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2	Calf chronology of the Franciscana dolphin (Pontoporia blainvillei): birth, onset of feeding, and duration of lactation in coastal waters of
3	Argentina
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Birth, lactation and maternal investment have been described and measured in many species of terrestrial mammals and marine mammals that nurse their young on land, such as pinnipeds, aquatic mustelids and polar bears (Siniff & Ralls, 1991; Derocher & Stirling, 1995; Trillmich & Weissing, 2006). Because of their fully aquatic life cycle, information about the early stages of life history in cetaceans is very limited. Most of the information is based on captive individuals of a few species such as the bottlenose dolphin (Tursiops truncatus) (West et al., 2007), the beluga (Delphinapterus leucas) (Robeck et al., 2005) or by indirect measures (i.e. post-mortem data from pregnant females and its fetus) from fetuses and/or from reproductive parameters of adults of several species (Perrin et al. 1977; Kasuya & Brownell, 1979; Lockyer et al., 2001;

Murphy et al., 2009). Also, information on mother-calf association has been obtained from photo-identification techniques (Grellier et al., 2003)

Lactation is a crucial stage in the life history of mammals and a defining characteristic of its reproduction that involves substantial investment of energy and nutrients by the mother (Oftedal, 1984). It is one of the most variable reproductive parameters among mammals, ranging in marine 2 3 mammals from less than one week (four days in the hooded seal, Cystophora cristata, Bowen et al., 1985) to more than two years (in some species of toothed whales such as the sperm whale, *Physeter macrocephalus*) with nutritional and behavioral consequences for both mother and 4 5 offspring (Oftedal, 1997; West et al., 2007; Pomeroy, 2011). The franciscana dolphin (Pontoporia blainvillei) is a small cetacean endemic to the coastal waters of Southwestern Atlantic Ocean from Brazil to 6 Argentina (Crespo et al., 1998; Bastida et al., 2007; Danilewicz et al., 2009). Due to the continued incidental mortality throughout most of its 7 8 geographic distribution, the franciscana may be the most threatened small cetacean in the western South Atlantic Ocean and has recently been 9 classified as Vulnerable (A3d) by the IUCN (Secchi et al., 2003; Reeves et al., 2008). 10 The biology and ecology of franciscana is mainly based on studies of juveniles and adult specimens entangled in gillnets (Kasuya & Brownell, 1979; Danilewicz et al., 2002; Ott et al., 2002; Di Beneditto et al.. 2009). However, franciscana mothers with calves are very rarely entangled or 11 found stranded, which has limited the information on lactation duration and weaning (Kasuya & Brownell 1979; Harrison et al., 1981). Some 12 13 information is available on birth, lactation and initial events of independent feeding from a direct study of calves (Rodríguez et al., 2002). The 14 goal of this study was characterize size at birth, duration of lactation, and onset of independent feeding in franciscanas based on a long-term data

from calves that stranded along the northern coast of Argentina from Rio Salado in Bahía Samborombón (35°44' S, 57°21' W) to Mar del Plata (38°00' S, 57°33' W) (Fig. 1). 2 Stranded (alive and dead fresh) and entangled franciscana calves were collected in cooperation with the Fundación Mundo Marino Rehabilitation 3 Centre (FMMRC) and local, artisanal fishermen. Only specimens within the first year of life (neonates to calves up to 105 cm long [standard 4 5 length]) were used. Neonates had an umbilical cord or traces of milk in their stomach, indicating that they were not an aborted fetus. The upper limit (105 cm) for standard length was based on the published maximum length of specimens known to be < 1 year old (Kasuya & Brownell, 6 1979; Negri, 2011) and from unpublished information on age composition of franciscana from the Northern Buenos Aires Province and nearby 7 8 areas (n=26; Fig. 2A; Kasuya & Brownell, 1979; Negri, 2011). Specimens that were known or suspected to be older than 1 year were not used. The age of the that 26 specimens was estimated by counting growth-layer groups (GLGs) following standard histological techniques (Pinedo & 9 10 Hohn, 2000; Botta et al., 2010). Six fetuses were included to estimate the size at birth using the Borjesson & Read (2003) overlap criterion, defined as the mean of the overlapping fetal (n=6) and calf (n=15) sizes, including the largest non-overlapping fetus and the smallest non-11

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overlapping calf (Figure 2B).

- Following the feeding regimes proposed by Rodriguez et al. (2002), calf stages were categorized as:
- 1. *Strict lactation*: represented by calves with gastric milk only.

- 2. Beginning of predation/weaning: specimens with mixed gastric milk and solid prey remains.
- 3. *Weaning:* specimens with prey remain only and total length up to 105 cm.
- 3 Calving chronology and lactation period were determined using the date of collection of each specimen.
- 4 Prey items (otoliths, cephalopod beaks or skeletal remains of invertebrates) recovered from stomach contents were identified to the lowest
- 5 possible taxon with the aid of a marine species reference collection (Marine Mammal Laboratory, Universidad Nacional de Mar del Plata,
- 6 Argentina). Biomass and total length of fishes and cephalopods were reconstructed from length mass regressions previously used by Rodríguez
- 7 et al. (2002). Only complete otoliths with little or no erosion were used for this analysis. Absolute number of prey (N), frequency of occurrence
- 8 (FO%) of each taxon, numerical abundance (NA%) and estimated biomass (W%) contributed by each prey were estimated for each dolphin. The
- 9 Index of Relative Importance (IRI) of Pinkas et al. (1971), with the Castley et al. (1991) volumetric modification (IRI = [%N+%W] * %FO), was
- 10 computed and expressed as the percentage IRI. Biomass (W%) and total length of fishes and cephalopods were reconstructed from length -
- weight regressions previously used by Rodríguez et al. (2002).
- From 1995-2011, a total of 91 franciscana calves were collected. Fifteen newborn calves, that define the *calving season*, were found over the
- course of early October to late January (124 days), with the highest frequency of occurrence in November (Table 1, Figure 3). Seventy three
- 14 (80.2%) of the calves examined contained traces of milk and/or food allowing classification into feeding stages: 1) Forty-two contained
- exclusively milk, that defined the *strict lactation* period, 2) 25 contained a mixture of milk and solid food, that defined the *beginning of*

predation/weaning and 3) six contained exclusively solid food, indicating weaning. Evidence of milk was found in the stomachs of calves from the northern coast of Argentina from early October to mid April (195 days) suggesting a lactation period of about 6-7 months (Table 1, Figure 3). 2 3 The strict lactation period occurred from October to February (135 days). The beginning of predation/weaning period occurred over 165 days between November and April with the highest frequency in February (Table 1 and Figure 3). The small number of animals assigned to the 4 weaning period did not allow that stage to be well defined (Table 1). Schematic depiction of the calving chronology of Franciscana dolphins in 5 Argentina is summarized in Figure 4. 6 Eleven prey species were identified in the *early predation* period (Table 2). The striped weakfish (*Cynoscion guatucupa*) was the most common 7 8 prey at this stage, followed by the long finned-squid (Loligo sanpaulensis) and, or lesser importance, white croakers (Micropogonias furnieri), banded croakers (Paralonchurus brasiliensis) and crustaceans. 9 10 Along the northern coast of Argentina, the presence of calves is relatively frequent, with a high incidence in Bahia Samborombón and Cabo San Antonio (Figure 1). The birth period of the franciscana extends over ca. four months, from early October to late January, with the highest 11 12 frequency in November. The presence of live stranded calves with umbilical cords combined with milk in the stomach was considered clear 13 evidence of calving, as this excluded any fetuses that might occur due to abortions during maternal entanglement. Also, past studies of 14 rehabilitated franciscana (reviewed by Danilewicz et al., 2002) and unpublished reports in FMMRC have shown that the umbilical cord remained

attached from 48-72 hr after birth, indicating that these individuals were recovered no more than one week after birth. This period coincides with the calving season reported for Uruguay and southern Brazil (Brownell, 1984; Pinedo et al., 1989, Danilewicz, 2003). 2 3 Mass and length at birth are usually estimated by comparing the sizes of the largest fetuses and smallest calves (Whitehead & Mann, 2000, Boejesson & Read, 2003). In our study, mean standard length and mass of neonatal franciscana specimens from northern Argentina were 64 cm 4 5 (range 61-69 cm) and 3.06 kg, respectively, whilst the length and mass at birth were 60.7 cm and 3.10 kg using the Borjesson & Read (2003) overlap method, which is somewhat lower than previous values (70-85 cm) estimated from Uruguay and Brazil (Kasuya & Brownell, 1979; 6 Harrison et al., 1981; Ramos et al., 2000; Danilewicz, 2003), probably due to the small sample size of the previous studies, geographical 7 8 differences in the size of franciscana (Ramos et al., 2000; Botta et al., 2010; Barbatto et al., 2011) or a combination of both. In odontocetes, 9 relative birth mass (proportion of maternal mass) can range up to 20% (Whitehead & Mann, 2000). Given that an adult female mean body mass of 36.5 kg along the northern coast of Argentina (Rodríguez et al., 2002), the mean weight of 3.3 kg determined in this study represents a relative 10 birth mass of 9.6%. 11 12 Based on the range of dates for calves with milk in their stomachs (strict lactation and beginning of predation/weaning stages), we estimated a 13 lactation duration of at least seven months (or 28 weeks) from October to April for the northern coast of Argentina. However, we cannot exclude 14 the possibility of a longer lactation period, because nearly 20% of the calves had empty stomachs (i.e., had fully digested the last meal of either 15 milk and/or prey). Some of the specimens considered as weaned calves could still be suckling even though we did not observe traces of milk.

Harrison et al., (1981) and Kasuya & Brownell (1979) estimated lactation duration as at least 8 months, or between 8 and 9 months, respectively, for franciscana in Uruguay. Both studies were based on lactating females and their ovarian characteristics. In southeastern Brazil, the lactation 2 3 period was also estimated between 7.5 and 8.5 months (Ramos et al., 2000). Environmental, nutritional and social influences may affect the timing of the reproductive cycle (Pomeroy, 2011). Variation in the duration of 4 5 maternal dependence and lactation among different species may be related to the need to acquire social and cognitive skills, foraging tactics and/or predator-avoidance strategies (Hayssen, 1993). There are two main lactation strategies in marine mammals with different nutritional 6 consequences for both mother and young. Relatively short lactation periods involve high energy transfers to suckling young, allowing rapid 7 8 growth and shorter dependence periods (few days or weeks, Oftedal, 1997). Among cetaceans, this pattern is typical of some mysticete whales 9 (Oftedal, 1997; Pomeroy, 2011). The alternative strategy is prolonged lactation lasting many months or even years, with less intense energy 10 transfer, slower growth, and longer dependency of young on their mothers. This strategy is used by less migratory species of cetaceans (Oftedal, 1997). 11 12 Franciscana spend at least seven months (or 28 weeks) in lactation representing 5% of the life of the species with an estimated longevity of 12 13 (Secchi et al., 2003). Our results are similar to the reproductive parameters estimated in other small coastal odontocetes with short lifespan, such 14 as the harbor porpoise (*Phocoena phocoena*). This species has an estimated 32 week lactation period, which represents around 7.5% of the 15 expected lifespan (Oftedal, 1997). Short lifespan, reproductive seasonality and lactation of less than a year have also been suggested for the

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vaquita (*Phocoena sinus*) (Hohn et al., 1996). Therefore, franciscana has lactation duration similar to other small, coastal dolphins with a short lifespan. Moreover, Peddemors et al. (1989) suggest that coastal cetaceans are characterized by lower fat concentration in milk, similar to the fat composition of the milk of franciscana (8-15%; Caon et al., 2008) and other coastal and river dolphins, such as the Amazon River dolphin (*Inia* geoffrensis; Rosas & Lehti, 1996), bottlenose dolphin (West et al. 2007), and Indo-Pacific humpback dolphin (Souza plumbea) (Peddemors et al., 1989) that vary between 8.2-15.6%. Our results indicate a short period of exclusive milk intake. Based on the elapsed time between the first record of neonates and the first hard 6 remains in stomach contents (Table 1), this phase of the lactation may last around 40 days during which calves more than double (ca. 2.2-times birth weight) their body mass and reach circa 30% of the mother mass. During the next phase of approximately five months, the calves complement milk intake with solid food (fish, squid and crustaceans), indicating that weaning may be gradual. The early foraging by franciscana calves may represent a learning process prior to attainment of independent feeding. Calves in the mixed feeding or transition period exceed 78 cm total length and 8.2 kg body mass. Early intake of solid food was also observed in the short-beaked 12 common dolphins (Delphinus delphis) in the Northeast Atlantic Ocean, where the onset of solid food ingestion started between 3-6 months of age (Brophy et al., 2009). Kasuya & Brownell (1979) and Pinedo (1994) suggest that early consumption of solid food by franciscana may explain the 14 early assimilation of calcium observed in erupting teeth (Pinedo & Hohn, 2000). Early predation on fish and squid by franciscana calves of northern Argentina has also been suggested based on the early accumulation of mercury and cadmium in their tissues (Gerpe et al., 2002) as in

other marine mammals (Gerpe et al., 2009) and also on patterns of gillnet entanglement (Rodríguez et al., 2002). Weaning size in franciscanas from northern Argentina (> 91 cm and > 11 kg; Table 1) is similar to previous estimates for Uruguay (Kasuya & Brownell, 1979). 2 3 The most important teleost prey of franciscana calves are juveniles of two commercial fishes (stripped weakfish and the whitemouth croaker), which have spawning and nursery grounds in the study area (Acha et al., 1999; Jaureguizar et al., 2003; FAO, 2005). Although our results 4 5 confirm that calves have a less species-rich diet than juvenile and adults (Rodríguez et al., 2002), their predominate are the same as in older age classes, indicating minor ontogenetic shifts in prey consumption. The size of consumed prey (circa 2-4 cm) is much smaller than age classes 6 fished commercially (>25cm; FAO, 2005) and also smaller than adult prey size (4-8 cm; Rodríguez et al., 2002). 7 8 This study confirms that the franciscana calving season in coastal waters of northern Argentina occurs during austral spring-summer. The size 9 and mass of neonates are lower than previously estimated, but our results are based on a much larger sample size than previous reports. Lactation 10 duration was estimated to be at least seven months, primarily based on direct observations of milk in stomach contents. Our results also confirm that the transition period from a diet solely of milk to independent feeding is characterized by a diet of low species diversity and small prey size. 11 12 Our study provides new information on the early life history of this threatened species. Live strandings of franciscana calves in northern 13 Argentina are probably a consequence of maternal entanglement in coastal gillnets. This impact needs to be considered when assessing 14 franciscana by-catch throughout its geographical distribution and in the development of conservation strategies and management actions for this 15 species.

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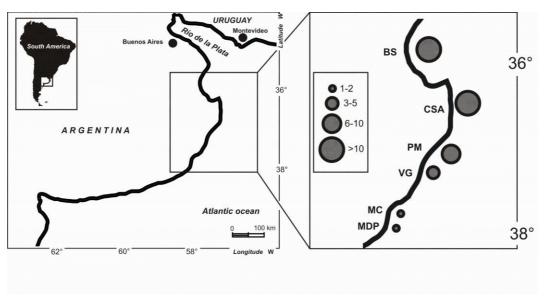
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Figure 1. Study area showing the sites where stranded or entangled franciscana calves were found: Bahia Samborombón (BS; including Rio

- Salado and General Lavalle localities), Cabo San Antonio (CSA; including the localities of San Clemente, Las Toninas, Santa Teresita, San
- 5 Bernardo, Mar de Ajó, Nueva Atlantis and Punta Médanos), Pinamar (PM), Villa Gesell (VG) Mar Chiquita, (MC) and Mar del Plata
- 6 (MDP). Circle size indicates the number of calves found.

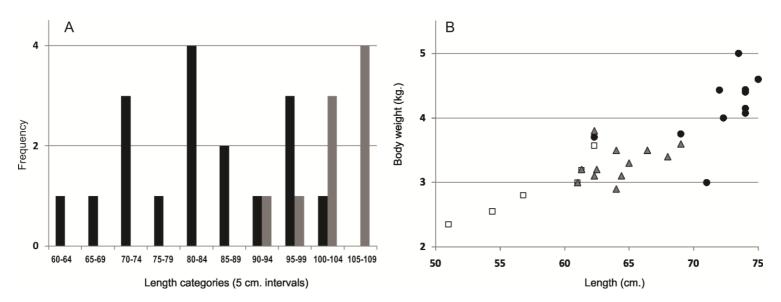


Figure 2. A: Body length frequency for franciscana of 0 years of age (black) and 1 year of age (grey) along the northern coast of Argentina. B: Length-mass relationship of franciscana up to a standard length of 75 cm including fetuses (empty square), neonates with umbilical cords (grey triangles) and suckling calves without umbilical cords (black circles).



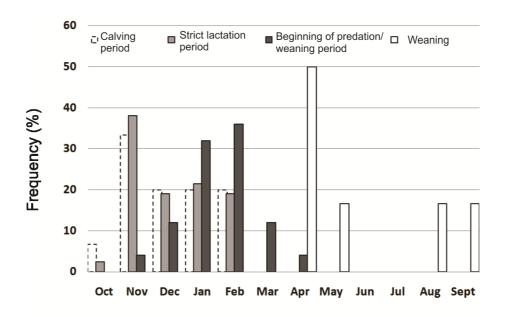


Figure 3. Percent frequency of calf feeding stages for franciscana in northern Argentina.

Strict lactation period Beginning of predation/weaning Calving period D Ν 0 M S Α M

No data

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3 **Figure 4.** Chronology of calf feeding stages during the first year postpartum.

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Weaning

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Tables 2

represent a small sample size and chronologically incomplete information distribution.

Table 1. Chronology of calf feeding stages during the first year postpartum with standard length and body mass for each stage.. *Weaned calves

			Calf Feeding Stages	
	Calving season	Strict lactation	Beginning of predation	Weaning *
Mean	November 23	December 10	February 3	-
SD	37.7	36.2	32.1	-
N	15	42	24	7
Min (early) date	October 1	October 1	November 10	April 4
Max (late) date	January 30	February 15	April 15	September 18
Month Mode	November	November	February	-
Mean	64.0	70.8	87.8	99.6
SD	3.1	8.1	11.9	6.2

N	1	.5	42	24	7
Mi	i n 6	51	61	78	91
Ma	ax 6	59	94	98	108
Me	ean 3	.3	4.8	10.6	14.1
SD	0	.2	2.3	1.6	1.8
N	1	.4	42	19	7
Mi	in 2	.9	2.9	8.3	11.3
Ma	ax 3	.6 1	11.5	13.6	15.8

Table 2. Prey analysis for franciscana calves from the northern coast of Argentina during the weaning period. N, total number of prey, N%, percentage by number; W%, percentage by mass; FO%, percentage frequency of occurrence; IRI, the index of relative importance and IRI%, percentage of IRI. In the right side shows mean and standard deviation (SD) of the prey size.

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			F00/	770/	TT 10 /		TDI 0/	Prey length	ı (in mm)
Common name	Scientific name	N	FO%	N%	W%	IRI	IRI %	Mean	SD
TELEOSTS									
Striped weakfish	Cynoscion guatucupa	202	43.48	46.98	33.13	3482.91	82.19	24.74	7.36
White croacker	Micropogonias furnieri	16	17.39	3.72	2.65	110.81	2.62	34.05	16.17
Banded croacker	Paralonchurus brasiliensis	10	13.04	2.33	1.55	50.59	1.19	18.96	9.27
King weakfish	Macrodon ancylodon	21	4.35	4.88	0.32	22.64	0.53	19.14	11.00
Rough scad	Trachurus lathami	1	4.35	0.23	0.22	1.97	0.05	31.19	17.56
Deep-bodied pipefish	Leptonotus blainvillanus	1	4.35	0.23					
CEPHALOPODS									
Long finned-squid	Loligo sanpaulensis	14	8.70	3.26	62.12	568.51	13.42	120.92	20.53

CRI	ווקי	ΓΔ	CF	Δ	21
\ .I\ !		_		$\overline{}$	1 N.)

Marine shrimp	Artemesia longinaris	3	4.35	0.70
Marine shrimp	Pleoticus muelleri	18	4.35	4.19
Small white shrimp	Peisos petrumkevitchi	9	8.70	2.09
Oppossum shrimp	Neomysis americana	135	8.70	31.40

TOTAL 430