Reproductive biology of male franciscanas (*Pontoporia blainvillei*) (Mammalia: Cetacea) from Rio Grande do Sul, southern Brazil*

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Abstract—The reproductive biology of male franciscanas (*Pontoporia blainvillei*), based on 121 individuals collected in Rio Grande do Sul State, southern Brazil, was studied. Estimates on age, length, and weight at attainment of sexual maturity are presented. Data on the reproductive seasonality and on the relationship between some testicular characteristics and age, size, and maturity status are provided. Sexual maturity was assessed by histological examination of the testes. Seasonality was determined by changes in relative and total testis weight, and in seminiferous tubule diameters. Testis weight, testicular index of maturity, and seminiferous tubule diameters were reliable indicators of sexual maturity, whereas testis length, age, length, and weight of the dolphin were not. Sexual maturity was estimated to be attained at 3.6 years (CI 95%=2.7–4.5) with the DeMaster method and 3.0 years with the logistic equation. Length and weight at attainment of sexual maturity were 128.2 cm (CI 95%=125.3–131.1 cm) and 26.4 kg (CI 95%=24.7–28.1 kg), respectively. It could not be verified that there was any seasonal change in the testis weight and in the seminiferous tubule diameters in mature males. It is suggested that at least some mature males may remain reproductively active throughout the year. The extremely low relative testis weight indicates that sperm competition does not occur in the species. On the other hand, the absence of secondary sexual characteristics, the reversed sexual size dimorphism, and the small number of scars from intrasexual combats in males reinforce the hypothesis that male combats for female reproductive access may be rare for franciscana. It is hypothesized that *P. blainvillei* form temporary pairs (one male copulating with only one female) during the reproductive period.

The franciscana (*Pontoporia blainvillei*) is a small dolphin endemic to the coastal waters of the southwestern Atlantic Ocean. The distribution of this species ranges from Golfo Nuevo (42°35′S; 64°48′W), Chubut Province, Argentina (Crespo et al., 1998) to Itaúnas (18°25′S; 30°42′W), Espírito Santo, southeastern Brazil (Moreira and Siciliano, 1991) (Fig. 1).

The franciscana's coastal habitat makes it vulnerable to being caught as incidental catch in gill nets and trammel nets throughout most of the species range (e.g., Praderi et al., 1989; Corcuera et al., 1994; Secchi et al., 2003). Because of its vulnerability as bycatch, the franciscana has been considered the most impacted small cetacean in the southwestern Atlantic Ocean (Secchi et al., 2002). In the Rio Grande do Sul coast, southern Brazil, this species has been subject to an intense bycatch in gill nets for at least three decades (Moreno et al., 1997; Secchi et al., 1997; Ott, 1998; Ott et al., 2002). The annual mortality of franciscanas in this region was estimated to range from several hundred up to about a thousand individuals (Ott et al., 2002). Simulations
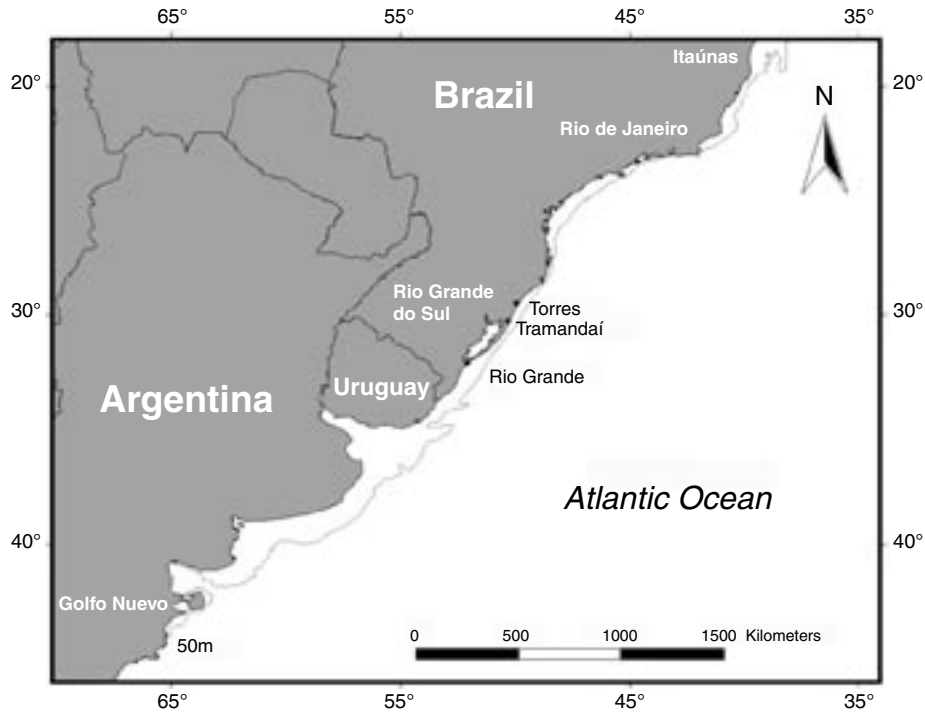
Figure 1

Map of the study area showing the locations along the southern coast of Brazil
where franciscanas were caught as bycatch between 1992 and 1998.

studies on the effects of incidental captures on francis-
canas in Rio Grande do Sul were carried out by using
available data on vital rates, stock size, and bycatch
estimates (e.g., Secchi, 1999; Kinas, 2002). All these
studies showed that there is a decline in franciscana
abundance in this region.

Although the reproductive biology of the female fran-
ciscanas have been studied in detail in Uruguay (Ka-
suya and Brownell, 1979; Harrison et al., 1981), Rio
Grande do Sul (Danilewicz et al., 2000; Danilewicz,
2003), and Rio de Janeiro (Ramos, 1997), there are few
data about male reproduction. Kasuya and Brownell
(1979) presented information on male reproduction for
Uruguay, although their small sample size precluded
them from estimating age and size at attainment of
sexual maturity.

In the Rio Grande do Sul coast, franciscanas are
known to reproduce seasonally; births occur from Oc-
tober to early February (about 75% from October to De-
cember). Because the gestation period was estimated to
last about 11.2 months, mating and conception may take
place between November and early March (Danilewicz,
2003). Seasonal changes in testicular size and activity
have been used to infer or corroborate mating seasons
in some cetacean species (e.g., Neimanis et al., 2000).
Nevertheless, it is not known if male franciscanas also
undergo seasonal changes in the testicular activity.

In this study, we describe the reproductive biology of
male franciscanas from Rio Grande do Sul and present
evidences for the species' mating system.

Materials and methods

Sampling procedures

Data and samples collected from 121 specimens inci-
dentally caught (88%) or beached (12%) along the Rio
Grande do Sul coast between 1992 and 1998 were used
for the analysis on reproduction of male franciscanas.
The sampling of the incidentally caught animals was
carried out through the monitoring of the commercial
fishery fleet from Rio Grande (32°08′S; 52°05′W) and
Tramandaí/Imbé (29°58′S, 50°07′W). Stranded dolphins
were sampled from systematic beach surveys conducted
in an area with an extension of 270 km of sandy beaches,
between Torres (29°19′S, 49°43′W) and Lagoa do Peixe
(31°15′S, 50°54′W).

Not all information could be collected from each car-
cass; therefore sample sizes varied among parameters.
Standard length (SL, n=118) was measured by following
the guidelines established by the American Society of
Mammalogy (1961). The animals were weighed (n=97)
and teeth were extracted and preserved dried or in a
1:1 mix of glycerin and alcohol (70%). Testes and epi-
didymis were removed and fixed in 10% formalin.

Age determination

Age was estimated by counting the growth layer groups
(GLGs) in thin, longitudinal sections of teeth (n=47).
The teeth were decalcified in nitric acid or in RDO

(a commercial mixture of acids) and sectioned on a freezing microtome. The 15–20 μm sections were stained with Mayer's hematoxylin and mounted on microscope slides with Canadian balsam or in glycerin. Poor and off-center sections were discarded in favor of new preparations. Three readers counted independently the number of growth layer groups in both the dentine and cementum. When reader estimates differed, the sections were reexamined together and a best estimate was agreed upon. In this study, we considered one GLG to represent one year of age, which is the accepted model for the franciscana (Kasuya and Brownell, 1979; Pinedo, 1991; Pinedo and Hohn; 2000).

Reproduction

In the laboratory, the testes were separated from the epididymis, weighed to the nearest 0.01 g (n=107), and measured in three dimensions (length and two diameters perpendicular to each other in the middle of the testis) to the nearest 0.1 mm (n=104). The mean of these two diameters was called mean testis diameter. The weight of one of the gonads could not be recorded on some occasions (n=8) and we assumed that both testes had the same weight. Then, relative testis weight was determined as the ratio of the combined testis weight to the animal weight.

A 1-cm^3 subsample of each testis from the central portion of the organ was removed and examined by using standard histological preparations. The tissue was embedded in paraffin, sectioned in 4–10 μm thick slides through a manual microtome, and stained with hematoxylin and eosin (H&E). Male sexual maturity status was determined by examining the testicular sections at a magnification of 100×. In this study, we followed the classification criteria suggested by Hohn et al. (1985):

1. Immature—seminiferous tubules containing mainly spermatogonias. Abundant interstitial tissue present between the seminiferous tubules and lumen totally closed.
2. Pubertal—seminiferous tubules containing spermatogonias and spermatocytes. Less interstitial tissue present between the seminiferous tubules than in immature animals. The lumen is partially opened.
3. Mature—seminiferous tubules containing spermatogonias, spermatocytes, spermatids and, in many cases, spermatozoa. Interstitial tissue almost nonexistent between the seminiferous tubules. The lumen is totally opened.

The diameters of ten random circular seminiferous tubules were measured for each specimen (n=93) with a scale present in the lens of the microscope in order to calculate the seminiferous tubule mean diameter. A maturity index (MI) was calculated as the ratio of the combined testes weight by the combined testes length ($\Sigma W/\Sigma L$).

An analysis of the variation along the year of the values of relative and combined testes weight, and seminiferous tubule mean diameter, was employed to assess reproductive seasonality. Values of these parameters were compared between months when mating and conception occur ("reproductive months": November–March) and months when they not occur ("nonreproductive months": April–October). In order to increase the sample size of mature animals collected in reproductive months, data on testes weight from mature male franciscanas from Uruguay were included in the analysis (data supplied by Kasuya[1]).

The mean age at attainment of sexual maturity (ASM) was estimated through the DeMaster (1978) method and the logistic regression.

The DeMaster (1978) equation computes the mean as

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

where f_a = the fraction of sexually mature animals in the sample with age a;
  j = the age of the youngest sexually mature animal in the sample; and
  k = the age of the oldest sexually immature animal in the sample.

The variance of the DeMaster method estimate is calculated as

$$\mathrm{var}(ASM) = \sum_{a=j}^{k} \left[(f_a(1-f_a)/N_a - 1) \right],$$

where N_a = the total number of animals aged a.

The logistic regression approach fits a sigmoid curve representing the probability that a franciscana of age a is sexually mature to the distribution of sexually mature and immature animals by age as

$$Y = 1/(1+e^{a+bx}) \quad \text{or} \quad \ln(1/Y-1) = a + bx,$$

where x = the age of the dolphin;
  b = the slope of the regression; and
  a = the intercept.

To obtain the age when 50% of the animals are sexually mature (Y=0.5), the last equation is simplified as $ASM = -a/b$.

Mean length and weight at sexual maturity was also estimated by the DeMaster (1978) method, by substituting age for length and weight, respectively. The method was slightly modified, as suggested by Ferrero and Walker (1993), and was calculated as

$$LSM = \sum_{C\min}^{C\max} L(f_t - f_{t-1}),$$

where $C\max$ = the length or weight class of the largest or heaviest sexually immature animal;

$C\min$ = the length or weight class of the smallest or lightest sexually mature animal;

L = the lower value of the length or weight class t; and

f_t = fraction of mature animals in the length or weight class t.

The specimens were pooled into length and weight intervals of 4 cm and 4 kg, respectively.

The estimated variance of this method is also modified and is calculated as

$$\mathrm{var}(MS) = w^2 \sum_{C\min}^{C\max} \big[(f_i(1-f_i)/N_i - 1\big],$$

where N_i = the number of specimens in the length or weight class t; and

w = the interval width, a constant equal to 4 in these cases.

For estimating age, length, and weight at sexual maturity, pubertal animals were grouped together with immature animals.

Results

The weight and length of the left testes ranged from 0.23 to 10.42 g ($\bar{x}$=2.60 g) and from 15.7 to 59.7 mm ($\bar{x}$=33.6 mm), respectively. The weight and length of the right testes ranged from 0.17 to 9.98 g ($\bar{x}$=2.62 g) and from 17.9 to 60.0 mm ($\bar{x}$=34.5 mm), respectively. The relationship of testes weight and testes length resulted in significant regression ($P<0.0001$) and correlation (r^2=0.91; F=823.9; $P<0.0001$; y=0.000012$x^{3.33}$). The male with the heaviest relative testes weight was 141.6 cm in length and 31.2 kg in weight, and its combined-testes weight was 20.1 g, which is 0.064% of its total weight. The mean of the relative testes weight from 23 mature males was 0.036% of their total weight.

The testes of the franciscana are characterized by a high lateral symmetry. There was no statistically difference in weight (t=−0.09; P=0.93; n=71) and length (t=−0.4; P=0.69; n=100) between testes of the same animal. A strong correlation was found between left and right testes length (b=0.95; F=1073.0; r^2=0.92; $P<0.0001$; n=100; y=1.232 $x^{0.95}$) and between left and right testes weight (b=0.99; F=7262.8; r^2=0.99; $P<0.0001$; n=71; y=1.02$x^{1.0}$), where x and y represent values of the left and right testis, respectively.

Seminiferous tubule diameter

A nonlinear regression demonstrated positive allometry ($b>0.333$) of the seminiferous tubule diameter to the combined testicular weight (b=0.39; 95% CI=0.35–0.44) (Fig. 2), and a strong correlation between these two variables (F=343.6; r^2=0.86; $P<0.0001$; y=59.4$x^{0.39}$).

The relationship between the seminiferous tubule diameter and testes length is shown in Figure 3 and the relationship between the seminiferous tubule diameter and standard length is shown in Figure 4. In immature males, there was almost no increase in the seminiferous tubule diameter with the increase of standard length (0.26 μm/cm) and total weight (0.5 μm/kg). In mature males, however, seminiferous tubule diameter was significantly correlated with standard length (b=1.06; F=4.4; r^2=0.18; P=0.048; y=1.4775x−43.572) and there was no correlation with total weight (b=0.23; F=1.28; r^2=0.07; P=0.27; y=1.6132x+108.54).

The differences of the seminiferous tubule mean diameters were statistically significant between immature, pubertal, and mature male franciscanas (ANOVA, Fs=255.4; df=87; $P<0.001$).

Combined-testes weight and length, and sexual maturity

There was almost no increment in mass of the combined-testes weight in immature dolphins. An increment of only about 2.0 g in the combined-testes mass was observed in animals of 70.0 to 125.0 cm in length. For dolphins about 120.0–130.0 cm in length, the combined-testes mass suddenly increased (Fig. 5), indicating the
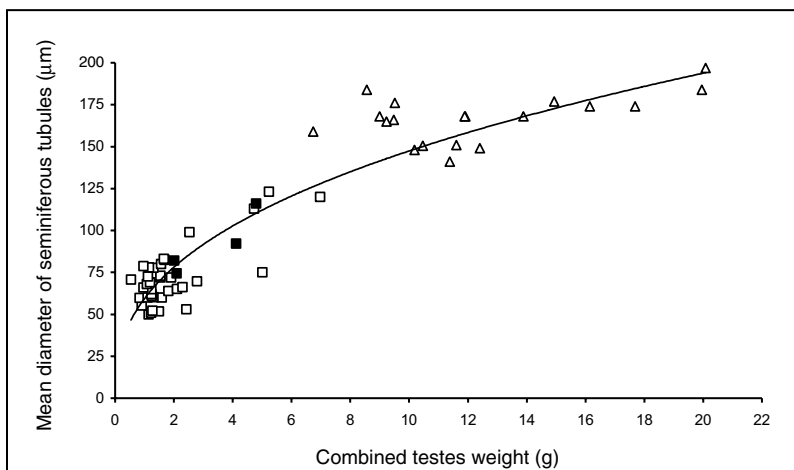

Figure 2

Relationship between combined-testes weight and mean seminiferous tubule diameter in immature (open boxes), pubertal (filled boxes), and mature (triangles) male franciscanas (*Pontoporia blainvillei*) from Rio Grande do Sul (n=59).

attainment of sexual maturity. The rate of testes-mass gain was 0.05 g/cm for immature and 0.28 g/cm for mature dolphins. All animals with combined-testes weight higher than 5.0 g were sexually mature, and this finding may indicate that this parameter can be used as a reliable indicator of sexual maturity in male franciscanas from Rio Grande do Sul (Table 1). However, the large variation in testes weight after the attainment of sexual maturity precludes a correlation between testes mass and standard length.

Although testes length increases progressively as standard length increases (Fig. 6), there is no abrupt increase in testes length at the moment of attainment of sexual maturity, as observed in the testes mass. A nonlinear regression (exponential) best fits this relationship ($y = 4.5444\,e0.0163x$). As opposed to testes mass, there is a considerable overlap in the values of testes length of immature, pubertal, and mature franciscanas (Table 1), which makes testes length a less reliable predictor of sexual maturity than testes mass.

Age, length, and weight at sexual maturity

Forty-seven specimens in the sample provided information on age and reproductive status (35 immature or pubertal, and 12 mature). The oldest immature animal was 5 years old and the youngest mature was 2 years old (Table 1). Average age at attainment of sexual maturity was estimated to be 3.6 years by the DeMaster method (SD=0.47; 95% CI =2.7–4.5) and 3.0 years by the logistic equation $Y = 1/(1+ e^{0.74-2.23x})$. The age structure of the sample studied is presented in Figure 7.

Sexual maturity in relation to standard length was estimated for 110 males. The smallest mature and the largest immature males were 120.5 and 137.5 cm long, respectively. The average length at sexual maturity was 128.2 cm (SD=1.49; 95% CI=125.3–131.1 cm). Sexual maturity in relation to total weight was estimated for 90 males. The lightest mature and the heaviest immature males were 20.3 and 29.7 kg, respectively. The average weight at sexual maturity was 26.4 kg (SD=0.88; 95% CI=24.7–28.1 kg).

Index of testicular maturity

The differences of the mean index of testicular maturity between immature (0.03), pubertal (0.04), and mature (0.11) dolphins were statistically significant (ANOVA, Fs=210.0 df=101, P<0.001). There was almost no overlap in the values of this index between mature specimens
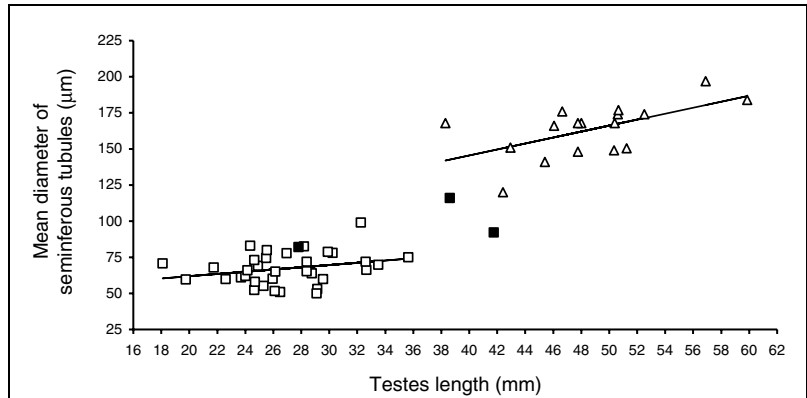

Figure 3

Relationship between testes length and mean diameter of seminiferous tubules in immature (open boxes), pubertal (filled boxes), and mature (triangles) male franciscanas (*Pontoporia blainvillei*) in Rio Grande do Sul (n=54). Data from pubertal animals are not included in the curves.
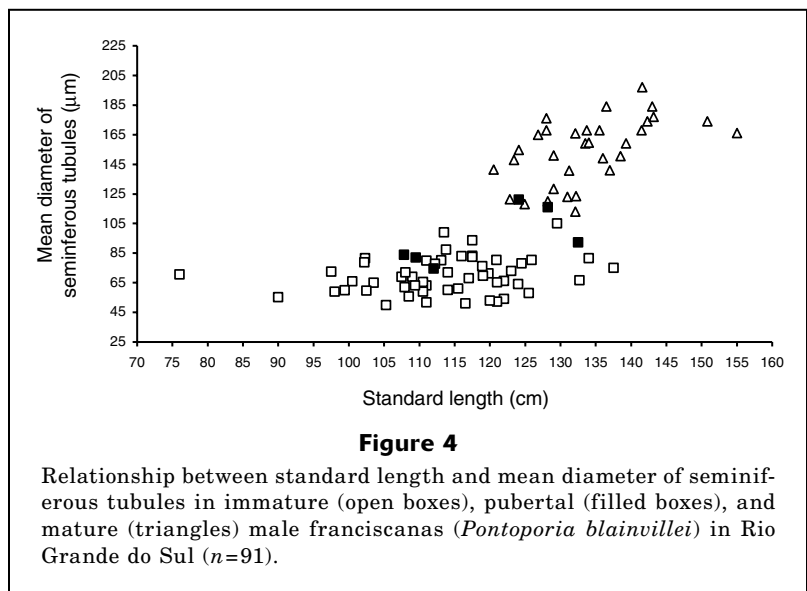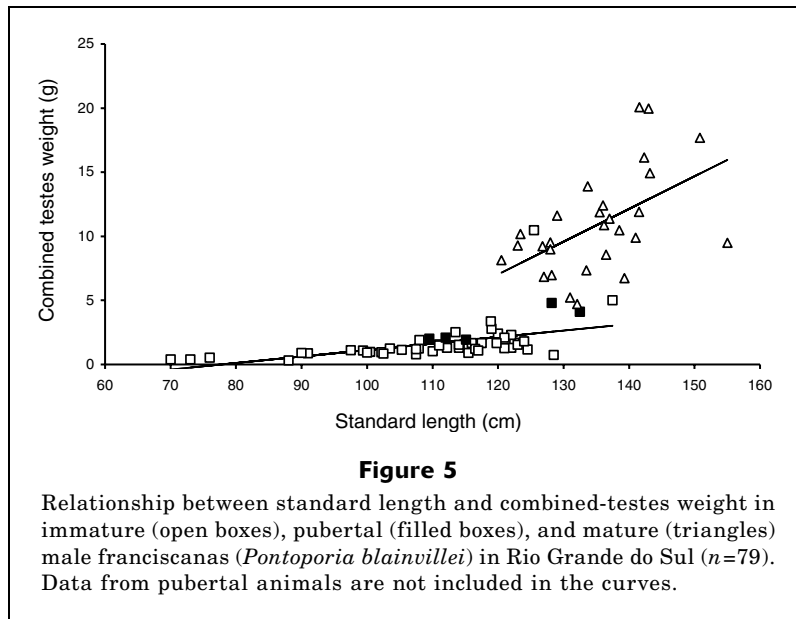

Figure 4

Relationship between standard length and mean diameter of seminiferous tubules in immature (open boxes), pubertal (filled boxes), and mature (triangles) male franciscanas (*Pontoporia blainvillei*) in Rio Grande do Sul (n=91).

and immature and pubertal specimens (Table 1). All males with an index value higher than 0.07 were sexually mature. These results indicate that the index of testicular maturity is a very good indicator of sexual maturity for franciscanas.

Reproductive seasonality

The null hypothesis that the combined and relative testis weight would be higher in the months when the females are reproductively active is rejected. No increase in the testes weight was observed during the months when most mating occurs (Fig. 8). There were no statistically significant differences in the combined-testes weight (ANOVA, Fs=2.28; df=34; P=0.48; n=35) and relative

Figure 5

Relationship between standard length and combined-testes weight in immature (open boxes), pubertal (filled boxes), and mature (triangles) male franciscanas (*Pontoporia blainvillei*) in Rio Grande do Sul (*n*=79). Data from pubertal animals are not included in the curves.

testes weight (ANOVA, F_s=2.42; df=29; P=0.76; n=30) between reproductive and nonreproductive months.

The analyses of the variation in the diameter of the seminiferous tubules throughout the year also did not indicate that the testes undergo seasonal changes. However, it is important to view this result with caution because the sample size of mature males (and therefore the information on tubules diameter collected in reproductive months) was small (*n*=3). However, the presence of spermatids or spermatozoa (or both) in the seminiferous tubules may be also regarded as a direct evidence of testicular activity. Three mature males (11%) in the sample presented seminiferous tubules with spermatids or spermatozoa (or both) and were collected in nonreproductive months (May, June, and August). Although the epididymis of

Table 1

Summarized information on age, length, mass, and testicular characteristics for male franciscanas (*Pontoporia blainvillei*) in the Rio Grande do Sul at different sexual maturity stages.

Characteristics and maturity state	*n*	Mean	Standard deviation	Range
Age (years)				
Immature	31	1.29	1.01	0–5
Pubertal	4	2.0	0.82	1–3
Mature	12	3.8	1.14	2–6
Standard length (cm)				
Immature	62	111.2	13.62	70.0–137.5
Pubertal	7	118.5	9.75	107.8–132.5
Mature	37	133.7	7.71	120.5–155.0
Total mass (kg)				
Immature	53	19.0	5.6	4.95–29.7
Pubertal	6	21.4	4.62	17.1–28.0
Mature	30	29.9	5.22	20.25–41.5
Mean diameter of seminiferous tubules (μm)				
Immature	54	69.6	12.2	50.0–105.0
Pubertal	6	95.0	19.2	74.5–121.2
Mature	33	154.1	21.7	113.0–197.0
Combined testes mass (g)				
Immature	63	1.59	0.84	0.33–4.78
Pubertal	7	2.73	1.28	1.30–4.8
Mature	37	10.24	3.94	4.27–20.08
Testes length (mm)				
Immature	62	27.2	4.9	15.7–35.5
Pubertal	7	32.6	6.2	25.0–41.0
Mature	35	45.4	5.6	31.6–59.7
Index of testicular maturity				
Immature	61	0.03	0.01	0.01–0.06
Pubertal	7	0.04	0.01	0.02–0.06
Mature	36	0.11	0.03	0.05–0.18

a subsample of 10 mature males were examined histologically, we did not find any sign of spermatozoa.

Discussion

The high bilateral uniformity in testicular weight and length presented by the franciscana is a characteristic shared with many other cetacean species. Studies on the striped dolphin, *Stenella coeruleoalba* (Miyazaki, 1977), the common dolphin, *Delphinus delphis* (Collet and Saint Girons, 1984), the sperm whale, *Physeter macrocephalus* (Mitchell and Kozicki, 1984), and the dusky dolphin, *Lagenorhynchus obscurus* (van Waerebeek and Read, 1994), among others, demonstrate the same pattern of testis symmetry. Given the similar dimensions of both testes in franciscanas, it is possible to extrapolate the combined-testes weight by weighing only one testis without introducing bias in the analysis. It is recommended, however, that the weight of the testes should be presented without the epididymis weight, as it was presented in the most extensive comparative study on the subject (Kenagy and Trombulak, 1986).

There is a negative allometry of the seminiferous tubule diameter in relation to testis length, standard length, and total weight. This pattern is accentuated in immature males, in which the tubule diameters remain almost unchanged with the increase of the other variables. The lack of values for tubule diameters in the testes weight interval (2.5–6.0 g) and testes length interval (34–42 mm) just before the attainment of sexual maturity (Figs. 2 and 3) indicates that the increase in tubule size in relation to sexual maturity must occur very quickly, probably when the tubules are between 85 and 125 μm in diameter.

Attainment of sexual maturity

Length and weight at attainment of sexual maturity of male franciscanas in Rio Grande do Sul are very similar to those values estimated in previous estimates for Uruguay (Table 2). In contrast to the present study, Kasuya and Brownell (1979) calculated mean length at sexual maturity for Uruguay as the
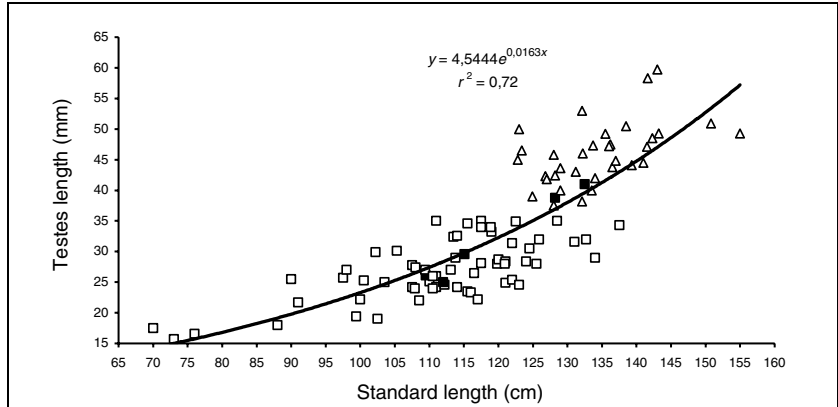

Figure 6
Relationship between standard length and testes length in immature (open boxes), pubertal (filled boxes), and mature (triangles) male franciscanas (*Pontoporia blainvillei*) in Rio Grande do Sul (n=99).
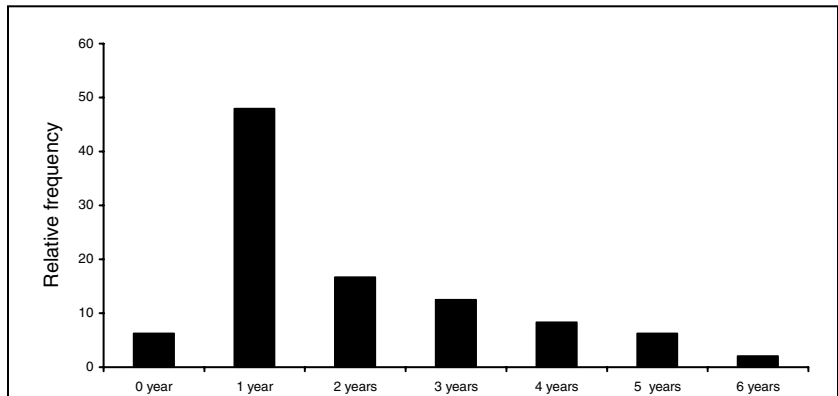

Figure 7
Age structure of male franciscanas (*Pontoporia blainvillei*) collected in Rio Grande do Sul (n=48).
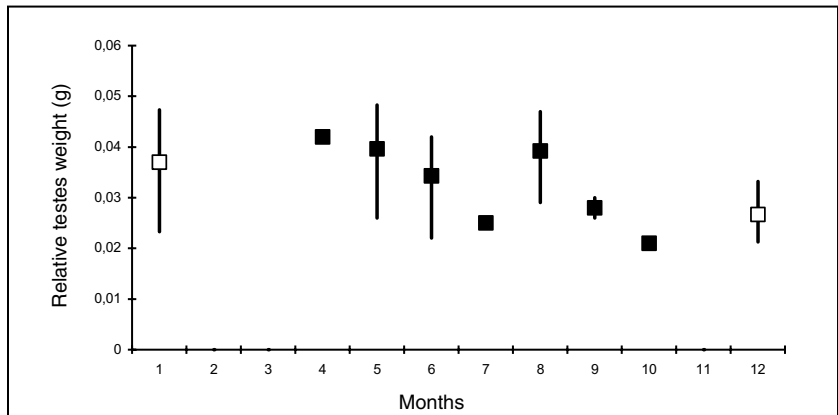

Figure 8
Relationship between month and relative testes weight in mature male franciscanas (*Pontoporia blainvillei*) (n=31) (1=January, 12=December; filled boxes=the nonreproductive months, open boxes=reproductive months). Bars indicate 25% and 75% percentiles.

Table 2

Comparison between average age, weight, and length at sexual maturity between male and female franciscanas from Rio Grande do Sul and Uruguay. The means of the animals from Rio Grande do Sul were estimated by using the DeMaster method (modified) and those from Uruguay were estimated by using a linear regression to determine the moment when 50% of the animals are mature.

	Rio Grande do Sul		Uruguay[1]	
	Males	Females[2]	Males	Females
Age	3.6	3.7	2–4	2.7
Weight (kg)	26.6	32.6	25.0–29.0	33.0–34.0
Length (cm)	127.4	138.9	131.4	140.3

[1] Data from Uruguay were compiled from Kasuya and Brownell (1979).

[2] Data from Danilewicz (2003).

length where 50% of the dolphins were mature through a linear regression. Applying this same approach, a LSM of 125.4 cm was estimated for Rio Grande do Sul—a value still very similar to the Uruguay estimate. Male franciscanas attain sexual maturity at less length and weight than do females in Rio Grande do Sul (Danilewicz, 2003), as observed previously in Uruguay (Kasuya and Brownell, 1979) and Rio de Janeiro (Ramos et al., 2000).

This is the first estimate of mean age at sexual maturity presented for male franciscanas. Kasuya and Brownell (1979) could not calculate ASM for Uruguay because of their small sample size (*n*=25). Nevertheless, Kasuya and Brownell suggested that sexual maturity is attained when males are between 2 and 4 years of age. Franciscanas from Rio de Janeiro were considered mature when they were older than 2 years of age and larger than 115.0 cm in length (Ramos et al., 2000). However, histological analysis of the testes was not performed and Ramos et al. employed indirect methods to determine sexual maturity. Nevertheless, despite the uncertainties produced by the use of different criteria to determine sexual maturity, it was evident that there was substantial difference in the size at maturity between males from Rio de Janeiro and those from Rio Grande do Sul and Uruguay. This difference is probably the result of the well-known distinct growth patterns of the franciscanas from these two regions (Pinedo, 1991) and does not necessarily reflect an early attainment of sexual maturity in males from the Rio de Janeiro population.

The trade-off between growth and reproduction is the best-documented phenotypic trade-off in nature (Stearns, 1992) and has been studied in a wide range of taxa. Because animals from Rio de Janeiro invest less in growth than do animals from Rio Grande do Sul, it is still an open question whether the franciscanas from the Rio de Janeiro have higher reproductive rates or start reproducing earlier than those from Rio Grande do Sul.

The oldest male and female franciscana ever aged were 16 and 21 years-old, respectively (Kasuya and Brownell, 1979; Pinedo, 1994). These ages contrasts with the age distribution found in the present study, where the oldest specimen analyzed was 6 years old. Similar to what is observed in catches for several other small cetacean species (e.g., Hector's dolphins—Slooten and Lad, 1991; harbor porpoise—Read and Hohn, 1995), a general feature of incidental catches for these species is the high entanglement rate of immature animals. In all fishing communities studied in Argentina, Uruguay, and Brazil, a large proportion (>50%) of the specimens caught were less than three years old (e.g., Kasuya and Brownell, 1979; Corcuera et al., 1994; Ott, 1998; Di Beneditto and Ramos, 2001). Although the precise reason for biased catch rates towards immature individuals is not well understood, it could be a combination of factors, including the imbalanced age-structure of local populations (where there are fewer older individuals because of an extensive history as bycatch) and a behavior-related higher vulnerability to bycatch for immature individuals (i.e., juveniles can be more inquisitive and have less ocean experience so that they rove into the area increasing the chances of being entangled). The typically low proportion of old animals in bycatches may explain the characteristics of the data used in this study.

Index of testicular maturity

An index of testicular maturity may be very useful in studies where it is necessary to know the sexual maturity of a large sample of animals without the need of histological analysis, which is time consuming and requires expertise. Although Hohn et al. (1985) recommended the investigation of the applicability of this indirect index of sexual maturity for male cetaceans, the research on this subject has shown no progress. To date, the index of sexual maturity has been calculated only for the common dolphin, *Delphinus delphis* (Collet and Saint Girons, 1984), and from the pantropical spotted dolphin, *Stenella attenuata* (Hohn et al., 1985). For both species, this index distinguished satisfactorily the mature from the immature and pubertal dolphins. Given the results presented, we also recommend the use of the index of testicular maturity as an alternative, nonhistological method, to determine the sexual maturity of male franciscanas. Males with index values lower than 0.05 can be safely classified as immature, and males with index values above 0.08 can be classified as mature. It is recommended that for animals with intermediate values their testes be analyzed histologically so that their reproductive status may be determined definitively.

Besides making intra- and inter-populational comparisons possible, the index of testicular maturity also permits interspecific comparisons because size differences between species are eliminated. The mean index of testicular maturity of mature franciscanas (0.12) is

considerably lower than mature pantropical spotted dolphins (1.9) (Hohn et al., 1985). This difference is a consequence of the relatively small increase of the testes weight of male franciscanas when sexual maturity is attained. Although male spotted dolphin show a marked increase of about 25-fold in testes weight at this moment, franciscanas show an increment in testes weight of about ninefold only.

Reproductive seasonality

The reproductive activities in male mammals are usually restricted to the periods when the females are in estrus (Lincoln, 1992). Reproductive seasonality in males has been reported for several cetacean species and populations through the identification of temporal variations in the testes weight and histological characteristics. In species where the reproductive period is restricted for a few months, as with the dusky dolphin (*Lagenorhynchus obscurus*) and the harbor porpoise (*Phocoena phocoena*), the testes weight presents marked fluctuations accompanying the reproductive period (Read, 1990; van Waerebeek and Read, 1994; Neimanis et al., 2000). Even in species with a diffuse reproductive period (i.e., with more than one peak for births per year) as in the case of dolphins of the genus *Stenella* in the tropical Pacific, it was possible to detect seasonal variation in the male reproductive rhythm (Perrin et al., 1976, Hohn et al., 1985).

Because of the known seasonality for births for franciscana (Kasuya and Brownell, 1979, Harrison et al., 1981, Danilewicz, 2003), it would be expected that the males would accompany the female rhythm, decreasing or even ceasing testicular activity in autumn and winter months. Kasuya and Brownell (1979) examined the seasonal change in testes weight in the months of January, June, and December. From our knowledge of the species' reproduction period, testes weight would be expected to be higher in December and January. However, the authors could not confirm this prediction and attributed the lack of seasonality to the small sample size of mature animals. Nevertheless, the lack of seasonality, even when the testes weight of the mature males from Rio Grande do Sul are included, may indicate what is occurring in the population, and not be a bias introduced by a small sample size.

In species that possess small testes, as in the case of the franciscana, the variation in the testicular activity may be better reflected by changes in the diameter of the seminiferous tubules and the rate of spermatogenesis rather than by changes in the testes weight. Nevertheless, the preliminary results about these characteristics (mature males with spermatids or spermatozoa [or both] in the seminiferous tubules in nonreproductive months and little monthly variation in the diameter of the seminiferous tubules) also do not support the hypothesis of a male reproductive seasonality. The combination of results presented here indicates that testicular activity is not completely interrupted in all males within the population, and that at least some of them

may remain capable of fertilizing females during the year. This conclusion is supported by the observation of pregnancies outside the normal gestation season and that the births resulting from these pregnancies were estimated to take place in September and in late March (Danilewicz, 2003).

The hormone and sperm production by the testes during periods when the females are not able to reproduce may represent an unnecessary energetic expense by the male (Dewsbury, 1982) and may be an explanation for the period of reproductive inactivity for males of several mammal species. In species with large relative testes weight, the maintenance of high levels of sperm production in the testes is a considerable energetic cost for the individual. However, as discussed earlier, this is definitely not the case for the franciscana. For this reason, we suggest that the small energy investment in producing sperm all over the year, due to the small testicular mass, may be an evolutionary advantage for male franciscanas in case of the appearance of off-season reproductive females.

Franciscana reproductive strategy

Although important advances in the knowledge of franciscana behavior in the wild have been made (e.g., Bordino et al., 1999; Bordino, 2002), there is no information on the species' reproductive behavior and its mating strategy remains unknown. Relative testis weight, sexual size dimorphism, and secondary sexual characteristics may provide indirect clues regarding mating strategy in franciscana and are discussed below.

Relative testis weight In mammals, there is a functional relationship between relative testis weight and the species' mating system (Kenagy and Trombulak, 1986). Testes are relatively small in species presenting monogamy or extreme poliginy (several females + few males), i.e., where a male copulates with all females of a group or harem. Comparative studies have demonstrated that males tend to be larger than females and show secondary sexual characteristics in species presenting extreme poliginy. On the other hand, the relative testis weight is high and the sexual size dimorphism is reduced or nonexistent in species where several males copulate with only one estrus female (polyandry). In this case, the evolution for a large testis is attributed to the sperm competition in a system where different males attempt to fertilize the same female and where a higher copulatory frequency and higher levels of sperm production are required (Harcourt et al., 1981; Kenagy and Trombulak, 1986).

Using the data on 133 mammal species, Kenagy and Trombulak (1986) presented a function describing the relationship between body weight and combined-testes weight without epididymis. Applying their equation for the adult male franciscanas, we discovered that mature franciscanas have testes 3 to 12 times lighter than expected (mean=6 times) for a mammal of its body weight. Indeed, among the 133 species analyzed,

the relative testes weight of the franciscana is heavier than that of gorilla (*Gorilla gorilla*), humpback whales (*Megaptera novaeangliae*), and fin whales (*Balaenoptera physalus*), indicating that sperm competition does not occur in franciscanas.

Sexual size dimorphism   Males are larger than females in most mammal species. Nevertheless, the reversed sexual size dimorphism (RSSD) (i.e., females are larger than males) is more common than previously thought and has been documented for 12 out of the 20 orders of mammals (Ralls, 1976, 1977). Among the odontocetes, four (Ziphidae, Pontoporiidae, Phocoenidae, and Delphinidae) out of the eight families present RSSD.

Although sexual selection may be the main reason why males are the larger sex in most mammal species, it has been systematically refused as an explanation in the cases where females are the larger sex (Ralls, 1976, 1977; Andersson, 1994). In species with RSSD, females do not mate with many males, they are not dominant, and are not more aggressive than males of the same species. Moreover, they do not show secondary sexual characteristics associated with intrasexual selection (e.g., horns in Artiodactyla and large canine teeth in Primates). Therefore, the occurrence of RSSD in mammals may be explained more satisfactorily by natural selection (Andersson, 1994).

Slooten (1991) proposed an interesting hypothesis for the occurrence of RSSD in cetaceans, suggesting that a minimum size may be necessary for a newborn cetacean to survive. In odontocetes, the smallest mean sizes at birth are about 70–80 cm. Because the size of the newborn is directly related to the size of the mother, in species of small dimensions the females would suffer a selective pressure to be a larger size, so that they could produce offspring with the minimum viable size. This hypothesis is reinforced by the fact that most of the odontocete species with RSSD (e.g*., Pontoporia blainvillei*, *Cephalorhynchus hectori*, *Cephalorhynchus commerssoni*, *Phocoena phocoena*, *Phocoena sinus*) are the smallest species within the group. Moreover, species presenting RSSD also have larger relative size at birth than the other species within the taxonomic group (Ralls, 1976).

The degree and direction of SSD (sexual size dimorphism) in mammals is the result of the difference of the sum of all selective pressures affecting the female's size and the sum of all selective pressures affecting the male's size (Ralls, 1976). Thus, it is very probable that more than one factor may act selectively on animals of both sexes in *Pontoporia*, molding the degree and direction of SSD. We propose that the requirement of a neonate minimum viable size (70–80 cm in length) is one of the main selective pressures acting on female franciscanas. It is important to emphasize that other factors may also be influencing SSD in franciscana, and in some species it was evident that different selective pressures could affect body size in opposing directions in males and females and in different age classes (Grant, 1986; Andersson, 1994). Among the factors that

may be simultaneously acting on franciscana body size are intrinsic genetic and physiological limitations, and the requirement of maintaining an optimum size for the species' ecological niche.

Secondary sexual characteristics   The presence and intensity of secondary sexual characteristics in males is a more precise indication of the degree of intrasexual selection than is body size (Andersson, 1994). In odontocete males, these characteristics are present in the form of "weapons," such as the tusk of the narwhal (*Monodon monoceros*) and the teeth in species of the genus *Grampus*, *Physeter*, *Berardius*, *Hyperoodon,* and *Mesoplodon* used in male-male combats (MacLeod, 1998). In species of these genus, the teeth were reduced in number, enlarged in size, and their form was modified (specially in males of Ziphiidae). The teeth of these species also lost their function in feeding because of a diet comprising almost exclusively cephalopods and were used uniquely in intrasexual combats. There is no evidence that the same evolutionary process occurred in male franciscanas because their teeth are very small and numerous (around 200), their diet is primarily fish, and the number of combat scars is apparently low. These characteristics support the hypothesis that male-male combat must be very rare or even nonexistent in franciscanas.

The sexual features presented in this study (extremely low testis weight, reversed sexual size dimorphism, absence of secondary sexual characteristics in males, and a low number of scars in males) indicate the absence of sperm competition in the franciscana, and these features differ drastically from those characteristics of odontocete species where males combat each other for copulation. This finding may indicate that franciscanas form temporary reproductive pairs during the reproductive period, where a male pairs and copulate with only one female. Recently, Valsecchi and Zanelatto (2003) provided molecular evidence suggesting that franciscanas may travel in kin groups that include mothers with their calves and the father of the youngest offspring. The authors also suggested that male franciscanas may prolong their bond with their reproductive partner, providing some form of paternal care. For a better understanding of franciscana social structure and mating system, the following suggestions are proposed: 1) an increase in the efforts of behavioral studies of free-ranging franciscanas; 2) quantification of the intraspecific teeth scars in franciscanas of different sexes and reproductive status in order to confirm the absence of intrassexual aggressions among males; 3) investigation of the relationship of relative testis weight, SSD, and reproductive strategies in cetaceans, by phylogenetic methods (see Harvey and Pagel, 1991) to understand the evolution of these characters in this group.

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