ultrasonography, which cannot examine the histology of gonads (Yu et al. 2016, Richard et al. 2017). In addition, *G. melas edwardii* is one of the cetacean species with the highest records of mass strandings on the coasts around the world, (except for catches in the northern hemisphere). These dramatic events involve large groups of animals; living dolphins are often put back into the sea but return again to the beach where they die after several hours of suffering (Oslo and Stephen 2002). There were many hypotheses that attempted to explain this phenomenon: loss of echolocation, auditory trauma, abnormal magnetic field, density dependence, and weather or oceanic events, among others (Trivers 1985; Odell 1987; Perrin and Geraci 2002; Duignan 2003; Walker et al. 2005; Bradshaw et al. 2006).

Previous studies on the northern subpopulation have shown that these animals have a matrilineal social organization in which neither males nor females leave the group where they were born into (Oslo and Stephen 2002); it is a structure that is also observed in orcas but rare in mammals (Amos et al. 1993). That is why it has been assumed that this beaching or stranding behavior relates to the species' social organization. Even though Oremus (2013) argues that if the social dynamics of kinship play a critical role in pilot whales stranding, it is expected to find that individuals in a stranding event are related, especially mothers and offspring should be close to each other when they end up on the beach. However, this study showed evidence of the "absent mothers", since many calves did not have an identifiable mother among the stranded pilot whales. Several scenarios could explain the lack of territorial cohesion, including the social bonds disruption among family members against real stranding. In fact, the separation of related pilot whales might actually be a causative factor in the stranding rather than simply a consequence.

CONCLUSIONS

Reproductive parameters such as LSM, ASM, fertility, and breeding interval are critical in the life history of any species and, in turn, enable us to study the social and demographic structures that help us understand sub-species dynamics. In this sense, this study allowed us to make inferences and draw comparisons about the reproductive biology of the females of *G. melas edwardii* in the Southwestern Atlantic Ocean, although it could be limited because the individuals come from one single mass stranding. Moreover, further studies are needed to fully comprehend the behavior and biology of this species.

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Authors' contributions: SFA, GMF and DSL designed the study. GMF, DSL, GNA and CEA performed the field work and collected samples. SFA and GMF prepared the figures, analyzed the data and wrote manuscript. All authors participated in revising the manuscript. All authors read and approved the final manuscript.

Competing interests: there is no competing interests.

Availability of data and materials: we have all data and materials.

Consent for publication: Not applicable.

Ethics approval consent to participate: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. No experiments were done on living animals in this study.

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