Reproductive biology of female franciscana dolphins (*Pontoporia blainvillei*) from Argentina

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The ovaries of 31 franciscana dolphins (19 immature and 12 mature) by-caught in coastal waters of Argentina were examined to describe the reproductive biology. No ovarian polarity was found, both ovaries were functional and showed similar amounts of corpora. A positive trend was observed between the number of corpora and age (3–8 years old), indicating that ovulation scars are detectable for at least 4 years, and a ovulation rate of 0.39 was found for the sampled population. Age, length and weight at sexual maturity were estimated at 3.92 ± 0.09 years, 133.47 ± 11.11 cm and 32.68 ± 2.72 kg, respectively. The annual pregnancy rate was 0.36 ± 0.02 (95% CI = 0.10–0.65). The proportion of lactating and resting females found were 0.25 and 0.33 respectively. These outcomes constitute the first reproductive and life history information on franciscana dolphins from the southernmost population and are important in relation with adequate conservation management plans for this small cetacean.

INTRODUCTION

Franciscana dolphin, *Pontoporia blainvillei* (Gervais & d'Orbigny, 1844), is a small and endemic cetacean of the South-western Atlantic Ocean. The species is restricted to shallow marine and estuarine coastal waters from Golfo Nuevo (42°35′S), Chubut Province, Argentina (Crespo *et al.*, 1998) to Itaúnas (18°25′S), Espírito Santo State, Brazil (Siciliano *et al.*, 2002). Along its entire geographic distribution, this dolphin has been categorized as Vulnerable by the IUCN (Reeves *et al.*, 2012). Mortality in fishing gear is likely the most important threat to the conservation of this small cetacean (Reeves *et al.*, 2012); a maximum of 360–539 dolphins are by-caught in the entire Buenos Aires province (Negri *et al.*, 2012). Moreover, recent studies have indicated that the abundance of franciscana dolphins from southern Brazil and Uruguay is declining due to unsustainable incidental mortality levels, suggesting that the species might be facing the highest

risk of extinction of any cetacean of the South-western Atlantic Ocean (Kinas, 2002; Secchi *et al.*, 2003).

Since the 1990s, the population structure of franciscana dolphin has been divided in two different geographic populations based on osteological characters and mitochondrial DNA (Pinedo, 1991; Secchi *et al.*, 1998). Recently, this division has been revised by Cunha *et al.* (2014), suggesting to split the franciscana dolphin population into two Evolutionarily Significant Units (ESUs). The North ESU is a small size population found from Espírito Santo (~18°S) to the centre of Rio de Janeiro and the South ESU is a larger size population ranged from somewhere in the centre of Rio de Janeiro to Argentina (42°S) (Pinedo, 1991; Cunha *et al.*, 2014). Using differentiation among genetic, morphometric and population parameters, Secchi *et al.* (2003) proposed four Franciscana Management Areas (FMAs) along the coast of South America: two coastal zones (FMAs I and II) in Brazilian waters, one zone (FMA III) that includes coastal waters of Southern Brazil and Uruguay, and one zone (FMA IV) in Argentine waters.

Reproductive studies about female franciscana dolphins are limited to Brazilian and Uruguayan populations (Kasuya & Brownell, 1979; Harrison *et al.*, 1981; Ramos *et al.*, 2000; Rosas & Monteiro-Filho, 2001; Danilewicz, 2003; Bertozzi,

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2009; Freitas da Silva, 2011). This highlights the need to conduct studies on reproductive aspects of female dolphins in FMA IV off Argentina. This study provides essential information to design new conservation management plans that fit the features exhibited for each population of franciscana dolphins. In this sense, the objectives of this work are to estimate reproductive aspects of female dolphins such as reproductive stage, size and age at sexual maturity, ovulation rate, and estimate the annual reproductive rate of female franciscana dolphins from Argentina. Furthermore, characteristics of the ovaries, reproductive resting and senescence and morphometric studies are presented.

MATERIALS AND METHODS

Sample data

Sample size of the ovarian morphometric parameters measured varied because complete data of each dolphin were not always available. Ovaries and data collected from 31 franciscana dolphins by-caught in artisanal fishing nets along the coast of Buenos Aires and Río Negro Provinces were used in the analyses. Dolphins were sampled from 1998 through 2011. The study area included localities of Buenos Aires Province from Necochea (38°37′S 58°50′W) to Bahía Blanca (38°44′S 62°14′W) and one locality of Río Negro Province, El Cóndor next to the Río Negro Estuary (41°03′S 62°48′W) recently identified as the southernmost breeding area reported to date for this vulnerable species (Failla *et al.*, 2012) (Figure 1). Carcasses of the dolphins collected during reproductive season (from November to March) and were kept frozen (at −21°C) until their postmortem analysis following standard procedures (Winchell, 1982). Mammary glands were examined to determine whether the female was lactating or not; the uterus was also examined to determine if a foetus was present. Biometric parameters measured were standard length (SL, N = 31) and total weight (TW, N = 29). SL was measured as a straight line from the tip of the rostrum to the fluke notch, and TW was obtained by a dynamo to the nearest 0.002 kg.

Age determination

Teeth were collected at mid-length of the left lower jaw of each animal and preserved in 70% alcohol. Age determination was obtained by counting growth layers groups (GLGs) in histological sections of teeth decalcified with the commercial acid mix RDO® and sectioned by cryostat at −21°C (see Pinedo & Hohn, 2000; Panebianco *et al.*, 2012 for further information on the technique used). On-centre histological sections were stained with Mayer's haematoxylin. Three different readers determined the age by countering the number of GLGs in the dentine and cementum. In this work, one GLG represents 1 year of age (Pinedo & Hohn, 2000).

Sexual maturity stage determination

The reproductive tract of each dolphin was removed and the ovaries were separated and fixed in 10% formalin solution. After fixation, ovaries were externally examined and then sliced at ~1 mm to assure that all *corpora* – *corpus luteum* (CL) and *corpus albicans* (CA) – resulting from an ovulation were counted (Danilewicz, 2003). Females were categorized as sexually immature when no *corpora* were present on the ovaries (Figure 2A). Females with at least one *corpus* in the ovaries were classified as sexually mature (Figure 2B). Females were defined as resting when they had at least one CA and showed no signs of gestation or lactation (no CL, no foetus, no milk in mammary glands, no enlarged uterine horn indicating recent parturition), as pregnant when both a CL and a foetus were found, and as lactating when milk was found in the mammary glands. Histological analyses were performed on the ovaries according to Harrison *et al.* (1981): cross-section slices of the ovaries were embedded in Histoplast® (Biopack), sectioned with a digital microtome into 4–7 μm thick sections and stained with haematoxylin-eosin to ensure that all corpora were counted.

Ovarian morphometric characteristics

The ovaries were weighed (±0.1 g) and measured (±0.01 mm) in their three larger orthogonal dimensions
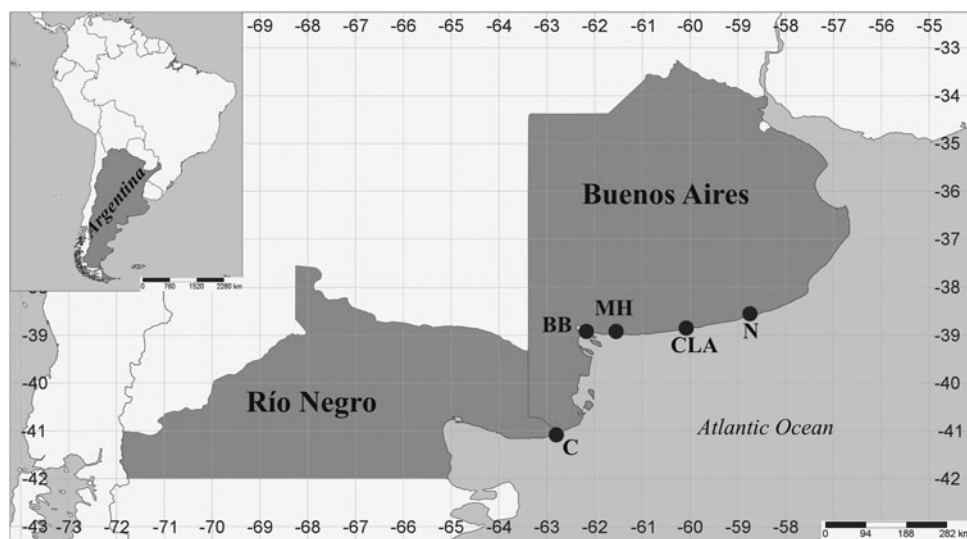

Fig 1. Geographic distribution of the study areas along the Southern coast of Argentina. References: N: Necochea; CLA: Claromecó; MH: Monte Hermoso; BB: Bahía Blanca; C: El Condor, Río Negro Estuary.
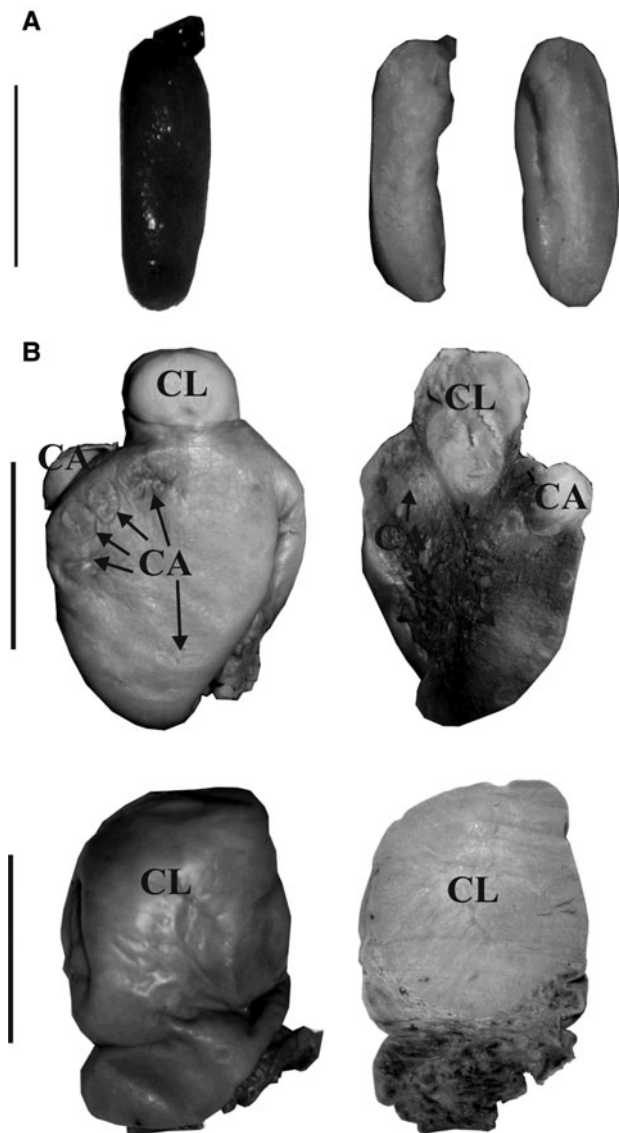
Fig. 2. External and internal macroscopic appearance of (A) immature and (B) mature ovaries of franciscana dolphins. The scars represent *corpora lutea* (CL) and *corpora albicantia* (CA). Scale bars: (A) 1 cm; (B) 2 cm.

(length, width and depth) with digital calipers before fixing them in 10% neutral buffered formalin solution, after which gonadal morphometric parameters were assessed (Table 1).

Length, weight and deepness of the right and left ovaries were statistically analysed by Wilcoxon matched-pairs-ranks test in order to verify the symmetry of the ovaries and the relationships between those variables were analysed by Spearman rank correlation. The number of *corpora* on each ovary was also recorded. The ovarian morphometric parameters (Table 1) correspond to each maturity group (Immature and Mature) were statistically compared by using Mann–Whitney U Test because parametrical statistical assumptions were not achieved (normality and homoscedasticity). Linear regressions between ovarian morphometric parameters: mean ovarian length (MOL), mean ovarian weight (MOW), mean ovarian deepness (MOD), relative ovarian weight (ROW) and index of maturity (IM) (Danilewicz *et al.*, 2004; Bertozzi, 2009; Panebianco *et al.*, 2012, Table 1) and SL were performed to investigate how development parameters vary according to sexual maturity stage and SL of the dolphins. Two different lineal regressions were fitted for mature and immature dolphins (the break points correspond to the mean length at sexual maturity (LSM) value of the 95% CI calculated by DeMaster method, see below), the regression slopes were compared with a Student t-test.

Attainment of sexual maturity

The age at sexual maturity (ASM) was estimated by using two different methods: the DeMaster method (1978) and the logistic regression method (Danilewicz, 2003).

According to DeMaster (1978), ASM was estimated as:

$$\text{ASM} = \sum_{a=j}^{k} a\big(f_a - f_{a-1}\big)$$

Where f_a is the fraction of animals sexually mature of the age class a, j is the age of the youngest sexually mature animal from the sample; and k is the eldest sexually immature dolphin from the sample.

The variance of ASM was expressed by the next equation:

$$\text{VAR(ASM)} = \sum_{a=j}^{k} \big[a\big(1 - f_a\big)/N_a - 1\big]$$

Where N_a is the total number of animals of age a.

The logistic regression method fits a sigmoid curve that represents the probability that a dolphin of age a was sexually mature. According to this approach the distribution of

Table 1. Ovarian morphometric parameters calculated for female franciscana dolphins, *Pontoporia blainvillei*, from Argentina.

Parameters	Acronyms	Description	Units
Right ovarian weight	ROW	Weight of the right ovary	g
Right ovarian length	ROL	Length of the right ovary	mm
Right ovarian depth	ROD	Depth of the right ovary	mm
Left ovarian weight	LOW	Weight of the left ovary	g
Left ovarian length	LOL	Length of the left ovary	mm
Left ovarian depth	LOD	Depth of the left ovary	mm
Mean ovarian weight	MOW	Mean weight of the ovaries of each dolphin	g
Mean ovarian length	MOL	Mean length of the ovaries of each dolphin	mm
Mean ovarian depth	MOD	Mean depth of the ovaries of each dolphin	mm
Relative ovarian weight	ROW	Relationship between the combined weight of the ovaries and the total weight of each dolphin	%
Index of maturity	IM	Relationship between the combined weight and length of the ovaries	g mm^{-1}

sexually mature and immature dolphins by age was:

$$Y = 1/(1 + e^{(-xb+a)}) \text{ or } \ln\left(\frac{1}{Y-1}\right) = a + bx$$

Where x is the age of the dolphin, b is the slope of the regression and a is the intercept. To obtain the age when 50% of the animals are sexually mature ($Y = 0.5$), the last equation is simplified as ASM $= -a/b$ by applying ln.

Length (LSM) and weight (WSM) at sexual maturity were also calculated using the equation of DeMaster (1978) modified by Ferrero & Walker (1995). The equation used for the calculation was the following:

$$\text{LSM/WSM} = \sum_{C_{\min}}^{C_{\max}} C(f_t - f_{t-1})$$

Where $C_{\max}$ represents the length/weight class of the sexually immature specimens with the highest length or weight, $C_{\min}$ is the length/weight class of the sexually mature specimens with the lowest length or weight, C is the smallest value of the length/weight class t, and f_t is the fraction of sexually mature animals from the length/weight class t. The calculation of the variance was expressed by the equation:

$$\text{VAR(LSM/WSM)} = w^2 \sum_{a=j}^{k} \left[f(1 - f_t)/N_t - 1 \right]$$

Where N_t is the total number of animals of the length/weight class t, and w^2 is the interval of classes, which in this case was 5 cm and 5 kg (Danilewicz, 2003).

Annual pregnancy rate (APR)

This parameter was estimated according to the Perrin & Reilly (1984) method as the proportion of sexually mature females that were pregnant adjusted by the gestation time in years (10.5 months – 0.88 years; Kasuya & Brownell, 1979) and its variance was estimated (Perrin & Reilly, 1984) by using the following equation:

$$\text{VAR(APR)} = \text{APR}(1 - \text{APR})/N$$

Where N is the number of reproductive females in the sample. The estimation of this parameter was performed only for the mature dolphins.

Population reproductive parameters

LACTATION PERIOD (LP)
LP was estimated as follows (Perrin & Reilly, 1984):

$$LP = Gp * \left(\frac{L}{P}\right)$$

Where LP is lactation period in months, Gp is gestation period in months, L is the proportion of sexually mature females that are lactating and P is the proportion of sexually

mature females that are pregnant. Gp was considered to be 0.88 months (Kasuya & Brownell, 1979).

LENGTH OF RESTING PERIOD (RP)
The resting period was calculated according to Perrin & Reilly (1984). The estimation included those females between ovulation cycles and those pregnant with embryos present.

$$RP = Gp * \left(\frac{R}{P}\right)$$

Where RP is resting period in months, Gp is gestation period in months, R is the proportion of sexually resting mature females and P is the proportion of sexually mature females that are pregnant.

Statistical analyses

All the statistical analyses were performed by using the statistical software Statistica 7.0 (Statsoft, Inc.) and InfoStat (Di Rienzo *et al.*, 2010). Data are given as mean $\pm$ SD and range. The level of statistical significance was set at $P \leq 0.05$.

RESULTS

Ovarian morphometric characteristics

Sexual maturity stage determination and ovarian morphometric characteristics

The sampler set contained 19 immature dolphins and 12 mature, of which three were pregnant, two lactating, one lactating and pregnant and six resting. The age of the dolphins ranged from 0+ to 8 years old (Figure 3).

The value of the morphometric variables of the right and left ovaries (length, weight and depth) did not vary significantly, thus indicating that the ovaries were symmetrical (Wilcoxon matched-pairs-ranks test, length ($T = 139$, N = 29), weight ($T = 64.5$, N = 26) and depth ($T = 84.5$, N = 24) $P \geq 0.1$). Due to this fact, we decided to pool together the parameters of the right and left ovaries and work with the MOL, MOW and MOD (Tables 1 & 2) parameters.

The total mass of the ovaries changed with both maturity stage (*I*: immature and *M*: Mature) and SL (Table 2, Figure 2). MOW (Range: $I = 0.10 - 1.10$ g, $M = 0.55 -$
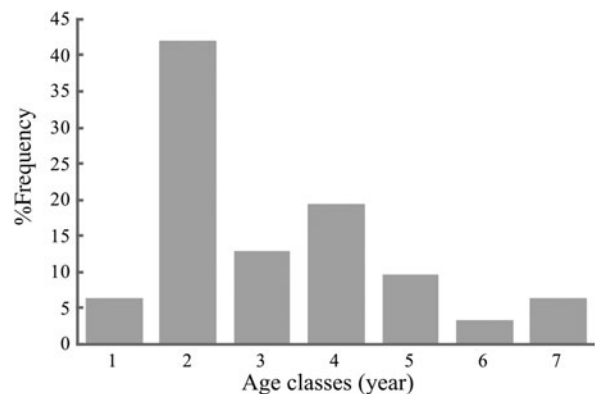

Fig. 3. Age distribution of female franciscana dolphins, *Pontoporia blainvillei*, incidentally captured from Southern Buenos Aires, Argentina.

Table 2. Summary of the ovarian morphometric parameters of female franciscana dolphins (*Pontoporia blainvillei*) from Argentina.

Variables		Immature					Mature				
		Mean	Median	SD	CI (95%)	N	Mean	Median	SD	CI (95%)	N
Left ovaries	Weight (g)	0.30	0.20	0.36	0.13 – 0.47	19	2.08	1.00	1.84	0.92 – 3.25	12
	Length (mm)	13.94	13.60	3.23	12.38 – 15.50	19	23.69	20.15	6.81	19.37 – 28.02	12
	Depth (mm)	3.73	3.40	1.94	2.66 – 4.80	15	8.12	7.90	2.52	6.42 – 9.81	11
Right ovaries	Weight (g)	0.26	0.20	0.21	0.16 – 0.36	19	2.62	1.25	2.34	1.13 – 4.11	12
	Length (mm)	14.44	13.70	3.07	12.96 – 15.92	19	20.58	18.80	6.62	16.38 – 24.79	12
	Depth (mm)	3.81	3.80	1.69	2.86 – 4.75	15	8.16	7.90	2.36	6.58 – 9.75	11
MOW		0.28	0.20	0.27	0.15 – 0.41	19	2.35	1.78	1.71	1.26 – 3.44	12
MOL		14.19	14.00	2.76	12.85 – 15.52	19	22.14	19.95	6.19	18.20 – 26.07	12
MOD		3.77	3.70	1.69	2.83 – 4.71	15	8.14	8.45	2.26	6.62 – 9.66	11
%ROW		0.003	0.002	0.002	0.002 – 0.004	19	0.013	0.011	0.008	0.007 – 0.018	10
IM		0.02	0.01	0.01	0.01 – 0.02	19	0.10	0.09	0.06	0.06 – 0.14	12

SD, standard deviation; CI, confidence interval; MOW, mean ovarian weight; MOL, mean ovarian length; MOD, mean ovarian depth; % ROW, percentage of relative ovarian weight; IM, index of maturity.

5.25 g), %ROW (Range: $I = 0.001 – 0.009$, $M = 0.004 – 0.027$), MOL (Range: $I = 8.45 – 19.50$ mm; $M = 14.40 – 34.50$ mm), MOD (Range: $I = 0.34 – 7.20$ mm; $M = 5.15 – 12.00$ mm) and IM (Range: $I = 0.001 – 0.009$; $M = 0.004 – 0.027$) varied according to maturity groups (Mann–Whitney U test, MOL ($U = 16$, $N_I = 19$, $N_M = 12$), MOD ($U = 8$, $N_I = 15$, $N_M = 12$) and IM ($U = 9$, $N_I = 19$, $N_M = 12$) $P \leq 0.001$). MOW and %ROW were the parameters that showed lower percentage of overlapping between maturity groups (10.7 and 15.1%), followed by MOD, IM and MOL (17.6, 19.2 and 19.6%). Resting female dolphins showed similar ovarian morphometric parameters values than mature dolphins (MOW, %ROW, MOL, MOD and IM) (Mann–Whitney U test, MOW: $U = 7$, $N_R = 4$, $N_M = 8$, $P = 0.15$; %ROW: $U = 5$, $N_R = 4$, $N_M = 6$, $P = 0.17$; MOL $U = 5$, $N_R = 4$, $N_M = 8$; MOT $U = 5$, $N_R = 4$, $N_M = 7$; IM $U = 12$, $N_R = 4$, $N_M = 8$; $P \geq 0.07$). A significant difference in the location of the CL was observed (Mann–Whitney U test, $P = 0.07$, ~55% of the left ovaries showed at least one *corpus* and this number was around 82% for the right ovaries, Figure 2). Additionally, 33% of mature females showed *corpora* in both ovaries simultaneously.

The attainment of maturity, which corresponds to the dotted line in the Figure 4, coincided with an increase in total ovarian mass and length. Mature dolphins showed significantly higher slopes – of the linear regression analysis – than immature dolphins for all ovarian morphometric parameters excepting MOD for which the lineal regression for mature dolphins was not significant (Table 3).

Attainment of sexual maturity

The oldest immature female was 4 years old and the youngest mature female was 3 years old, thus suggesting that the age at attainment of sexual maturity is between 3 and 4 years old. In fact, the ASM was estimated at 3.92 ± 0.09 years (95% CI = 3.33 – 4.51) by using the DeMaster method and 3.42 years by the logistic regression method. Those ASM values were close to the average age of the three females (3.67 years) collected in the year when they attained sexual maturity, i.e. with only one CL.

The length of the immature and mature dolphins ranged from 72.70 to 129.70 cm and from 136.00 to 160.50 cm respectively; LSM was estimated at 133.47 ± 11.11 cm (95%

CI = 126.93 – 140 cm). The immature and mature dolphins weighed from 3.58 to 30.50 kg and from 26.50 to 52.00 kg respectively, WSM was calculated at 32.68 ± 2.72 kg (95% CI = 29.41 – 35.95 kg).

Annual pregnancy rate (APR)

APR for female franciscana dolphins from FMA IV was 0.36 ± 0.02 (95% CI = 0.10 – 0.65). The proportion of mature, lactating, pregnant and resting females was 0.39, 0.25, 0.33 and 0.33 respectively, and one female was simultaneously pregnant and lactating.

No evidence of senescent females was found since the oldest females (8 years old) were pregnant or lactating and both showed CL in their ovaries. Based on the ASM, the number of corpora in the ovaries and the relationship between the number of corpora and the age of the dolphins (Linear regression, $y = 1.13x – 2.72$, $r^2 = 0.66$, $F_{(1,10)} = 17,11$, $P = 0.003$, N = 11), the ovulation rate is 0.39 for sampled population. Neither the gestation period nor length at birth were estimated because of the low number of neonates collected – two, in December and February (SL = 75.8 ± 4.4 cm, TW = 5.0 ± 3.4 g).

Two additional population parameters were estimated, Lactation Period (LP) was estimated at 7.95 months and Length of Resting Period (RP) at 10.5 months (Figure 5).

DISCUSSION

No ovulation asymmetry was found in female franciscana dolphins from FMA IV and the evidence supports that both right and left ovaries are functional. This outcome is consistent with that observed by Rosas & Monteiro-Filho (2001) for franciscana dolphins belonging to FMA II, although ovarian asymmetry was described in dolphins from FMA III (Brownell, 1984; Danilewicz, 2003), where only the left ovary was active. The lack of asymmetry was also evident as both ovaries were able to ovulate from the attainment of sexual maturity onwards – the by-caught dolphins analysed in this work accumulated similar amounts of *corpora* in both ovaries (Table 4). This fact has been previously described as a general trend in cetacean species (Dabin *et al.*, 2008).

Table 3. Linear regression of the ovarian morphometric parameters as a function of standard length, and the statistical significance of linear regression slope comparison according to sexual maturity stage.

Parameters	Immature					Mature					ST significance
	$b \pm$ SE	$a \pm$ SE	r^2	P	N	$b \pm$ SE	$a \pm$ SE	r^2	P	N	
MOL	0.17 ± 0.03	-3.89 ± 2.71	0.73	< 0.01	19	0.54 ± 0.18	-57.09 ± 26.46	0.47	0.01	12	*
MOW	$0.01 \pm 3.5 \times 10^{-3}$	-1.18 ± 0.37	0.48	< 0.01	19	0.18 ± 0.04	-24.60 ± 5.34	0.72	< 0.01	12	***
MOD	0.08 ± 0.02	-4.61 ± 2.55	0.46	< 0.01	15	0.06 ± 0.11	0.12 ± 15.25	0.03	0.61	11	
IM	$6.4\text{E-}04 \pm 1.8 \times 10^{-4}$	-0.05 ± 0.02	0.43	< 0.01	19	$5 \times 10^{-3} \pm 2 \times 10^{-3}$	-0.63 ± 0.29	0.39	0.03	12	*
ROW	$8.6 \times 10^{-5} \pm 2.6 \times 10^{-5}$	$-0.01 \pm 2.8 \times 10^{-3}$	0.39	< 0.01	19	$7.7 \times 10^{-4} \pm 2.1 \times 10^{-4}$	-0.1 ± 0.03	0.59	< 0.01	11	**

MOL, mean ovarian length; MOW, mean ovarian weight; MOD, mean ovarian depth; %ROW, percentage of relative ovarian weight; IM, index of maturity. Regression model: $y = bx + a$; SE, standard error; ST, Student t-test significance: $*P < 0.05$; $**P < 0.001$; $***P < 0.001$; $****P < 0.0005$. Statistical parameters: MOL: $t_{27} = 2.02$, $P = 0.02$; MOW: $t_{22} = 0.17$, $P = 0.43$; IM: $t_{26} = 2.17$, $P = 0.02$; ROW: $t_{26} = 3.23$, $P < 0.01$.

The analyses on ovarian morphometric parameters revealed that ovaries clearly increase in size as a function of SL. Similar trends have been reported by Ramos *et al.* (2000), Danilewicz (2003) and Rosas & Monteiro-Filho (2001), for FMAs Ib, IIIa and IIb respectively (Cunha *et al.*, 2014), thus suggesting that this is a common feature among all populations of *Pontoporia blainvillei*. This feature has also been observed in several cetacean species (Marsh & Kasuya, 1984; Hohn *et al.*, 1996).

Annual ovulation rates can be calculated when the sample is large enough according to age classes (Marsh & Kasuya, 1984, 1986; Perrin & Donovan, 1984), although observations on smaller samples – as in this work – can only indicate a trend in the number of ovulations in relation to age. In this context, we found that the number of CL and CA increased with the age from puberty (3–4 years old) to at least 8 years old for the dolphins, indicating that CL and CA are detectable in the ovaries for at least 4 years in this species. Read & Hohn (1995) also found a similar positive trend between the number of *corpora* and age for harbour porpoise, *Phocoena phocoena*, from the Gulf of Maine. Harrison *et al.* (1981) published similar results for franciscana dolphins from Uruguay, although and according to his findings, the CAs do not persist throughout life in this species, as occurs in certain large whales (Perrin & Donovan, 1984). The former authors have demonstrated that older females from Uruguay (161–175 cm SL) did not accumulate a growing number of CA with increasing age. Based on the accumulation pattern of the *corpora* scars in the ovaries, the ovulation rate and the presence of a simultaneously pregnant and lactating female (Table 4), we suggest that franciscana dolphins might be *monoestrous* and breed annually (Ovulation rate: 0.39). We found that some females might also ovulate twice a year (Table 4), but only one of these ovulations might result in pregnancy. Several studies have estimated fecundity rates from CA counts and discussed these in relation to the age of the female (Marsh & Kasuya, 1984; Berta & Sumich, 1999; Chivers, 2002). Harrison *et al.* (1981) reached a similar conclusion (i.e. annual breeding and monoestrous) based on ovarian characteristics of *Pontoporia blainvillei* from Uruguay by using similar methodology. Later, Danilewicz (2003) confirmed the same outcome for specimens from Rio Grande do Sul, Brazil. Despite this valuable information, it is worth pointing out that multiple ovulations have been reported, particularly in young adult females, as well as the existence of accessory CL; consequently, CA count may differ from the actual number of ovulatory events (Harrison *et al.*, 1972; Dabin *et al.*, 2008). In addition, a recent study on postmortem observations on the ovaries of a captive bottlenose dolphin, *Tursiops truncatus*, has proposed that only the CAs resulting from a gestation would be persistent, which would imply that the others had completely healed out – or become completely resorbed (Brook *et al.*, 2002).

Individual variation of the accumulation rate of *corpora* was observed in the present study; this is a common feature in cetaceans and is a reflection of differences in the annual ovulation rate between individuals as well as variation in the attainment of sexual maturity (Kasuya & Marsh, 1984; Read & Hohn, 1995; Brook *et al.*, 2002; Murphy *et al.*, 2010). Many other factors might affect ovulation rates by affecting hormonal production, such as climate, e.g. living in temperate *vs* tropical waters through variation in light intensity and day-
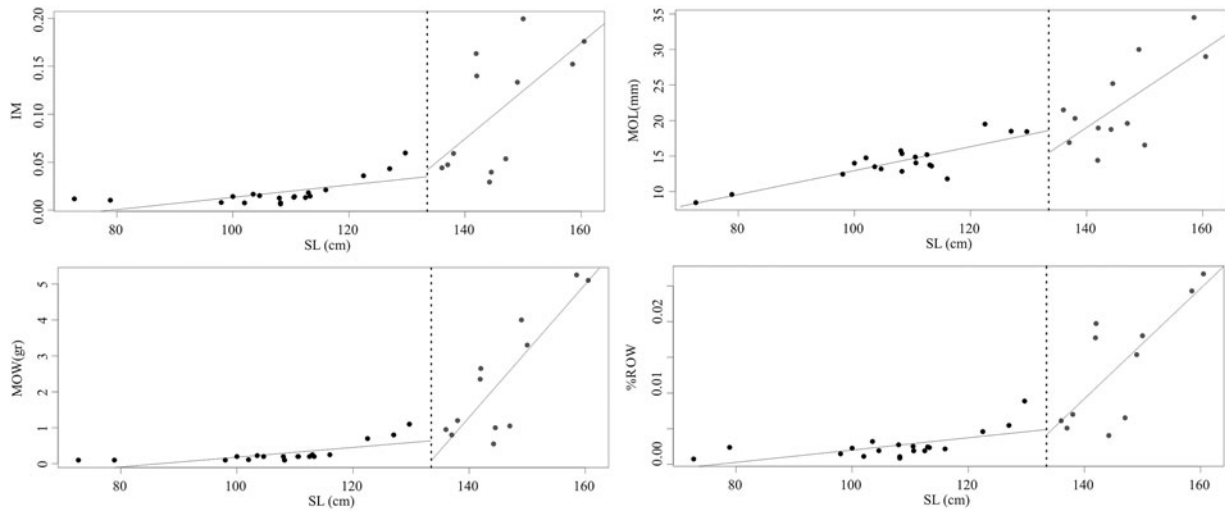
Fig. 4. Ovarian morphometric parameters as a function of standard length of female franciscana dolphins from Argentina. Dotted line corresponds to the mean Length at Sexual Maturity value. Black circles correspond to immature dolphins and grey circles to mature dolphins.
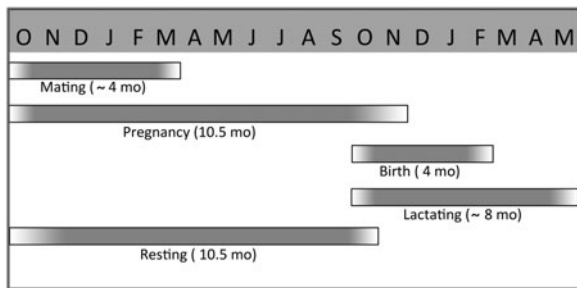

Fig. 5. Reproductive chronology of female franciscana dolphins. The seasonality of the reproductive events was modelled on the data presented in this work (Pregnancy, Resting and Lactating periods) and from previous work (Mating and Birth period according to Danilewicz, 2003). Bars represent the mean time of each reproductive event and the gradient pattern the time lag that may occur at the beginning and end of each event shown.

Table 4. Distribution of *corpora lutea* and *albicantia* in the ovaries of franciscana dolphins from Argentina.

Reproductive status	Month[a]	Age	CL		CA	
			Left ovary	Right ovary	Left ovary	Right ovary
R	11	5	0	0	0	2
R	3	4	0	0	1	3
L	2	8	0	1	0	7
P-L	2	4	1	2	0	0
R	11	5	0	0	1	1
L	11	3	0	1	0	0
L	11	4	0	1	0	0
R	8	4	0	0	2	2
L	2	7	0	1	4	0
P	12	4	0	1	0	0
R	11	5	0	0	0	2

[a]The month when the dolphins were by-caught. Age in GLGs. R, resting; L, lactating; P, pregnant and P-L, pregnant and lactating females; CL, number of *corpora lutea*; CA, number of *corpora albicantia*.

length as perceived by the hypothalamus, free-living *vs* captive condition, stress, and species-specific differences in mating systems (Dabin *et al.*, 2008). It is also important to note that our analyses only included dolphins that were by-caught, resulting in a bias sample – older franciscana dolphins (larger than 160 cm) and dolphins with calves are very rarely entangled or found stranded (Negri *et al.*, in press) – due to this further analysis is needed in order to obtain more accurate outcomes about ovulation pattern.

Cetaceans' species that exhibit annual reproduction included small odontocetes like the Dall's porpoise, *Phocoenoides dalli*, and harbour porpoise, and baleen whales, such as the large baleen minke whale, *Balaenoptera acutorostrata* (Lockyer, 1984; Hohn & Brownell, 1990; Clapham, 1992; Ferrero & Walker, 1999). Many small cetacean species have relatively young ages at sexual maturity as well as short lifespans. Species that do not have a strictly annual reproduction, such as the dwarf sperm whale, *Kogia sima*, and the large humpback whale, *Megaptera novaeangliae*, show longer gestation periods than those species with a strictly annual reproduction, as do franciscana dolphins from Argentina. References to the presence of senescent females' dolphins are scarce. Marsh & Kasuya (1984) found an association between follicle abundance and the incidence of

follicular atresia with senescence in pilot whales, *Globicephala* sp., over 50 years old. Later, Myrick *et al.* (1986) also found evidence of senescence in old spotted dolphins.

The ASM found in this work was lower than the one found by Corcuera & Monzón (1990) (ASM = 5–7, N = 12) and Corcuera (1996) (ASM = 4.3–4.4, N = 27) for franciscana dolphins from FMA IV. LSM was also lower in this work than the value found by Corcuera in 1996 (133.47 cm *vs* 140 cm). Nevertheless, ASM and LSM values were close to those found by Danilewicz (2003) in franciscana dolphins from Rio Grande do Sul (FMA III, 3.5–3.7 years old and 138.9 cm, N = 72) and São Paulo and Paraná (FMA II, 3.6–4 years old and 128–130 cm, Freitas da Silva, 2011, N = 35), whereas the same parameters were higher than those found in dolphins from Rio de Janeiro (3 years old and 130 cm; Ramos *et al.*, 2000). An earlier study on dolphins collected in Uruguay showed lower ASM (2.7 years old), but a higher LSM (140 cm) (Kasuya & Brownell, 1979). These

variations in LSM and ASM have been noticed in previous studies where it has also been demonstrated that those parameters could vary with decreasing population abundance caused by incidental mortality in small odontocetes (Kasuya, 1985; Read & Gaskin, 1990). Nevertheless, we cannot exclude differences in the methodologies used and the low number of samples as possible sources of variation in the estimated values of ASM and LSM. Comparison of age at attainment of sexual maturity within a population over time is useful in identifying fluctuations in reproductive patterns; mainly in populations under human pressure such as franciscana dolphins, the most threatened small cetacean in the South-western Atlantic Ocean due to incidental mortality (Reeves *et al.*, 2012). Even though previous studies have demonstrated that ASM and LSM vary – i.e. diminish with decreasing population abundance caused by incidental mortality – in small odontocetes (Kasuya, 1985; Read & Gaskin, 1990), we cannot exclude differences in the methodologies used and the low number of samples as possible sources of variation in the estimated values of ASM and LSM. Based on the variation of ASM and LSM and ovarian weight between FMA II, III, IV and FMA I (Ramos *et al.*, 2000; Rosas & Monteiro-Filho, 2001; Danilewicz, 2003; Freitas da Silva, 2011; this study) we suggest that those differences supported the existence of the two distinctive ESU described by Cunha *et al.* (2014). Note that the existing information about reproductive biology of franciscana dolphins is relatively old and scarce which enhanced the value of the information presented in this work.

We found a lower APR in comparison with that found by Danilewicz (2003) for franciscana dolphins from Brazil (0.66) and harbour porpoise (0.95; Read & Hohn, 1995) whereas it was similar to the one found for spotted dolphins, *Stenella attenuata* (0.33; Myrick *et al.*, 1986). We also found a low proportion of lactating females and high proportion of resting females (0.33), indicating that pregnancy is likely to fail or not occur, so females must wait until the next mating season (Figure 5). This low pregnancy rate could be explained by sampling bias due to lower vulnerability of larger females and females with calves to by-catch (Danilewicz *et al.*, 2004; Negri *et al.*, in press). A different reason that may explain the difference between the results found here with those previously reported is due to sampling period that coincided with the breeding season of the species, the resting females recovered may have not ovulated yet during that specific mating period. This may account for the lower pregnancy rate estimated in the current study compared with more northern management areas for this species. Further studies are likely to provide new insight into habitat use of franciscana dolphins from Argentina.

Harrison *et al.* (1981) and Kasuya & Brownell (1979) estimated lactation period duration for franciscana in Uruguay as at least 8 months, based on lactating females and their ovarian characteristics respectively. The lactation period was also estimated between 7.5 and 8.5 months in franciscana dolphins from South-eastern Brazil, included in the North ESU (FMA II; Ramos *et al.*, 2000), and at least 7 months in dolphins from northern Buenos Aires from South ESU (FMA IVa; Rodríguez *et al.*, 2002; Denuncio *et al.*, 2013). We achieved a similar outcome (7.95 months, estimated according Perrin & Reilly, 1984) to the ones found in other small coastal odontocetes with a short lifespan, such as the harbour porpoise and vaquita, *Phocoena sinus* (Read & Hohn, 1995; Hohn *et al.*, 1996). Therefore, franciscana dolphins have a lactation period duration similar to other small, coastal dolphins with a short lifespan.

Knowledge of the natural history of a species is vital for understanding its biology, conservation status and for ensuring its continued survival. Framed within the evolutionary theory of life history of mammals the concept of 'slow-fast continuum' arose (Stearns, 1992). This slow-fast continuum concept, driven by body size and ecology of a species (Harvey & Purvis, 1999), explains the great variation in life history strategies observed throughout the mammalian orders, including cetaceans. Small odontocetes, such as the harbour porpoise, are located closer to the fast end of the slow-fast continuum as they reach sexual maturity relatively early, have a low life expectancy (around 20 years) and very high reproductive rates (∼0.9) (Read & Hohn, 1995; Hohn *et al.*, 1996). Those small cetaceans represent one extreme of life history strategies compared with larger cetaceans (Read & Hohn, 1995). Species with a 'fast' life history strategy are expected to show a short calving interval and a large offspring size per lifetime, although it was not the case for harbour porpoise and franciscana dolphin, when only a few offspring are produced per lifetime (Read, 1990; Danilewicz, 2003).

Estimation of reproductive parameters of females and understanding their life history strategy (Perrin *et al.*, 1977; Kasuya, 1985; Myrick *et al.*, 1986) are important for monitoring the possible impacts that exploitation, habitat degradation, pollution and other factors may have on a population. This study provides novel data about reproductive aspects of female franciscana dolphins, including the first information obtained on its southern limit distribution, which can aid conservation management of this species in Argentina.

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