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Article

Macroscopic and Histological Descriptive Gonadal Study in Different Stages of Sexual Maturity of the Burmeister's Porpoise *Phocoena spinipinnis* from Peru

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Abstract: The morphology and maturation process of gonads of 70 Burmeister´s porpoises, with body lengths ranging 135–183 cm (n= 34 females) and 64.5-182 cm (n= 36 males) were described. Samples were collected in six ports of central and northern Peru in 1987-1999. In the field, females were classified as immature, mature (resting, lactating, pregnant) and males as immature, pubescent and mature based on, respectively, the presence of ovarian corpora and the relative quantity of semen in cut epididymides. The ovaries of P. spinipinnis are ovoid or bean-shaped and flattened, with corpora modifying surface appearance. In the laboratory, ovaries were examined macro- and microscopically, measured, weighed and sliced in 1-3 mm sections. The number of corpora ovarica (lutea, albicantia, atretica) found in each ovary as well as their macroscopic and microscopic characteristics were documented in some detail. The follicles, their oocytes and nucleus were measured. Follicular development in P. spinipinnis is predominantly left-sided, but occurs in both ovaries in 16.3% of females, mainly in those with numerous corpora. Macroscopically, several corpora atretica with luteinization had characteristics similar to those of corpora albicantia, making microscopic determination essential. Inconclusive evidence of recent ovulation was found in January. Two of three immature females showed good follicular development in March and April. The body length at 50% sexual maturity in females was estimated at 152.7 cm. There was no evidence of reproductive senescence.

The testes of P. spinipinnis are elongated and cylindrical. Of the 36 males examined macroscopically, 7 were immature, 5 pubescent and 24 mature. The histological analysis determining the presence and abundance of Sertoli cells, spermatogonia, spermatocytes, spermatids and spermatozoa, the relative amount of interstitial tissue, the elongation and mean diameter of the seminiferous tubules and the relative size of the lumen allowed to confidently determine sexual maturity status. The field evaluation of maturity based on the presence of sperm in the epididymides is a useful but, in 8.3% of cases, not exact method. For males the body length at 50% sexual maturity was estimated at 157 cm. No histological evidence of male reproductive seasonality was found. Spermatogenesis was perceptible year-round and tubule diameters had non-specific variations for each month.

Keywords: Burmeister's porpoise; reproduction; sexual maturity; testes; ovarian corpora

1. Introduction

The Burmeister´s porpoise *Phocoena spinipinnis* Burmeister, 1865, is probably the most common inshore-living coastal small cetacean in temperate subequatorial waters of South America and particularly in the Southeast Pacific where it is distributed from Bahía de Paita in northern Peru south to Valdivia, Chile (Brownell and Praderi, 1982; Reyes, 2018). Locally known as 'marsopa espinosa' it is the only representative of Phocoenidae found

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in Peruvian waters. This porpoise is also one of the most commonly captured small cetaceans along the Peruvian coast, ranking second in terms of catches by artisanal fisheries (Read *et al.*, 1988; Van Waerebeek and Reyes, 1990, 1994a,b), hence the Peruvian population has long been considered at risk (Culik, 2004; Félix *et al*., 2018; Reyes, 2018). Between 1995 and 1999, from 30 ports monitored in central and northern Peru, 25 had evidence of cetacean exploitation, with the Burmeister´s porpoise occupying the first place (Van Waerebeek *et al*., 1999). Species composition of strandings is a useful proxy for relative removal levels, as the large majority of beach-cast small cetaceans near fishing towns in Peru are thought to be fisheries related. Of a total of 942 specimens (873 identified) of eight cetacean species tallied via beachcombing efforts along the Peruvian coast in the period 2000-2017, 66.3% were *P. spinipinnis* (Van Waerebeek *et al.,* 2018).

The large majority of the published literature on Phocoenidae biology concerns the harbour porpoise *Phocoena phocoena* from the Northern Hemisphere, reflecting major differences in resources allocated to marine mammal research. For instance, merely on the anatomy of the urogenital and reproductive systems of *P. phocoena*, Kastelein *et al.* (1997) listed 15 studies and their summary was far from exhaustive. Also, Fisher and Harrison (1970), Harrison and McBrearty (1973-1974) and Gaskin *et al*. (1984) reported important studies on gonadal morphology and histology in Phocoenidae, primarily *P. phocoena*. Other studies describe the histology of testes for immature, pubertal and mature harbour porpoises, and length at sexual maturity (Sorensen and Kinze, 1994), histological features for resting mature individuals (Learmont *et al*. 2014) and a gonadal study of *P. phocoena* from the North and Baltic Seas which provided basic macroscopic and microscopic data about sexual maturity, gonadal and spermatogenesis/folliculogenesis (Kesselring *et al*., 2018). The most recent study for Phocoenidae described maturity and growth parameters of *P. phocoena* from Japanese waters (Matsui *et al*., 2021).

Research of *Phocoena spinipinnis* is limited and has predominantly focused on its general biology, distribution, strandings, external features, cranial and skeletal morphology, and its conservation status (*e.g.*, Brownell and Praderi, 1982, 1984; Corcuera *et al.* (1995), Goodall *et al.*, 1995; Reyes and Van Waerebeek, 1995; Van Waerebeek *et al*., 2018). A comprehensive molecular genetic (nuclear and mt-DNA) study confirmed significant differences between Pacific and Atlantic populations (Rosa *et al*., 2005). Other aspects studied in Peru include diet (García-Godos *et al*., 2007), parasitology (Sarmiento and Tantaléan, 1991; Reyes and Van Waerebeek, 1995), skeletal, skin and viral diseases (Montes *et al*., 2004; Van Bressem *et al*., 1993, 1998, 2006, 2007a,b) and sightings (Van Waerebeek *et al*., 2002). There is minimal published information on the reproductive parameters of the Atlantic population of *P. spinipinnis*, mostly limited to the body size of small calves and mature animals (Brownell and Praderi, 1982; Goodall *et al*., 1995; Corcuera *et al*., 1995).

A first analysis of the natural history of the Peruvian Burmeister´s porpoise, for the period 1983-1989, still stands as the only published study on reproduction for the species (Reyes and Van Waerebeek, 1995). Average body length at attainment of sexual maturity was estimated at 159.9 cm for males and 154.8 cm for females. The peak of the mating season was observed in the summer (February and March), the gestation period was estimated at 11-12 months and the pregnancy rate was 60%. During that study a surprising 34% of pregnant females were also lactating, suggesting annual reproduction. Length at birth was about 86 cm. The mean weight and length of a mature testis was 300 g and 129 mm (Reyes and Van Waerebeek, 1995).

As *P. spinipinnis* is a highly cryptic species that is infrequently and only briefly sighted at sea (Van Waerebeek *et al*., 2002) there is little opportunity to obtain observations of reproductive behaviour and calves that could help shed further light on reproductive parameters. The main alternative is via direct anatomical studies, including detailed macroscopic and microscopic examinations of reproductive organs. While the presence of ovarian corpora in females can be reliably determined in the field, a precise diagnosis of sexual maturity in males requires histological examination of the testes, which is more accurate than gross examination (Hohn *et al.*, 1985).

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The present study describes the macroscopic and histological characteristics of the ovaries and testes of Burmeister's porpoises of different body sizes and sexual maturity, based on samples collected on the Peruvian coast in the period 1988-1995. The majority of samples were obtained after the 1983-1989 sampling period on which Reyes and Van Waerebeek (1995) was based. Our study yields essential knowledge of the normal, healthy gonad morphology and maturation process as a comparative base for future cross-sectional studies of reproductive biology of *P. spinipinnis,* to recognize pathologic cases, as well as provide a practical diagnostic tool to determine sexual maturity status.

2. Materials and Methods

2.1. Ovarian morphology

Left and right ovaries were collected from 34 female Burmeister'porpoises and preserved in 10% buffered formalin. Details are presented in Table 1. Sexual maturity status of each female was determined macroscopically, under field conditions, based on the presence of minimally one corpus in at least one ovary, a foetus or evidence of lactation (Perrin and Donovan, 1984; Read, 1989; Reyes and Van Waerebeek, 1995). The presence or absence of lactation was determined in the field through external examination (palpation) of mammary glands. During necropsy, both cornua of the bicornuate uterus of porpoises were opened and carefully examined for the presence of small foetuses (Reyes and Van Waerebeek, 1995). In the laboratory the ovaries were examined macro- and microscopically. Each formalin-fixed ovary was examined, photographed, weighed to the nearest 0.1 g and measured (length, width, height) to the nearest 0.05 mm, using a vernier calliper. Preserved ovaries were sliced by hand to obtain 1-3 mm thick sections, connected by the hilar region. The characteristics of the *corpora albicantia*, *lutea, atretica* and follicles were examined in each section. In the section where the *corpus* and the largest follicle had greater dimensions, their thickness (perpendicular to the ovarian epithelium) and their maximum length (parallel to the ovarian epithelium) were measured. The presence of *corpora lutea* and/or *corpora albicantia* were used to define sexual maturity (Sergeant, 1962; Ferrero and Walker, 1993).

For the histological study, selected sections of the ovarian cortex were dehydrated through a graded alcohol series, cleared in xylene, embedded in Paraplast and sectioned at 4μm. The sections were subsequently stained with Harris Hematoxylin and Eosin (H and E) according to Allen (1995). Tissue preparations were examined on glass slides using an optical research microscope. The ovarian structures, including 60 diameters of each follicle type (primordial, primary, secondary, tertiary and Graafian were measured and their oocytes and nucleus). The ovarian *corpora* morphology and condition of *corpora lutea* and *albicantia* (young, medium and old) were described (Marsh and Kasuya, 1984). The different types of atresia, a degenerative process causing oocytes to disappear before ovulation (Geneser, 1997) were described. Terminology for the ovarian morphology follows Perrin and Donovan (1984) and Marsh and Kasuya (1984).

2.2. Testicular morphology

Freshly collected testes of 36 males were examined macroscopically, weighed and measured (n=12) before being preserved in 10% buffered formalin. In the field, sexual maturity was determined if seminal fluid was clearly visible upon cutting the epididymides (Reyes and Van Waerebeek, 1995). Small testes that were not previously measured or weighed had their weights and lengths taken after fixation. Microscopical characteristics of the testes were examined and maturity status (immature, pubertal and mature) was determined in all 36 male porpoises according to Collet and Saint Girons (1984), Hohn *et al*. (1985) and Sorensen and Kinze (1994).

The histological method was equivalent as that described for the ovaries. The presence of Sertoli cells, spermatogonia, spermatocytes, spermatids and spermatozoa indicative of spermatogenesis, as well as the relative quantity of interstitial tissue, lumen size and seminiferous tubules elongation were documented. The interstitial tissue was

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categorized according to the amount of tissue present: little, moderate or abundant. Tubular lumen size was scored as none, small or large. Tubules with no lumen were those that were densely packed with tissue and spermatogonia. Small lumina had less tissue present leaving a small but clear open space in the centre. Large lumina contained essentially no intra-tubular tissue. Seminiferous tubules diameter were measured using an ocular micrometre and taken as the mean of at least 25 representative, circular cross-sections of tubules, for each testis. When most tubules sections appeared subcircular, two diameters were taken, one perpendicular to the other, then averaged. The thickness of the *tunica albuginea* was measured 10-fold for each testis.

2.3. Estimation of body length at attainment of sexual maturity

The body length at attainment of sexual maturity (LSM) at 50% of individuals was calculated by using lineal estimation (DeMaster, 1984; Kasuya and Brownell, 1979; Reyes and Van Waerebeek, 1995) after plotting the mature proportion of females and males versus each length-class of 10 cm.

3. Results

3.1. Females

The standard body lengths of 32 of the 34 females examined (Table 1) varied between 135.0 cm and 183.0 cm ($\bar{X}$ = 163.7 cm, SD = 11.1). The lengths of two females could not be measured. Examination of the gonads revealed that the sample included three immature and 31 mature individuals. Externally, the ovaries of the Burmeister's porpoise are similar to those of other odontocetes with an ovoid, flattened shape and a cream-coloured epithelium that varies in appearance (Fig. 2, 3 and 4) according to the reproductive status and the presence of *corpora*. The length and weight of the ovaries are presented in Table 2.

The internal structure of *P. spinipinnis* ovaries is typical of mammals. A simple cuboidal epithelium covering the ovary and the *tunica albuginea* present under the epithelium are easy to distinguish (Fig. 1A) as well as the cortex with its ovarian stroma between the follicles (Fig. 1B). The medulla sits more centrally (Fig. 1C) and envelopes the *ovarian hilum* where blood vessels and nerves enter the parenchyma. Between the *hilum* and the medulla were irregular tubules lined by a simple columnar epithelium with small lumen called *rete ovarii* (Fig. 1D). Diameters of each follicle, oocyte and oocyte nucleus were measured and are provided in Table 3. Atretic follicles (without luteinization) were found in all females while luteinizing *corpora atretica* were limited to mature specimens only.

3.1.1. Immatures. Of the 34 specimens examined, 3 were immature with body lengths of 135.0, 143.0 and 150.6 cm ($\bar{X}$ = 142.8 cm, SD =7.8). All immatures were collected between March and April.

3.1.1.1 Macroscopic characteristics. The ovaries of the immature specimens were small, ovoid, occasionally bean-shaped, flattened, with a smooth surface (Fig. 2) and weighed between 0.8 and 1.4 g ($\bar{X}$ = 1.11 g, SD = 0.22). Their length ranged from 18.3 to 21.7 mm; the width from 6.8 to 13.9 mm and their thickness from 7.4 to 11.8 mm. In two porpoises the left ovary was larger. *Corpora lutea* or *albicantia* were not observed and follicles were smaller than 3.75 x 2.70 mm.

3.1.1.2 Microscopic characteristics**.** The cortex of the left and right ovaries presented abundant healthy primordial follicles. Follicular development was noticeable in both ovaries, but was greater in the left. Atretic follicles and small fibrous *corpora* were distinguished but no luteinizing *corpora atretica* were found.

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Table 1. Collection data and reproductive status of female Burmeister´s porpoises from Peruvian waters. I (immature), M (mature), P (pregnancy), L (lactation).

Collection data			Reproductive status			
Specimen number	Body length (cm)	Date	I	M	P	L
RBC-029	135.0	27/03/1993	X			
MFB-751	143.0	08/03/1995	X			
MFB-482	149.2	25/01/1994		X	X	
MFB-167	150.6	24/04/1993	X			
KOS-259	152.5	28/11/1993		X	X	
MFB-474	153.5	19/01/1994		X		
MFB-493	153.5	18/02/1994		X	X	
MFB-493	153.5	18/02/1994		X	X	
DMI-148	155.0	23/06/1994		X		
KOS-270	155.5	04/12/1993		X		
JCR-1472	159.0	02/07/1988		X	X	X
MFB-429	159.5	28/11/1993		X	X	X
MFB-162	160.0	22/04/1993		X	X	
KVW-1936	162.0	15/12/1989		X	X	
JAS-043	163.5	01/03/1995		X	X	X
MFB-473	164.0	16/01/1994		X		X
MFB-084	164.5	20/03/1993		X	X	X
SZ-017	165.0	18/04/1990		X		
MFB-130	165.1	15/04/1993		X	X	
MFB-430	165.5	28/11/1993		X	X	
JCR-1815	168.5	20/09/1990		X		X
JAS-050	169.0	31/03/1995		X	X	
MFB-718	170.0	13/08/1994		X	X	
MFB-168	170.3	24/04/1993		X	X	X
RJD-004	172.0	11/11/1995		X	X	
KVW-1931	172.5	15/12/1989		X	X	
JCR-1485	173.0	05/04/1989		X	X	X
MFB-457	173.5	04/12/1993		X		
AGG-090	175.5	21/08/1991		X	X	X
JAS-033	177.0	25/09/1994		X		X
JCR-1628	179.5	22/04/1990		X		X
JCR-1830	179.5	21/09/1990		X	X	
KVW-1958	183.0	18/12/1989		X	X	
JAS-048	-	23/03/1995		X	X	
AGG-755	-	07/08/1993		X		

3.1.2. Matures

The standard body length of 29 of the 31 mature females varied between 149.2 cm and 183.0 cm ($\bar{X}$ = 165.88 cm; SD = 8.99). They were collected at different times of the year.

3.1.2.1 Macroscopic characteristics**.** Externally, the ovaries of the mature females were usually easy to distinguish from immature ones by the presence of a yellowish *corpus luteum* or by one or more *corpora albicantia* visible as white scars on the surface of the ovary (Fig. 3 and 4). The size and weight of the ovaries, as well as their external surface, varied substantially depending on their reproductive state (Table 2). Except for a few cases, the largest follicles were seen in the left ovaries.

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Table 2. Metric data for ovaries of Burmeister´s porpoises from Peruvian waters. Ovary 1 = left, Ovary 2 = right; *: Females without record of ovaries' left-right position. (-) = Without measurement or ovary not collected.

Collection data			Ovary 1				Ovary 2			
Specimen	Total	Date	Weigt	Lengh	Wid	Thicknes	weigt	Lengh	Wid	Thickness
	cm.		g	Mm	mm	mm	g	mm	mm	Mm
*RBC-029	135.0	27/03/93	1.2	21.3	6.8	8.6	0.9	18.8	9.6	7.4
MFB-751	143.0	08/03/95	1.4	21.7	13.9	7.4	1.2	20.7	12.4	57.5
MFB-482	149.2	25/01/94	16.3	39.1	31.6	23.6	0.8	18.8	10.0	8.5
MFB-167	150.6	24/04/93	1.2	20.8	9.6	11.8	0.8	18.3	8.4	10.0
KOS-259	152.5	28/11/93	13.4	32.3	38.8	21.3	1.0	20.0	10.7	7.7
MFB-474	153.5	19/01/94	1.0	21.5	10.9	7.9	3.6	28.5	17.6	14.9
MFB-493	153.5	18/02/94	13.9	38.7	35.2	22.2	1.1	22.8	12.4	8.0
*DMI-148	155.0	23/06/94	13.8	31.1	33.8	24.2	-	-	-	-
KOS-270	155.5	04/12/93	6.0	33.4	23.7	12.5	1.7	24.4	12.4	10.0
JCR-1472	159.0	02/07/88	2.6	19.4	6.3	10.0	-	-	-	-
MFB-429	159.5	28/11/93	12.1	28.3	37.5	20.5	0.6	18.5	9.2	7.0
MFB-162	160.0	22/04/93	19.1	44.0	33.6	25.2	-	-	-	-
KVW-1936	162.0	15/12/89	10.9	44.2	26.0	21.0	1.5	27.5	12.8	8.1
JAS-043	163.5	01/03/95	11.3	42.5	16.4	8.5	-	-	-	-
MFB-473	164.0	16/01/94	13.8	31.6	30.9	20.1	1.1	18.8	10.7	8.8
MFB-084	164.5	20/03/93	11.9	30.0	40.2	18.7	1.0	20.2	11.8	7.5
SZ-017	165.0	18/04/90	4.4	26.9	20.9	13.0	6.3	30.7	19.8	18.4
MFB-130	165.1	15/04/93	16.6	54.0	28.4	22.2	2.4	26.8	14.4	10.7
MFB-430	165.5	28/11/93	10.7	29.3	37.9	15.7	2.8	26.5	17.2	11.2
JCR-1815	168.5	20/09/90	6.6	34.2	24.2	15.7	1.0	21.5	11.8	8.2
JAS-050	169.0	31/03/95	17.4	50.9	27.8	24.9	1.7	22.9	14.2	8.8
MFB-718	170.0	13/08/94	11.6	36.9	25.4	20.8	1.4	27.0	19.4	6.3
MFB-168	170.3	24/04/93	19.8	52.7	30.3	25.5	1.6	25.0	13.3	7.7
RJD-004	172.0	11/11/95	18.8	44.0	34.2	23.2	-	-	-	-
KVW-1931	172.5	15/12/89	17.3	45.5	32.3	20.6	1.0	20.5	11.6	7.9
JCR-1485	173.0	05/04/89	13.9	28.8	40.8	21.4	1.5	25.5	14.4	8.0
MFB-457	173.5	04/12/93	6.3	33.0	23.7	14.0	1.6	20.5	15.0	8.3
*AGG-090	175.5	21/08/91	15.1	51.2	31.4	17.9	1.5	23.3	13.8	7.0
JAS-033	177.0	25/09/94	14.8	29.0	24.8	25.0	1.0	14.9	12.2	8.4
JCR-1628	179.5	22/04/90	4.2	34.3	20.4	10.3	3.0	29.5	15.6	11.0
JCR-1830	179.5	21/09/90	20.6	53.5	28.8	26.5	2.0	23.6	13.8	11.3
KVW-1958	183.0	18/12/89	17.0	50.4	31.9	18.3	1.1	23.3	9.6	10.4
JAS-048	-	23/03/95	9.7	38.8	25.6	19.4	1.0	18.7	10.2	8.9
*AGG-755	-	07/08/93	10.1	38.1	40.2	12.2	-	-	-	-

Table 3. Diameters range (μm) of follicular structures.

Type of follicle	Oocyte	Nucleus of oocyte	Follicle diameter
Primordial follicles	26.3-50.6	12.2-26.3	32.4-64.8
Primary follicles	30.4-68.9	14.2-28.4	46.6-101.3
Secondary follicles	44.6-117.5	20.3-44.6	99.2-184.3
Tertiary follicles	56.7-147.8	24.3-42.5	165-465
Graafian follicles	87.1-170.1	42.5-52.7	1050-3225

Atresia with luteinization resulted in orange-brown irregularly shaped masses, round or fusiform that occurred in the ovary. The smallest of these *corpora* measured 0.95 x 0.75 mm, and the largest 13.10 x 2.65 mm. They were found in 54.8% of the mature individuals but not in the three immatures. Though rarely visible at the ovary surface, they sometimes left very small scars. Among mature females, 17 of 31 presented *corpora atretica* with luteinization (Table 4), with 29 the highest number of such *corpora* found in a left ovary (JAS-033; 177 cm body length). Also, of these 17 mature females, 3 presented *corpora atretica* with luteinization in the right ovary, with a maximum of 4 *corpora* (SZ-017; 165.0 cm body length).

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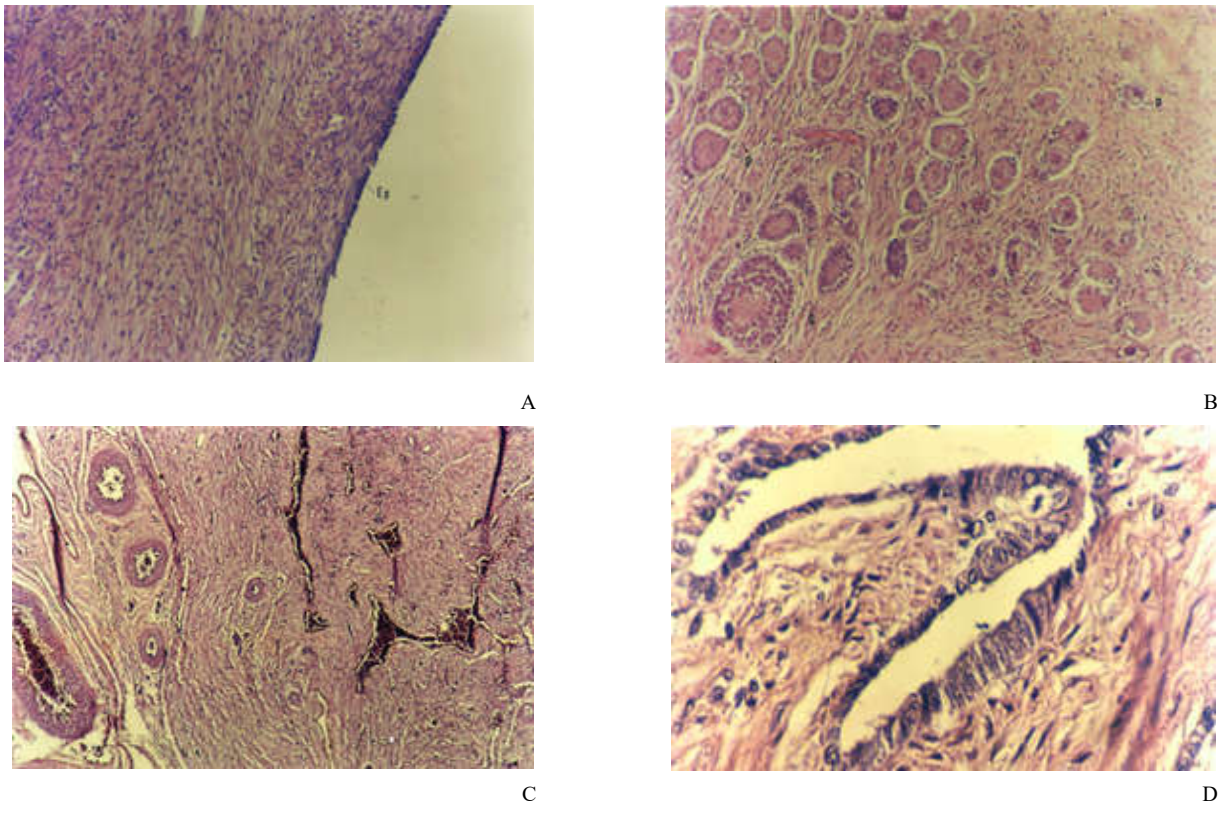

Figure 1. Photomicrographs of ovarian morphology in *Phocoena spinipinnis*. A -The epithelium of the ovary (Ep) and the *tunica albuginea* (TA) (JAS-050, left ovary) appears as a capsule of dense connective tissue, whose collagen fibers are oriented parallel to the surface of the ovary. This tissue is poorly vascularized. 100x magnification. **B -** Ovarian stroma (MFB-167, right ovary), where the primordial follicles (p), primary follicles (P) and secondary follicles (S) can be observed. 100x magnification. **C -** Ovarian medulla (JCR-1472, left ovary). 40x magnification. **D -** Ovary rete (JAS-050, right ovary). It is made up of irregular tubules lined by a simple cylindrical epithelium. The lumen of these tubules is small. 400x magnification.

Figure 2. Ovaries of an immature female *P. spinipinnis*. The small ovaries were ovoid [upper] or bean-shaped [lower], flattened, and with a smooth surface.

Macroscopically, all *corpora lutea* were characterised by a relatively large size (Fig. 3A and B) and by the typical yellowish parenchyma provided by the lutein pigment. Twenty-six females had only one *corpus luteum*, measuring between 11.45 x 8.70 mm to 33.2 x 25.2 mm. In 22 of them, the *corpus luteum* was present in the left ovary while in one female (SZ-017) it occurred in the right ovary and in 3 others the laterality of the ovary was not registered.

In 21 of pregnant females, 20 presented a single *corpus luteum* and one (JCR-1472, with 23 cm foetus) did not, however a corpus luteum was presumably present in its right ovary, which was missing. Its left ovary with only 3 *corpora albicantia* indeed indicated low activity. Maximum dimensions of the *corpora lutea* ranged 28.20 x 15.20 mm to 33.2 x 25.2

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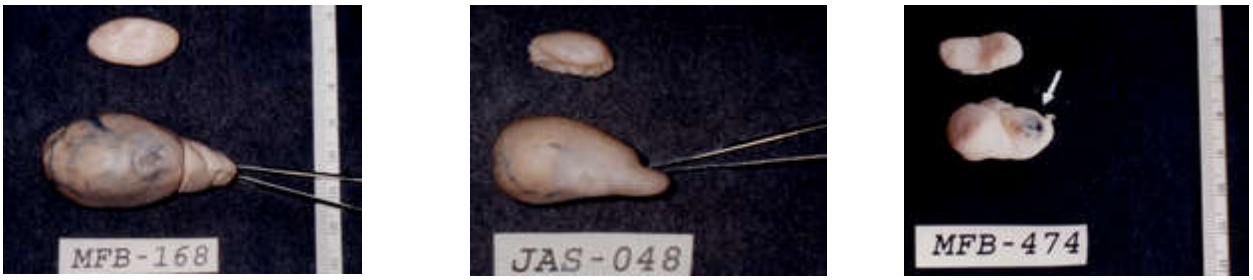
mm. The largest *corpus luteum* belonged to a porpoise 160 cm in length (MFB-162), with a foetus (male, 191 mm) collected in April.



A B C

Figure 3. In *Phocoena spinipinnis,* there is a marked trend of activity in a single ovary (83.87%, 26/31), mainly in the left one (84.62%, 22/26). **A, B -** ovaries (below) where only the left (enlarged) one showed activity; **C** - ovaries of the only animal that showed activity only on the right. Note the hole [arrow] in the right ovary from the recent rupture (ovulation) of a Graafian follicle.

The left ovary of female KOS-270 had 2 *corpora lutea* in involution (Table 4). They measured 4.70 x 3.95 mm and 8.50 x 7.20 mm and showed a wide whitish centre composed of hyaline material. A large, young *corpus albicans was* present in the right ovary. KOS-270 was neither pregnant nor lactating.

Macroscopically, the shape, size and colour of *corpora albicantia* varied widely. Externally they could be recognized as scars left on the ovarian *tunica albuginea*, sometimes accompanied by a soft pigmentation on the surface (Fig. 3 and 4). Despite this, when sectioning, for many ovaries the determined number of *corpora albicantia* increased since some presented barely perceptible scars externally. These corpora were mostly found in the left ovaries (Fig. 3A and B) but some right ovaries had also *corpora albicantia* (Fig. 4, Table 4).

A B C

Figure 4. Activity in both ovaries of *P. spinipinnis*. **A -** Left ovary [below] with numerous *corpora albicantia* scars; **B -** Right ovary [upper] with numerous *corpora albicantia* scars. Left ovary [below] shows a corpus luteum; **C -** Ovaries of female with the highest number of *corpora albicantia* found (n=28) in left [below] and right [upper] ovaries.

Young *corpora albicantia* slightly protruded from the ovarian *tunica albuginea*, leaving a visible scar. They assumed various shapes and were generally pale orange to pale yellow in colour. The smallest young *corpus albicans* measured 3.30 x 1.95 mm and the largest 15.95 x 8.30 mm.

Most mature *corpora albicantia* were not protuberant but appeared as a visible scar. Their colour ranged from a pale orange, pale yellow or cream to whitish. The smallest mature *corpus albicans* measured 1.70 x 0.80 mm and the largest 9.70 x 8.90 mm. Old *corpora albicantia* did not protrude from the surface nor left a visible scar. They were generally light cream coloured and sometimes orange or pale yellow.

Of 31 mature females, 27 had *corpora albicantia,* most of these in the left ovaries (Table 4). Porpoise SZ-017 showed a total of 28 CA in both ovaries combined (Fig. 4C). The highest number of *corpora albicantia* in the left ovary was 24 (MFB-457; 173.5 cm) (Fig. 4A) and

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13 in the right ovary (MFB-430; 165.5 cm) (Fig, 4B). Five of the 27 females presented *corpora albicantia* also in the right ovary and one animal (MFB-474) presented exclusively 2 *corpora albicantia* in the right ovary (Fig. 3C).

Table 4. Total ovarian *corpora* counts and relative quantity of follicles for female Burmeister´s porpoises from Peruvian waters. Ovary 1=left, Ovary 2=right; *=Animals without record of ovaries sidedness. [Open space]= without measurement or ovary absent; CL=*Corpora lutea*: Y=young, M=mature, I=in involution; CA = *Corpora albicantia*: Y=young, M=mature, O = old; CAL = *Corpora atretica* with luteinization. Relative quantity of follicles (pd = primordials; P = primaries; S = secundaries; T = terciaries; G = Graaf): ++++ = abundant; +++ = numerous; ++ = regular; +- = a few; + = rarely; - = not found.

Collection data			Ovary 1							Ovary 2							Relative Quantity of Follicles				
Specimen code	Body length	Date	CL			CA			CAL	CL			CA			CAL					
			Y	M	I	Y	M	O		Y	M	I	Y	M	O		pd	P	S	T	G
*RBC-029	135.0	27/03/93															++++	++	+-	+	-
MFB-751	143.0	08/03/95															++++	+++	+	+	++
MFB-482	149.2	25/01/94		1													+++	++	-	-	-
MFB-167	150.6	24/04/93															++++	++	++	++	+-
KOS-259	152.5	28/11/93		1		1											+++	+++	+	+	-
MFB-474	153.5	19/01/94											1	1			+++	+++	+	+	-
MFB-493	153.5	18/02/94		1													+++	+++	+-	+-	-
*DMI-148	155.0	23/06/94		1		2											+++	++++	+	+-	-
KOS-270	155.5	04/12/93			2	5	2	15	11				1				+	+	+	+	-
JCR-1472	159.0	02/07/88				1	1	1									++++	+-	++	+-	-
MFB-429	159.5	28/11/93		1													+++	+++	+	+-	-
MFB-162	160.0	22/04/93		1													++++	++	+-	+	-
KVW-1936	162.0	15/12/89		1			2										+++	+++	+-	+	-
JAS-043	163.5	01/03/95		1		1	3	2	4								++	++	+	-	-
MFB-473	164.0	16/01/94		1			1										+++	+++	+-	+-	+
MFB-084	164.5	20/03/93		1			5		4								+++	++	-	+	-
SZ-017	165.0	18/04/90				2	4	13	13		1		8		1		+	+	-	-	-
MFB-130	165.1	15/04/93		1			1	6	2								++	++	+-	-	-
MFB-430	165.5	28/11/93		1			2	1						8	5	4	+++	++	-	-	-
JCR-1815	168.5	20/09/90			1		2										++++	+++	+-	+-	-
JAS-050	169.0	31/03/95		1			6	14	6							3	+	+	-	-	-
MFB-718	170.0	13/08/94		1		1	8	1	2								+++	+-	-	-	-
MFB-168	170.3	24/04/93		1			9										++	++	+	+-	-
RJD-004	172.0	11/11/95		1		1	8										++	+	-	-	-
KVW-1931	172.5	15/12/89		1			1		4								+++	+-	-	+-	-
JCR-1485	173.0	05/04/89		1		3	3	1	1								++	++	-	-	-
MFB-457	173.5	04/12/93				3	4	17	2				1	1			+-	+	-	-	-
*AGG-090	175.5	21/08/91		1			5	2	4								+++	++	+	+++	-
JAS-033	177.0	25/09/94		1			6	3	29								+-	+-	+-	-	-
JCR-1628	179.5	22/04/90					7	6							7	2	+	+	+	-	-
JCR-1830	179.5	21/09/90		1			8	2	5								+++	+-	+	-	-
KVW-1958	183.0	18/12/89			1	1	5		4								+-	+	-	-	-
JAS-048		23/03/95		1			1										+++	++	+	+	-
*AGG-755		07/08/93	1				1	9	3								++	+-	-	-	-

Females with a *corpus luteum* or evidence of a recent ovulation (*e.g.* ruptured follicle in right ovary of MFB-474) did not have Graafian follicles or healthy developing follicles. However, some animals (*e.g.*, KVW-1931) with a developed *corpus luteum* had well-preserved developing follicles (mostly secondary ones), with only some slight separations between the granulosa cells, which may indicate slow degeneration of developing follicles.

Seven mature females showed a marked decrease in the number of primordial and Graafian follicles, an increase in fibrous tissue in the cortex, and a higher number of *corpora* (between *lutea, albicantia* and *atretica* with luteinization) except for KVW-1958 (KOS-270, n= 36 *corpora*; SZ-017, n=46; JAS-050, n=27; MFB-457, n=28; JAS-033, n=9; JCR-1628, n=29 and KVW-1958, n=11). In these animals, the relative amount of atretic follicles and fibrous *corpora*, which were located towards the medulla, was notably lower than those with a smaller number of *corpora*.

3.1.2.2 Microscopic characteristics. Ovaries generally presented numerous primordial follicles and maturing follicle atresia. The histological appearance of atresia is highly

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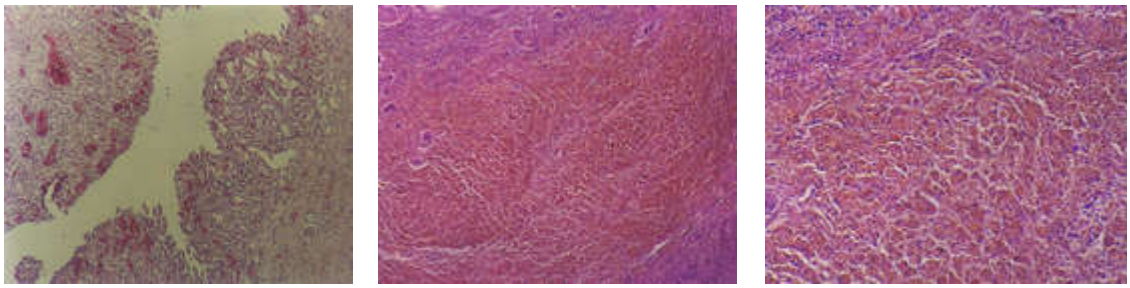
variable depending on the stage of development of the follicle and the advancement stage of the atresia. Atresia could only be evidenced by microscopic analysis. In one individual (MFB-474) collected in mid-January, the right ovary (Fig. 3C) presented 1 follicle that had recently ovulated (5.75 x 8.30 mm),whose walls were folded and clusters of small capillaries and scattered blood (*corpus hemorrhagic*) were evident (Fig. 5A). There were also 5 atretic follicles in the same ovary, which according to histological evidence began their atresia, showing the separation of the granulosa cells from the basement membrane, and theca interna cells also slightly separated from each other (dimensions, in mm, of 5 largest atretic follicles: 6.10 x 4.90; 4.30 x 3.95; 3.90 x 4.40; 4.50 x 2.95; 6.70 x 5.50). In another individual (JCR-1815) its left ovary presented 11 large atretic follicles that macroscopically resembled maturing follicles, presenting numerous flaking granulosa cells towards the antrum (dimensions, in mm, of largest atretic follicles: 2.30 x 4.00; 3.00 x 2.20; 2.40 x 3.30; 2.20 x 1.45; 2.60 x 2.10; 2.45 x 1.85; 3.80 x 2.00; 4.15 x 3.25; 3.05 x 2.60; 4.10 x 3.20; 5.80 x 4.40.

In some *corpora fibrosa* residues of a collapsed zona pellucida were seen inside fibrocollagenous connective tissue. These corpora occurred in various sizes, the largest measuring 1.45 x 1.05 mm. Some ovaries presented apparent follicles that, when examined histologically were in fact follicular cysts, presenting fibrous tissue in place of the follicular cell layer. It was impossible to make this distinction macroscopically as the cysts were usually tiny.

Corpora atretica with thin band shapes (sometimes with tiny ramifications at their ends) or spindle-shaped, showed histologically fibrous connective tissue and, among this, groups of cells similar to the luteal cells of the theca, some still conserved and others already degenerated presenting vacuolated cytoplasm and pyknosis in their nucleus (Fig. 5B and 5C). In some, mostly round, *corpora atretica*, less fibrous connective tissue was seen, as well as granulosa cells, in disorder with luteinization, arranged towards the centre of the *corpus* enclosed by the still visible basal membrane, and around the cells of the theca folliculi with luteinization. Some of the cells of these *corpora* showed signs of atresia. None of the *corpora atretica* with luteinization presented vascularization, some blood vessels were present close-by but were never their own.



A B C

Figure 5. Microphotographs of ovaries in *P. spinipinnis*. **A -** Recently ruptured follicle (5.75 x 8.30 mm) (MFB-474, right ovary). Its walls are folded and clusters of small capillaries and scattered blood can be observed towards the center. 40x magnification. **B -** Luteinized *corpus atreticum* (KOS-270, left ovary, 40x magnification) and **C**- The same luteinized *corpus atreticum* of KOS-270 with 100x magnification.

Microscopically, in the central region of the *corpus luteum* there were large, swollen, pale staining cells (36.5 to 56.7 μm in diameter) with one or two round nuclei (averaging 16.2 μm in diameter), which are known as luteal cells of the granulosa (Fig. 6A and 6C). On the periphery of the *corpus* are smaller cells, of dark staining (Fig. 6C), concentrated mainly along the fibrous septa, known as thecal luteal cells. In the well-formed (mature) *corpora lutea*, a small central area occupied by collagen fibers was present, surrounded by a large area containing the luteal cells of the granulosa, with sparser clusters of thecal luteal cells, scattered in the periphery. Fibrous septa or trabeculae of fibrous connective tissue with good vascularization separated the luteal cell masses from the granulosa cells (Fig. 6B). In the growing (young) *corpora lutea* an empty centre was observed with clusters

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of red blood cells (evidence of recent bleeding) mixed with polymorphs. The walls of the *corpus* were slightly folded and formed by the luteal cells of the granulosa, already forming a thick layer (although these cells were not yet so large or very close), and by the luteal cells of the theca in the periphery. The absence of fibrous septa was also noted (Fig. 6C). The involute *corpora lutea* had a fibrous centre that was still small but larger than the mature ones, the fibrous connective tissue septa thicker than the mature septa, and several smaller luteal cells compared to the mature *corpora*, with several of their cytoplasms and nuclei showing lysis (Fig. 6D).

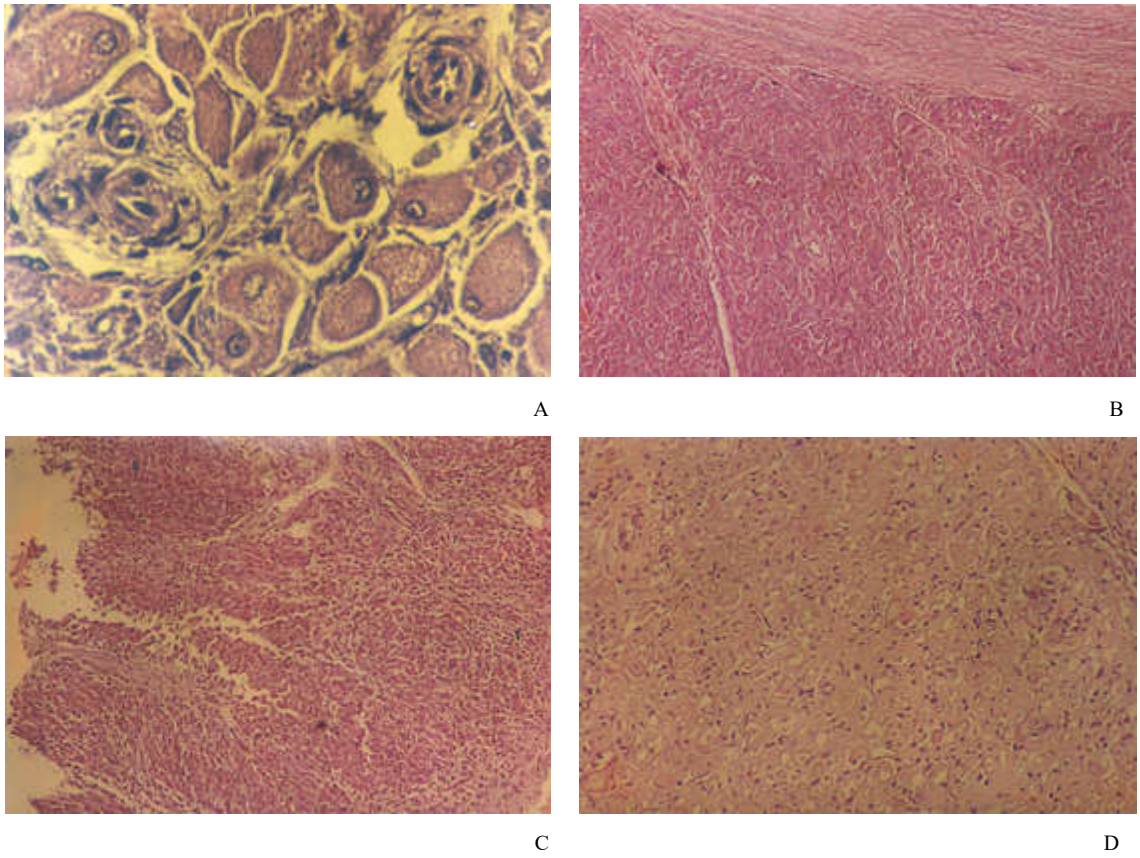

Figure 6. Microphotographs of *corpora lutea* in *P. spinipinnis*. **A** - Granulosa luteal cells (JAS-048, left ovary). 400x magnification. **B** - Mature *corpus luteum* (MFB-482, left ovary). 40x magnification. **C -** Young *corpus luteum* (AGG-755, left ovary). Granulosa lutein cells (G) and small theca lutein cells (T) can be seen. 40x magnification. **D -** Involuting *corpus luteum* (JCR-1815, left ovary). 100x magnification.

Histologically, in the young *corpus albicans* the trabecular arrangement of the anterior corpus *luteum* was still recognizable. Towards the centre there were clusters of scattered fibres, the luteal cells of the granulosa were replaced by clusters of fibrous cellular tissue, showing numerous fibroblasts. Among these clusters of tissue some degenerating luteal cells of the granulosa were still visible and small groups in the periphery of luteal cells of theca also in degeneration. The peripheral limits of the corpus were clearly visible, presenting vascularization mainly in this area (Fig. 7A).

The connective tissue of the mature *corpus albicans* was less abundant than in the young *corpora albicantia*, showing clusters of blood vessels of different sizes, scattered throughout the *corpus.* In a few mature *corpora,* towards the periphery, they presented residues of luteal cells, the trabeculae were less visible but their peripheral limits were still perceptible (Fig. 7B).

In the old *corpora albicantia* there was little connective tissue while thick-walled blood vessels of various sizes formed lumps or compact groups. Trabeculae were not seen and

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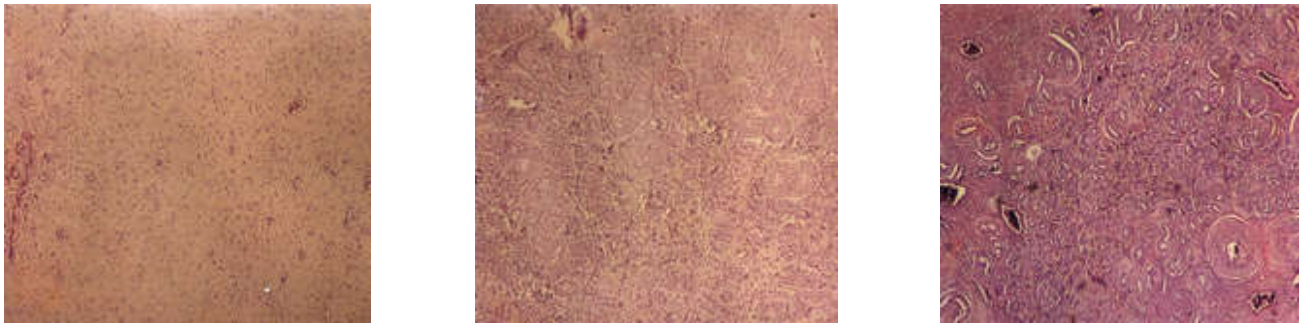
the limits of their periphery were difficult to distinguish (Fig. 7C). The smallest old *corpus albicans* measured 1.00 x 1.90 mm and the largest 5.20 x 8.30 mm.



A B C

Figure 7. Microphotographs of *corpora albicantia* in *P. spinipinnis.* **A-**Young *corpus albicans* (AGG-755, left ovary). 40x magnification. **B-**Mature *corpus albicans* (MFB-718, left ovary). 40x magnification. **C -** Old *corpus albicans* (JCR-1472, left ovary). 40x magnification.

Some of the *corpora albicantia* in all these three categories presented a central area with lipid-filled cells interspersed with connective tissue, very similar to the luteal cells of the theca, and many of these cells were lysed.

3.2. Males

The testes of 36 specimens with standard body length varying between 122.0 and 182.0 cm, and a 64.5 cm foetus, were examined. Among these, 7 were immatures, 5 pubescents and 24 matures. Their lengths are presented in Table 5. The mean length of sexually mature males was 166.39 cm (SD= 10.40; n=24)

As in other odontocetes (Slijper, 1966) the testes of Burmeister's porpoises are elongated and cylindrical, with an external smooth, shiny and cream-colored surface (*tunica albuginea*). The internal structure of the porpoise testes is typical of mammals. Connective tissue includes the capsule or *tunica albuginea*, the trabeculae, a loose connective tissue of support between the seminiferous tubules (which include dispersed Leydig's cells whith a polygonal shape of about 12.15 x 16.20 μm in diameter) and the mediastinum testis.

The seminiferous tubules had a thin wall (*tunica propia*) and intratubular cells (Fig. 9B). The average diameter of the seminiferous tubules for each animal is recorded in Table 5. The *tunica propia* (Fig. 9B) showed a layer of fibroblasts, which did not form a continuous covering, and fibres surrounding the basal membrane or lamina. The intratubular cells included Sertoli cells and germ cells (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids). Three stages were seen: the young spermatids that have spherical and central nuclei with little pigmentation (Fig. 9B); mature spermatids, with round nuclei that are strongly pigmented (Fig. 9B); and spermatids close to turn into spermatozoa were elongated, with a fusiform nucleus, a small flagellum still within their cytoplasm, and attached to Sertoli cells. The intratesticular spermatic ducts included the straight tubule and finally in the mediastinum testis these tubules form the rete testis.

3.2.1. Immatures

Of the 36 males examined, 7 were immature with a standard body length varying between 122.0 and 144.0 cm. ($\bar{X}$ = 132.4 cm; SD = 7.8; n=6) excluding the foetus.

3.2.1.1. Macroscopic characteristics. Testes weighed without epididymis 3.0 to 8.7 g. ($\bar{X}$ = 5.16 g; SD = 1.7; n = 11) and were between 29.60 to 45.00 mm long; 11.90 to 20.60 mm broad and 10.80 to 17.50 mm thick (Table 3, fig. 8A). The testes of a 64.5 cm fetus weighed 0.5 and 0.4 g and measured 18.0 x 6.5 x 7.8 mm and 16.9 x 6.7 x 7.0 mm.

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Table 5. Metric data for male Burmeister's porpoises (*P. spinipinnis*) studied. Code a=testis 1 is the left and testis 2 is the right; code b= testis 1 is the left, testis 2 is the right, but for the average diameter of tubules and thickness of the *tunica albuginea*, its sidedness was not determined; code c = it was not known which was the left and which was the right and the average diameter of the tubules and thickness of the *tunica albuginea* could not be identified whether they belonged to gonads 1 or 2. Maturity stage (field observation): IM = Immature; MA = Mature; ND = not determined; IM? = Probably Immature, MA? = Probably mature. ** Maturity stage by histology: IM = Immature; PB = Pubescent; MA = Mature.

Collection data				Testis 1						Testis 2						
Code	Total Length	Date	Maturity stage at a glance*	Weigth	Length	Wide	Thickness	Mean tubule diameter	Tunica albuginea thickness	Weigth	Length	Wide	Thickness	Mean tubule diameter	Tunica albuginea thickness	Maturity stage by histology**
	cm.			g	mm	mm	mm	µm	µm	g	mm	mm	mm	µm	µm	
AGG-090	64.5	21/08/91	IM	0.5	18.0	6.5	7.8	68.1	164.0	0.4	16.9	6.7	7.0	71.0	164.0	IM
AGG-033	122.0	12/03/91	IM	4.9	36.9	19.0	10.8	60.6	180.0	4.4	37.4	16.2	12.8	56.8	204.0	IM
AGG-135	129.0	24/08/91	IM	4.2	37.9	15.0	12.7	56.0	155.0	3.4	38.1	11.9	12.2	56.1	168.0	IM
JAS-046	130.0	09/03/95	IM	3.1	29.65	13.3	12.20	54.2	189.0	3.0	29.60	13.20	12.05	51.0	198.0	IM
AGG-034	130.5	12/03/91	IM	6.4	38.9	19.0	14.4	51.7	150.0	5.8	39.1	17.5	15.3	50.6	194.0	IM
JCR - 903[a]	139.0	16/01/87	IM?	8.7	44.2	20.6	17.2	70.5	267.0	-	-	-	-	-	-	IM
JAS-145[a]	142.0	17/06/99	IM?	13.1	50.3	21.3	20.90	82.5	240.0	12.6	51.10	20.90	19.90	76.9	198.0	PB
AGG-037[a]	143.5	13/03/91	ND	13.2	53.1	22.2	15.5	73.6	214.0	-	-	-	-	-	-	MA
AGG-129	144.0	23/08/91	IM	6.5	45.0	17.4	17.5	61.8	188.0	6.4	42.2	17.5	15.5	53.8	180.0	IM
AGG-726	150.0	02/02/93	IM?	-	-	-	-	67.9	306.0	-	-	-	-	-	-	PB
MFB-528[c]	151.5	26/05/94	IM	78.5	96.0	44.0	22.0	158.8	730.0	85.5	95.0	49.0	30.0	-	-	MA
AGG-031[a]	152.0	12/03/91	ND	10.5	46.5	20.0	18.7	60.7	299.0	10.3	50.0	17.6	18.6	63.4	290.0	PB
AGG-049	152.0	22/03/91	ND	-	-	-	-	200.5	-	-	-	-	-	-	-	MA
AGG-081	153.0	21/08/91	IM	7.9	42.5	16.5	18.2	70.5	270.0	8.3	43.5	18.0	19.6	68.8	269.0	PB
JCR-1587	154.0	16/09/89	IM	-	-	-	-	71.9	298.0	-	-	-	-	-	-	PB
JCR-1932	155.0	20/03/91	MA?	10.4	50.6	21.5	17.8	81.8	219.0	-	-	-	-	-	-	MA
MFB-495[b]	155.5	19/02/94	MA?	109	100.0	49.0	32.0	178.9	742.5	119	101.0	48.0	34.0	-	-	MA
AGG-055	157.0	24/06/91	MA?	-	-	-	-	212.9	-	-	-	-	-	-	-	MA
MFB-181	159.1	29/04/93	MA	-	-	-	-	195.4	-	-	-	-	-	-	-	MA
JCR-1630	160.0	25/04/90	MA	-	-	-	-	203.9	-	-	-	-	-	-	-	MA
DMI-019[a]	160.0	24/03/94	ND	-	-	-	-	177.6	580.0	-	-	-	-	176.5	582.5	MA
MFB-526[b]	164.0	22/05/94	MA	250	135.0	65.0	39.0	214.7	-	217	132.0	63.0	41.0	-	-	MA
AGG-609	164.5	12/09/92	IM?	-	-	-	-	154.9	-	-	-	-	-	-	-	MA
AGG-134	171.0	24/08/91	MA	-	-	-	-	348.6	-	-	-	-	-	-	-	MA
JAM-002[a]	171.0	05/11/97	MA?	230	130.0	70.0	40.0	212.9	682.5	230	137.0	71.0	37.0	199.3	750.0	MA
MFB-672[b]	171.5	29/06/94	MA	205	122.0	68.0	40.0	223.9	-	170	122.0	60.0	35.0	-	-	MA
JCR-1792	172.0	23/08/90	ND	-	-	-	-	251.8	-	-	-	-	-	-	-	MA
MFB-161[b]	172.6	21/04/93	MA	-	144.0	-	-	265.5	785.0	-	144.0	-	-	-	-	MA
MFB-145[c]	173.1	16/04/93	MA	350	153.0	64.0	-	220.5	772.5	350	146.0	67.0	-	-	-	MA
MFB-461[b]	173.5	08/12/93	MA	235	132.0	63.0	45.0	220.9	815.0	198	125.0	59.0	36.0	-	-	MA
KVW-2438[a]	174.0	25/01/98	MA	108	93.4	38.0	53.7	159.2	515.0	111	96.5	47.0	46.6	163.7	612.5	MA
JCR-1627	175.0	22/04/90	MA	-	-	-	-	251.5	-	-	-	-	-	-	-	MA
KVW2427[b]	177.0	08/11/95	MA	-	-	-	-	200.8	610.0	-	-	-	-	-	-	MA
MFB-459[c]	178.5	08/12/93	MA	321	152.0	68.0	45.0	227.1	782.5	316	146.0	65.0	50.0	-	-	MA
JCR-1814[b]	180.0	20/09/90	MA	231	130.0	65.0	38.0	238.5	-	231	130.0	67.0	38.0	-	-	MA
JCR-1578	182.0	26/08/89	MA	-	-	-	-	215.4	662.5	-	-	-	-	-	-	MA

3.2.1.2. Microscopic characteristics. The thickness of the *tunica albuginea* of the six males measured 150.0 to 267.0 µm ($\bar{X}$ = 188.45 µm; SD= 31.1; n=11) and the seminiferous tubules were organized in numerous lobes, densely packed, with imperceptible elongation and circular in cross-section. The seminiferous tubules were narrow (mean diameter = 56.67 µm; SD = 5.8; n = 11; range 50.67 - 70.46 µm), embedded in the abundant interstitial tissue. A regular layer of Sertoli cells was found lining the tubules and a few centrally located spermatogonia or interspersed with Sertoli cells. Other germ cell states were not observed. The lumen was imperceptible (Fig. 8B). In the foetus the average thickness of the *tunica albuginea* was 164.0 µm, the mean diameter of the seminiferous tubules was 69.55 µm and the other histological characteristics were similar to those described in the six immature males.

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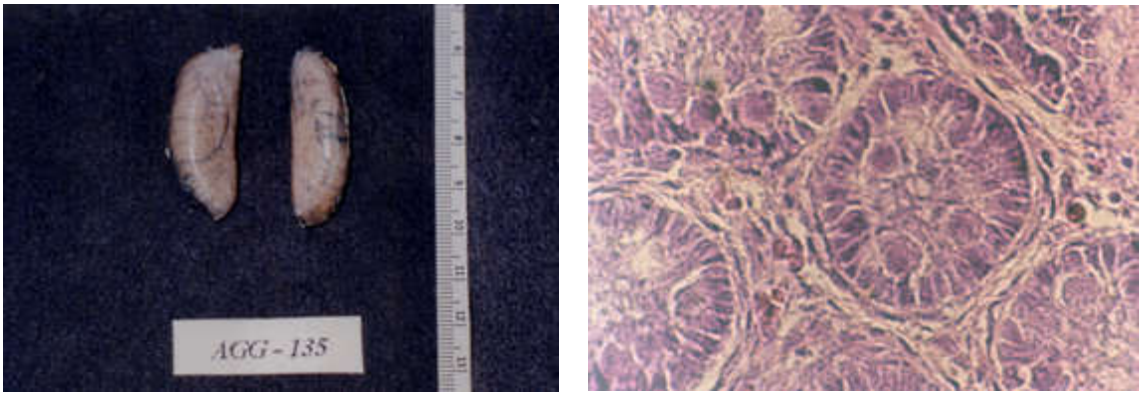

A B

Figure 8. Immature testes of Burmeister' porpoise. **A –** Testes (AGG-135) are elongated and cylindrical, with an external smooth, shiny and cream-colored surface. **B -** Testicular tissue microphotograph of AGG-135, testis 2. 400x magnification.

3.2.2. Pubescent

The standard body length of males which were considered pubescent in the field ranged from 142.0 to 154.0 cm ($\bar{X}$ = 150.2 cm; SD = 4.8; n = 5).

3.2.2.1 Macroscopic characteristics. When examined in the field, researchers typically described the maturity of pubescent males as 'indetermined' or 'immature', due to the paucity or absence of semen in the epididymides. The testes of the 5 pubescent porpoises were elongated and cylindrical like the immature ones. The length of the testes ranged from 42.50 to 51.10 mm, the width from 16.50 to 21.30 mm and the thickness from 18.20 to 20.90 mm (Table 3). The testes weighed without epididymis 7.9 to 13.1 g ($\bar{X}$ = 10.45 g; SD = 2.13; n = 6).

3.2.2.2 Microscopic characteristics. The thickness of the tunica albuginea ranged from 198.0 to 306.0 μm ($\bar{X}$ = 271.25 μm; SD = 36.6; n = 8). Most of the seminiferous tubules were packed and imperceptibly elongated, showing circular cross-sections (Fig. 9A). In some areas of the testis (mostly in the periphery) the tubules were looser, with some elongation and were ovoid, somewhat flattened, in cross-section. The tubules were still small in diameter ($\bar{X}$ = 70.35 μm; SD = 6.9; n = 8; range = 60.71 to 82.46 μm) and were immersed in moderate to little interstitial tissue (Table 5).

Sertoli cells were regularly distributed and alternated with a regular number of spermatogonia. The tubular lumen was mostly medium-sized, empty and well-defined. Small groups of primary spermatocytes were also found in some areas of the testis, their number was markedly increased towards the periphery of the testis.

In a single male (AGG-726) of 150.0 cm, only a few Sertoli cells, abundant spermatogonia, a regular number of primary and secondary spermatocytes, few spermatids and 4 spermatozoa were observed in a peripheral tubule. This was still considered pubertal because all the other tubules presented the histological characteristics of puberty (Collet and Saint Girons, 1984; Hohn et al., 1985; Sorensen and Kinze, 1994).

3.2.3. Matures

The sample included 24 mature specimens with SBL varying between 143.5 and 182.0 cm. ($\bar{X}$ = 166.39 cm; SD = 10.4).

3.2.3.1. Macroscopic characteristics. The adult testes weighed without epididymis from as low as 10.4 g (JCR-1932, 155cm, few semen) to 350.0 g ($\bar{X}$ = 189.49 g; SD = 99.82 ; n = 22). Their length ranged from 50.6 to 350.0 mm ($\bar{X}$ = 119.4 mm; SD = 28.17; n = 24), the width from 21.5 to 146.0 mm ($\bar{X}$ = 56.08 mm; SD = 14.52; n = 22) and thickness from 15.5 to 71.0 mm ($\bar{X}$ = 36.78 mm; SD = 9.87; n = 20) (Table 5).

3.2.3.2. Microscopic characteristics. The thickness of the tunica albuginea ranged 214.0 to 815.0 μm ($\bar{X}$ = 628.47 μm; SD = 183.17; n = 27). Most of the tubules presented

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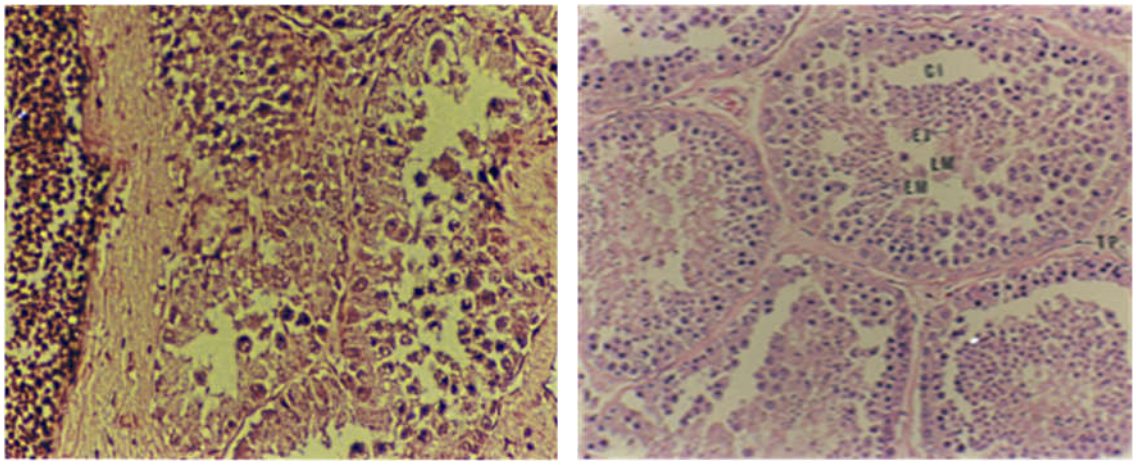
extensive elongation, few showed less elongation, in cross-sections they were mostly ovoid or subcircular, were flattened and several had wavy walls. The tubules ranged in diameter from 73.66 to 348.63 μm ($\bar{X}$ = 201.10 μm; SD = 53.4; n = 27) and there was little interstitial tissue between them.

Few Sertoli cells were seen in the tubules in proportion to the germ cells present. The cytoplasm of Sertoli cells was irregular in shape. All stages of spermatogenesis cells were present, being in sequential order by the stage of development from the basement membrane of the tubule to its lumen, which was large in most mature animals, with free spermatozoa observed in it (Fig. 9B). The relative proportion of intratubular cells varied from individual to individual and often even within the same testis.



A B

Figure 9. Microphotographs of testes of *P. spinipinnis*. **A -** Testicular tissue (from the periphery) of pubescent (AGG-726), where groups of primary spermatocytes can be observed. 100x magnification. **B -** Testicular tissue of mature male (MFB-161). The seminiferous tubules' wall is made up of a thin layer of connective tissue: *tunica propia* (TP) and a stratified epithelium resting on a basement membrane. Young spermatids (EJ) and mature spermatids (EM) are seen towards the lumen (LM). 100x magnification.

The determination of the sexual maturity status in males at the macroscopic level (presence of a perceptible quantity of semen in at least one epididymis) occasionally contrasted with the subsequent determination at the histological level. In 20/36 cases (55.5%) the results coincided; in 3/36 (8.3%) macroscopic examination led to an incorrect conclusion; 8/36 (22.2%) yielded doubtful results (especially in pubescent males) and in 5/36 of cases (13.8%) maturity status had not been determined macroscopically, mainly due to a lack of time at sample collection. This indicates that a histological analysis is significantly more accurate and that it can clarify the doubtful results that often were seen with gross examinations made under challenging field conditions.

3.3. Body length at sexual maturity

In this sample, 50% of females (n=29) had attained sexual maturity at an estimated length of 152.7 cm (Fig. 10). The largest sexually immature female was 150.6 cm while the smallest mature female measured 149.2 cm. Two of the three immature females showed good follicular development when they were captured (April 1993 and March 1995), suggesting upcoming ovulation at first time. These porpoises measured on average 146.8 cm.

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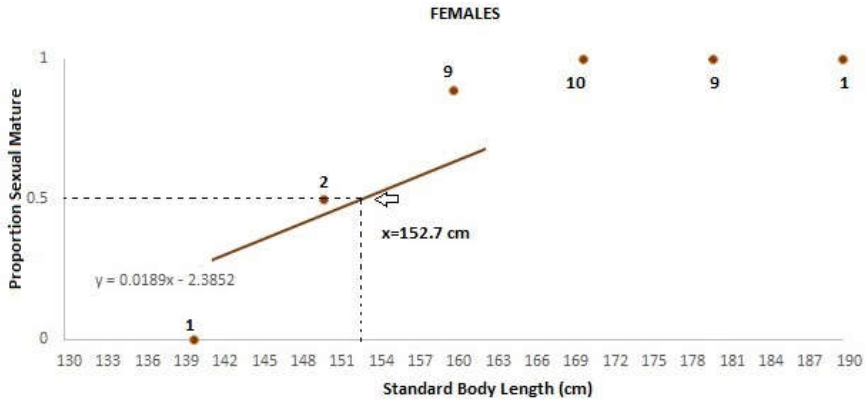

Figure 10. Proportion of sexually mature females according to body length and determination of the LSM *P*(0.50) by linear adjustment, showing trend line and equation.

In 24 males we estimated the body length at 50% sexual maturity at 157 cm (Fig. 11). The largest immature male measured 144.0 cm and the smallest mature male was 143.5 cm. The smallest male identified as pubescent in the field measured 142.0 cm, the largest 154.0 cm. Four smaller mature males (body lengths, in cm, 143.5; 151.5; 152.0 and 155.0) whose histological characteristics were not entirely like those of the larger mature males nonetheless showed all the stages of spermatogenesis, but mainly in peripheral tubules. These animals were considered as having just reached sexual maturity. Their sizes were similar to the values seen in pubescent males. All individuals larger than 155.5 cm presented full testicular activity with macroscopic and histological characteristics well differentiated from the smaller males.

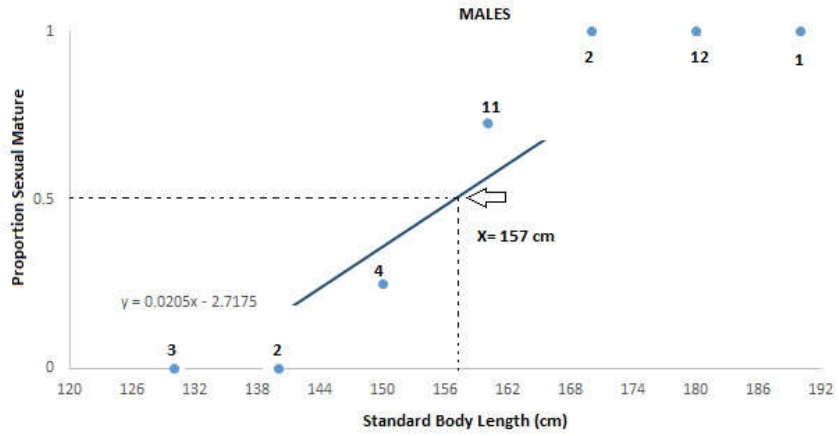

Figure 11. Proportion of sexually mature males according to body length and determination of the LSM *P*(0.50) by linear adjustment, showing trend line and equation.

4. Discussion

In 26/31 of Burmeister's porpoise females (83.9%) there was a marked trend of activity in a single ovary, mainly in the left (22/26, 84.6%), indicating that for some reason one ovary was slower in its follicular development than the other. But remarkably, in 16.13% of mature females both ovaries were active, mainly in those specimens with numerous *corpora*. This phenomenon may be related to the high reproductive need of a species that lives in the highly unstable ENSO habitat (El Niño-Southern Oscillation) (Fiedler, 2018) and/or to the adaptation to an unusually high mortality rate in Peruvian coastal fisheries for decades, at least since 1985, as mentioned above.

High activity in both ovaries ensure a high pregnancy rate which was estimated at 60% (Reyes and Van Waerebeek, 1995). Thus, in *P. spinipinnis* ovarian activity (oocyte release) is not limited to the left ovary, in contrast with *Phocoenoides dalli* in Japanese waters (Kasuya, 1978). For *P. phocoena*, Fisher and Harrison (1970) reported only one specimen

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with a *corpus albicans* in the right ovary, while Sorensen and Kinze (1994) in Denmark found a single female with 4 *corpora albicantia* in the right ovary. In the vaquita *Phocoena sinus* Hohn *et al.* (1996) described activity in the right ovary of an old female with numerous *corpora* in both ovaries. But those are exceptions as in most porpoises (Phocoenidae) activity is limited to a single ovary, most often the left (Gaskin *et al.*, 1984).

Because of the limited number of specimens, the fertile periods could not be confirmed histologically. However, evidence of recent ovulation was found in one specimen (MFB-474) in January, as evidenced by the presence of an haemorrhagic *corpus.* Besides, two of the three immature females showed good follicular development when they were captured (April 1993 and March 1995), suggesting upcoming first-time ovulation. These porpoises measured on average 146.8 cm a size very close to that of the smallest mature individual with a single *corpus luteum* (149.2 cm).

In this sample the LSM p(0.50) at 152.7 cm is smaller than the length at which 50% of females attained sexual maturity, estimated as 154.8 cm by Reyes and Van Waerebeek (1995) based on specimens captured in earlier years (1983-1989). No estimates are available for other populations of *P. spinipinnis* to compare with, but these values are surprisingly similar to the estimated LSM *P*(0.50) = 154.4 cm reported for female *P. phocoena* from Japanese waters (Matsui *et al*., 2021). Larger female asymptotic lengths were recorded for *P. phocoena* from the waters of Great Britain, the Bay of Fundy, West Greenland and Denmark, implying that female LSMs are also larger in these regions (Matsui *et al*. 2021).

It is quite possible that the body length (and age) at first ovulation in Burmeister's porpoise has decreased since the first sampling period (Reyes and Van Waerebeek, 1995) which would support the hypothesis of a density-dependent response effect as a result of the intense exploitation and the suspected reduction in the size of the population of *P. spinipinnis* in Peru (Reyes, 2018). Such an effect has been well-documented in commercially hunted whale populations (Wade, 2018).

Although several studies have been published on overexploitation in small cetaceans, only a few reports the occurrence of variation in the age and length at sexual maturity (Chávez, 1998). In Eastern Tropical Pacific populations of *Stenella longirostris* and *S. attenuata* that suffered high levels of mortality in tuna fisheries, several authors (Perrin and Henderson, 1984; Kasuya, 1976; Chivers and Myrick, 1993) found a decline in the average age and length at sexual maturity and suggested that this was a consequence of their population decrease. The mean body length at attainment of sexual maturity in the Peruvian dusky dolphin *Lagenorhynchus obscurus*, an odontocete that occupied the first place in previous years in terms of catches (Van Waerebeek and Reyes, 1994), is lower than that estimated for other populations of this species (Chávez, 1998; Van Waerebeek and Read, 1994). A gradual long-term decline of the prevalence of dusky dolphins in by-catches and strandings in Peru is indicative for the high pressure on the population (Van Waerebeek, 1994; Van Waerebeek et al., 2018).

Fisher and Harrison (1970) suggested that a *corpus albicans* derived from a pregnancy *corpus luteum* can be differentiated from a simple ovulation *corpus luteum* in phocoenids by histological examination. But in the present study, there was no histological evidence to support this. Only two *corpora albicantia* of the same state could be differentiated microscopically by the presence of pigmentation, which may have the same origin as that which occurs in *corpora atretica* with luteinization, due to destruction of luteal cells without this evidencing pregnancy or simple ovulation. Gaskin *et al*. (1984) also concluded that none of the *corpora albicantia* types can be specifically related to pregnancy or ovulation in *P. phocoena*.

The size of the *corpora albicantia* is not indicative to determine its status, as a mature *corpus albicans* can have the same dimensions as a young one, or be larger than a young one and smaller than an old one. This overlapping of sizes is due to the size of the *corpus luteum* that gave it its origin and although it is well known that a *corpus luteum* of pregnancy is larger than one of simple ovulation (Ivashin, 1984), aborted pregnancies would also generate numerous size variants. Thus the size of a *corpus albicans* is not indicative for its origin, whether from pregnancy or simple ovulation.

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A mature female (KOS-270) that was not pregnant nor lactating had two *corpora lutea* of different sizes in involution that looked very similar histologically. They had a large centre composed of hyaline material. Both were much smaller than the involuting *corpora lutea* observed in other females, suggesting the occurrence of two parallel ovulations, one of them with successful fertilization (the largest *corpus)* and an early abortion. The issue of multiple ovulations in phocoenids has not been satisfactorily resolved (Gaskin *et al*., 1984), and speculations were based on the presence of numerous *corpora albicantia* in very young females. In this study, on several occasions, the majority of *corpora albicantia* in the same state within an ovary had little difference in size, which could indicate parallel or close ovulations. Similarly, Harrison and McBrearty (1973-74) indicated that due to this characteristic, not all ovulations were related to successful pregnancies.

We have found a higher number of *corpora albicantia* (28 in a single individual) in *Phocoena spinipinnis* than in any other phocoenid, such as *P. phocoena* (maximum 16) (Sorensen and Kinze, 1994) and *Neophocaena phocaenoides* (maximum 11) (Harrison and McBrearty, 1973-74), which is consistent with the higher ovulation activity in both ovaries of *P. spinipinnis* compared to other porpoise species. Again, we suggest this to be an adaptation to high mortality, both anthropogenic and natural.

Seven mature females presented a notable decrease in the number of follicles, an increase in fibrous tissue in the cortex, a high number of *corpora* (*lutea, albicantia*, and atretic with luteinization), but a relatively lower number of atretic follicles and fibrous *corpora*. From the high number of *corpora albicantia,* it can be deduced that these females had a high number of ovulations and that they were older than the others. As expected, the reserve of primordial follicles was strongly diminished and therefore the developing follicles as well. However there was no evidence of reproductive senescence. The reduced relative amount of atretic follicles and fibrous *corpora* in these females, compared to the high relative amount of them in the younger females, suggests that throughout fertile life of the older females these have been reabsorbed until disappearing. This, we suggest, does not happen to the *corpora atretica* with luteinization which are more numerous in these seven mature females, and are thought to be permanent like the *corpora albicantia.*

Though the greatest diameters of seminiferous tubules occurred in April and August (Fig. 12), there are no convincing indications of a male reproductive seasonality. Spermatogenesis was perceptible year-round and tubule diameters had non-specific variations for each month. This result is consistent with Reyes and Van Waerebeek (1995) who did not observe significant seasonal variation in the mean testis weight of *P. spinipinnis,* while admitting that sample size was small. Thus, in contrast with other porpoises (Robeck and O'Brien, 2018) which exhibit a discrete male reproductive seasonality (whereby sperm production is limited to a 2-3 month annual period), in Peruvian *P. spinipinnis* it is diffuse with year-round production of mature spermatozoa and an apparent peak in late summer. This evidently favors a maximum reproductive potential and fertilization probability.

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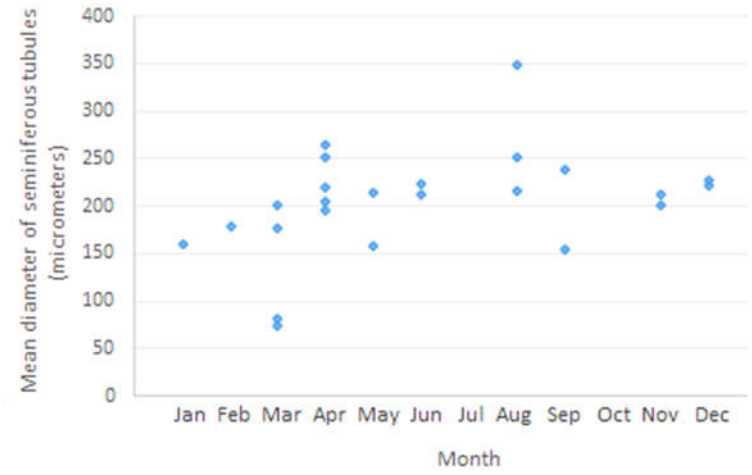

Figure 12. Mean diameter of seminiferous tubules versus collection month for 24 mature Burmeister's porpoises. Though the highest tubule diameters occurred in April and August, no clear seasonality was perceptible.

Two of the immature females were about to ovulate in March, if this coincided with the highest tubule diameters of males in April, fertility could have a maximum peak between these two months. But our data also indicate ovulation in January and as spermatogenesis is continuous, fertilizations could occur at any time of the year. Reyes and Van Waerebeek (1995) found a highly significant correlation ($p<0.0001$) between month of the year and size of foetuses and small neonates ($n=34$) indicating that the peak of the mating season and parturition occurred during summer. Gestation period was estimated at 11-12 months and the smallest neonates were seen in February (85.5 cm) and March (93 cm). Three outliers confirmed that at least some successful mating occurs out of the main season (Reyes and Van Waerebeek, 1995).

The body length at attainment of sexual maturity in males of 157 cm is less than the estimated 159.9 cm by Reyes and Van Waerebeek (1995) however that study was not based on histological observations. The macroscopic determination of male sexual maturity based on the presence of sperm in the epididymis, as practiced in the field, is a useful but occasionally (in 8.3% of cases) not exact method. For males with well-differentiated macroscopic gonadal characteristics, such as an appreciable quantity of sperm and large testes size and weight, classification was not problematic, but in pubescent individuals it was. In the present study, among four males that had just reached sexual maturity (histologically) only one had been considered 'possibly' mature in the field. One was considered immature and the other two were labelled as 'of unknown sexual maturity'. Besides, five other pubertal individuals were only recognized as such microscopically. These results can help future field researchers to more confidently classify small male porpoises with small testes and low quantities of semen in epididymides as pubescent.

Based on histological analysis Learmont *et al.* (2014) classified the males of *P. phocoena* of Scottish waters as immature, pubescent, active mature and resting mature (numerous sertoli cells, few germinal cells and various sizes seminiferous tubules). They had body lengths for immatures (84-130 cm), pubescents (119-153 cm), active matures (135-157 cm) and resting matures (116-160 cm), however these ranges were wide and greatly overlapping. We did not find similar histological characteristics as those reported in the study by Learmont *et al.* (2014) to characterize resting mature individuals; in our study the spermatogenesis was continuous. Kesselring *et al.* (2017) reported that various stages of spermatogenesis can be found in one tubular cross section during the mating season. We did not observe significant seasonal variation in the presence of germ cells in Peruvian *P. spinipinnis*.

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