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Macroscopic and histologic characteristics of sexual maturation in 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 *Phocoena spinipinnis*, with body lengths ranging 135 - 183 cm (n = 34 females) and 64.5 - 182 cm (n = 36 males) are described. Samples were collected in six ports of central and northern Peru from 1987 to 1999. In the field, sexual maturity was determined through macroscopic examination of gonads. Ovarian corpora indicated mature females (resting, lactating, pregnant). Semen in epididymides was considered evidence for mature males. The ovaries of Burmeister's porpoises 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. The number of *corpora ovarica* (*lutea*, *albicantia, atretica*) and their morphology were documented. The follicles, oocytes, and nuclei were measured. Follicular development was predominantly left-sided, but occurred 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 reported for January. Two of three immature females showed good follicular development in March and April.

The testes of Burmeister's porpoise are elongated and cylindrical. Of 36 males examined macroscopically, seven were immature, five pubescent and 24 mature. The histological analysis allowed to confidently determine sexual maturity status. Field evaluation of maturity based on the presence of sperm in the epididymides is a useful but in 8.3% of cases not an exact method. No histological evidence of male reproductive seasonality was found. Spermatogenesis was perceptible year-round and tubule diameters showed stochastic monthly variations. The body length at 50% sexual maturity was estimated at 157 cm and 152.7 cm, for males and females, respectively. There was no evidence of reproductive senescence.

This study is the first evaluation of the sexual maturation process in the Burmeister's porpoise, using both macroscopic observations and elaborate gonads histology. It provides important new information on the natural history of the species, including parameters (*e.g.*, mean body length at maturation, reproductive seasonality) highly relevant to its conservation.

Keywords:
density-dependent response, Eastern Pacific, ovarian corpora, *Phocoenidae*, reproduction, sexual maturity, testes

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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, particularly in the Southeast Pacific where it is distributed from Bahía de Paita in northern Peru south to Valdivia, Chile (Brownell & Praderi, 1982; Reyes, 2018). Locally known as 'marsopa espinosa' it is the only representative of Phocoenidae 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 & Reyes, 1990, 1994a, b), hence the Peruvian population has long been considered at risk (Culik, 2004; Rosa et al., 2005; 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). Besides, a high percentage (48.5%, n = 33) of Burmeister's porpoises examined in 1993 - 1995 had genital warts of papillomavirus etiology which, in some severe cases, may have compromised reproductive success (Van Bressem et al., 1996).

The large majority of the published literature on Phocoenidae biology concerns the harbor 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 harbor porpoise, 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 studies on gonadal morphology and histology in Phocoenidae, primarily *P. phocoena*. Other studies describe the histology of testes for harbor porpoises and length at sexual maturity (Sorensen & Kinze, 1994), histological features for resting mature individuals (Learmont et al., 2014) and a gonadal study of harbor porpoise 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 harbor porpoise from Japanese waters (Matsui et al., 2021).

Research on Burmeister's porpoise 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 & Praderi, 1982, 1984; Corcuera et al., 1995; Goodall et al., 1995; Reyes & 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 & Tantaleán, 1991; Reyes & Van Waerebeek, 1995), skeletal, skin and viral diseases (Van Bressem et al., 1993, 1998, 2006, 2007a, b; Montes et al., 2004) 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 & Praderi, 1982; Corcuera et al., 1995; Goodall et al., 1995).

A first analysis of the natural history of the Burmeister's porpoise in Peruvian waters, for the period 1983 - 1989, still stands as the only published study on reproduction for the species (Reyes & 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 & Van Waerebeek, 1995).

As Burmeister's porpoise 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 behavior 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).

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 1983 - 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 Burmeister's porpoise, to recognize pathologic cases, as well as provide a practical diagnostic tool to determine sexual maturity status.

Material and Methods

1. Ovarian morphology

Left and right ovaries were collected from 34 female Burmeister's 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 & Donovan, 1984; Read, 1989; Reyes & 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 & Van Waerebeek, 1995). In the laboratory the ovaries were examined macro- and microscopically. Each formalin-fixed ovary was 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 & 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 4 µm. The sections were subsequently stained with Harris hematoxylin and eosin 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) and their oocytes and nuclei were measured. The ovarian corpora morphology and condition of *corpora lutea* and *albicantia* (young, medium and old) were described (Marsh & Kasuya, 1984). The different types of atresia, a degenerative process causing oocytes to disappear before ovulation (Geneser, 1997) were characterised. Terminology for the ovarian morphology follows Perrin and Donovan (1984) and Marsh and Kasuya (1984).

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 & 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 the same as used 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 categorized according to the amount of tissue present: little, moderate or abundant. Tubular lumen size

Table 1. Collection data, reproductive status and metric data for ovaries of female Burmeister's porpoises *Phocoena spinipinnis* from Peruvian waters. Ovary 1 (left), Ovary 2 (right); except in (*) because sidedness was not registered. (-) = Without measurement or ovary not collected. I (immature), M (mature), P (pregnancy), L (lactation).

Collection data			Reproductive status				Ovary 1				Ovary 2			
Specimen number	Body length (cm)	Date	I	M	P	L	weight g	length mm	width mm	thickness mm	weight g	length mm	width mm	thickness mm
*RBC-029	135.0	27 Mar 1993	x				1.2	21.3	6.8	8.6	0.9	18.8	9.6	7.4
MFB-751	143.0	08 Mar 1995	x				1.4	21.7	13.9	7.4	1.2	20.7	12.4	57.5
MFB-482	149.2	25 Jan 1994		x	x		16.3	39.1	31.6	23.6	0.8	18.8	10.0	8.5
MFB-167	150.6	24 Apr 1993	x				1.2	20.8	9.6	11.8	0.8	18.3	8.4	10.0
KOS-259	152.5	28 Nov 1993		x	x		13.4	32.3	38.8	21.3	1.0	20.0	10.7	7.7
MFB-474	153.5	19 Jan 1994		x			1.0	21.5	10.9	7.9	3.6	28.5	17.6	14.9
MFB-493	153.5	18 Feb 1994		x	x		13.9	38.7	35.2	22.2	1.1	22.8	12.4	8.0
*DMI-148	155.0	23 Jun 1994		x			13.8	31.1	33.8	24.2	-	-	-	-
KOS-270	155.5	04 Dec 1993		x			6.0	33.4	23.7	12.5	1.7	24.4	12.4	10.0
JCR-1472	159.0	02 Jul 1988		x	x	x	2.6	19.4	6.3	10.0	-	-	-	-
MFB-429	159.5	28 Nov 1993		x	x	x	12.1	28.3	37.5	20.5	0.6	18.5	9.2	7.0
MFB-162	160.0	22 Apr 1993		x	x		19.1	44.0	33.6	25.2	-	-	-	-
KVW-1936	162.0	15 Dec 1989		x	x		10.9	44.2	26.0	21.0	1.5	27.5	12.8	8.1
JAS-043	163.5	01 Mar 1995		x	x	x	11.3	42.5	16.4	8.5	-	-	-	-
MFB-473	164.0	16 Jan 1994		x		x	13.8	31.6	30.9	20.1	1.1	18.8	10.7	8.8
MFB-084	164.5	20 Mar 1993		x	x	x	11.9	30.0	40.2	18.7	1.0	20.2	11.8	7.5
SZ-017	165.0	18 Apr 1990		x			4.4	26.9	20.9	13.0	6.3	30.7	19.8	18.4
MFB-130	165.1	15 Apr 1993		x	x		16.6	54.0	28.4	22.2	2.4	26.8	14.4	10.7
MFB-430	165.5	28 Nov 1993		x	x		10.7	29.3	37.9	15.7	2.8	26.5	17.2	11.2
JCR-1815	168.5	20 Sep 1990		x		x	6.6	34.2	24.2	15.7	1.0	21.5	11.8	8.2
JAS-050	169.0	31 Mar 1995		x	x		17.4	50.9	27.8	24.9	1.7	22.9	14.2	8.8
MFB-718	170.0	13 Aug 1994		x	x		11.6	36.9	25.4	20.8	1.4	27.0	19.4	6.3
MFB-168	170.3	24 Apr 1993		x	x	x	19.8	52.7	30.3	25.5	1.6	25.0	13.3	7.7
RJD-004	172.0	11 Nov 1995		x	x		18.8	44.0	34.2	23.2	-	-	-	-
KVW-1931	172.5	15 Dec 1989		x	x		17.3	45.5	32.3	20.6	1.0	20.5	11.6	7.9
JCR-1485	173.0	05 Apr 1989		x	x	x	13.9	28.8	40.8	21.4	1.5	25.5	14.4	8.0
MFB-457	173.5	04 Dec 1993		x			6.3	33.0	23.7	14.0	1.6	20.5	15.0	8.3
*AGG-090	175.5	21 Aug 1991		x	x	x	15.1	51.2	31.4	17.9	1.5	23.3	13.8	7.0
JAS-033	177.0	25 Sep 1994		x		x	14.8	29.0	24.8	25.0	1.0	14.9	12.2	8.4
JCR-1628	179.5	22 Apr 1990		x		x	4.2	34.3	20.4	10.3	3.0	29.5	15.6	11.0
JCR-1830	179.5	21 Sep 1990		x	x		20.6	53.5	28.8	26.5	2.0	23.6	13.8	11.3
KVW-1958	183.0	18 Dec 1989		x	x		17.0	50.4	31.9	18.3	1.1	23.3	9.6	10.4
JAS-048	-	23 Mar 1995		x	x		9.7	38.8	25.6	19.4	1.0	18.7	10.2	8.9
*AGG-755	-	07 Aug 1993		x			10.1	38.1	40.2	12.2	-	-	-	-

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 center. Large lumina contained essentially no intra-tubular tissue. Seminiferous tubules diameter was 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 times for each testis.

3. Estimation of body length at attainment of sexual maturity

The body length at attainment of sexual maturity at 50% (LSM *P*(0.50)) of individuals was calculated by using lineal estimation (DeMaster, 1984; Kasuya & Brownell, 1979; Reyes & Van Waerebeek, 1995) after plotting the mature proportion of females and males versus each length-class of 10 cm.

Results

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). 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 (Figs 1, 2 and 3), according to the reproductive status and the presence of *corpora*. The length and weight of the ovaries are presented in Table 1.

The internal structure of Burmeister's porpoise ovaries is typical of mammals. A simple cuboidal epithelium covers the ovary. The *tunica albuginea* present under the epithelium is easy to distinguish (Fig. 4A) as well as the cortex with its ovarian stroma between the follicles (Fig. 4B). The medulla sits more centrally (Fig. 4C) and envelopes the ovarian *hilum* where blood vessels and nerves enter the parenchyma. Between the *hilum* and the medulla are irregular tubules lined by a simple columnar epithelium with small lumen called *rete ovarii* (Fig. 4D). The diameters of each follicle, oocyte and oocyte nucleus are provided in Table 2. Atretic follicles (without luteinization) occurred in all females while luteinizing *corpora atretica* were limited to mature specimens.

Figure 1. Ovaries of an immature female Burmeister's porpoise *P. spinipinnis*. The small ovaries were ovoid [upper] or bean-shaped [lower], flattened, and with a smooth surface.

1.1. Immature females

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

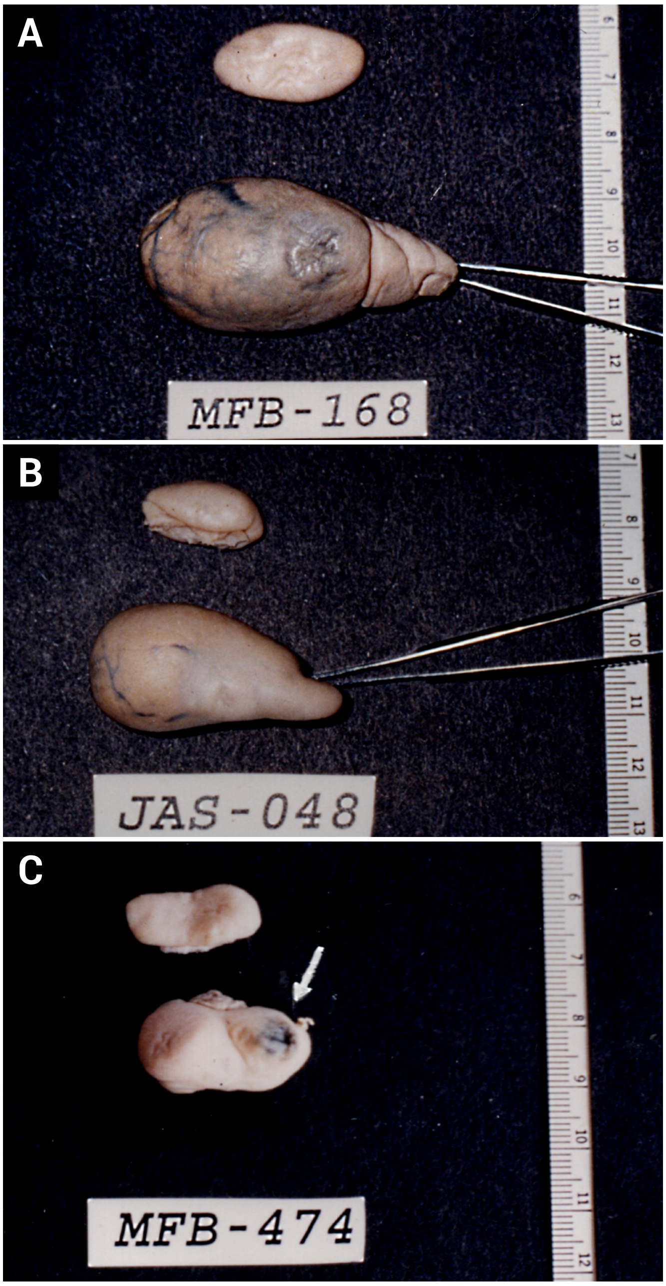

Figure 2. Activity in a single ovary of Burmeister's porpoise *P. spinipinnis*. There is a marked trend of activity in a single ovary (83.87%), mainly in the left one (84.62%). A,B) - ovaries (below) where only the left (enlarged) one showed activity; C) ovaries of the only animal that showed activity only in the right one. Note the cavity [arrow] in the right ovary from the recent rupture (ovulation) of a Graafian follicle.

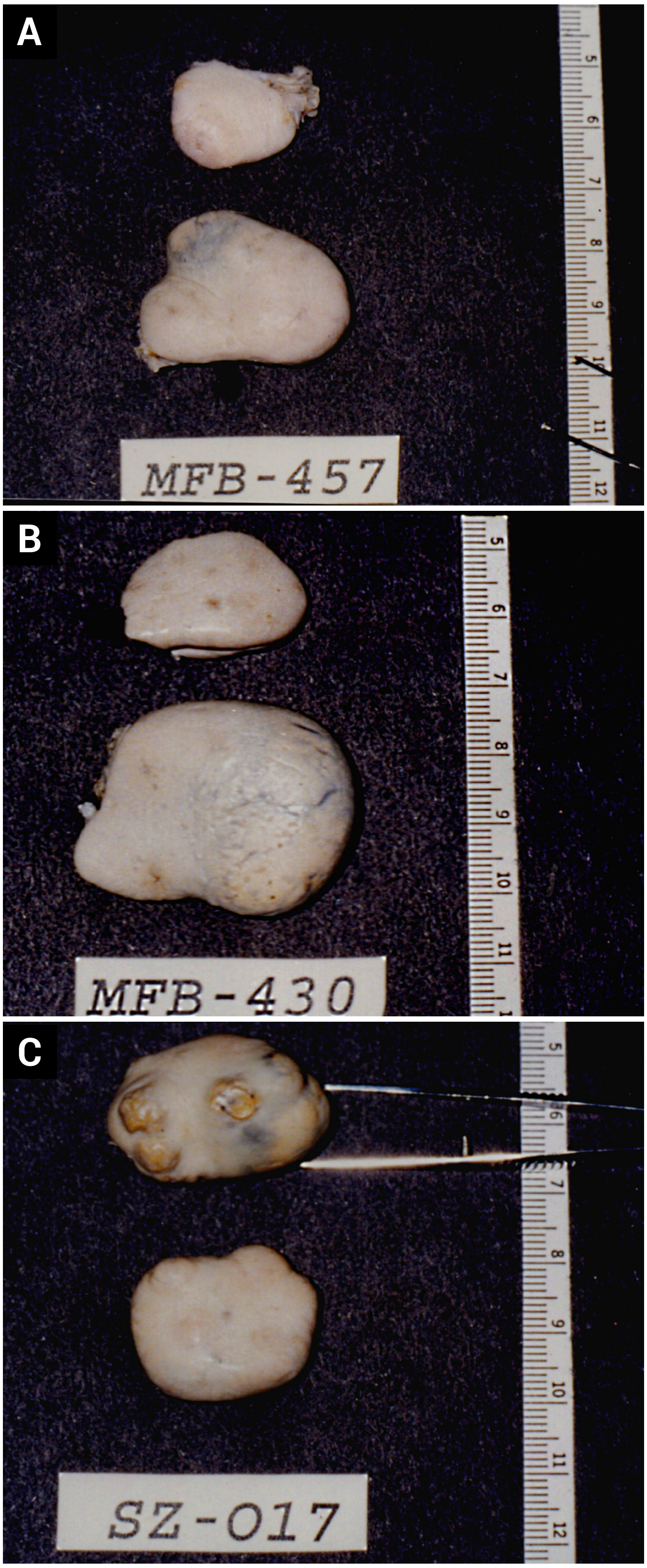

Figure 3. Activity in both ovaries of Burmeister's porpoise *P. spinipinnis*. A) Left ovary [below] with numerous *corpora albicantia* scars; B) Right ovary [upper] with numerous *corpora albicantia* scars. Left ovary [below] shows a *corpus luteum*; C) Ovaries of female with the highest number of *corpora albicantia* found (n = 28) in left [below] and right [upper] ovaries.

Table 2. Diameters range (µm) of follicular structures of female Burmeister's porpoises *Phocoena spinipinnis* from Peruvian waters.

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

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.

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.

1.2. Mature females

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.

1.2.1. Macroscopic characteristics

Externally, the ovaries of the mature females were usually easy to distinguish from the 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 (Figs 2 and 3). The size and weight of the ovaries, as well as their external surface, varied substantially depending on their reproductive state (Table 1). Except for a few cases, the largest follicles were seen in the left ovaries.

Atresia with luteinization resulted in orange-brown irregularly shaped masses, round or fusiform, present 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 immature ones. 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 3), 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, three presented *corpora atretica* with luteinization in the right ovary, with a maximum of four *corpora* (SZ-017; 165.0 cm body length).

All *corpora lutea* were characterised by a relatively large size (Figs 2A 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 and 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. The laterality was not registered for the remaining three.

Table 3. Ovarian *corpora* counts and relative quantity of follicles. Ovary 1 (left), Ovary 2 (right); except in (*) because sidedness was not registered, [open space] = without measurement or ovary absent; CL = *corpora lutea*: Y = young, M = mature, I = in involution; CA = *corpora albicantia*: O = old; CAL = *corpora atretica* with luteinization. (pd = primordial follicles, P = primary follicles, S = secundary follicles, T = terciary follicles, G = Graaf follicles): ++++ = abundant; +++ = numerous; ++ = regular; +- = a few; + = rarely; - = not found.

Specimen number	Ovary 1							Ovary 2							Relative Quantity of Follicles				
	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															Y	++	+-	+	-
MFB-751															++++	+++	+	+	++
MFB-482		1													+++	++	-	-	-
MFB-167															++++	++	++	++	+-
KOS-259		1		1											+++	+++	+	+	-
MFB-474											1	1			+++	+++	+	+	-
MFB-493		1													+++	+++	+-	+-	-
*DMI-148		1		2											+++	++++	+	+-	-
KOS-270			2	5	2	15	11				1				+	+	+	+	-
JCR-1472				1	1	1									++++	+-	++	+-	-
MFB-429		1													+++	+++	+	+-	-
MFB-162		1													++++	++	+-	+	-
KVW-1936		1			2										+++	+++	+-	+	-
JAS-043		1		1	3	2	4								++	++	+	-	-
MFB-473		1			1										+++	+++	+-	+-	+
MFB-084		1			5		4								+++	++	-	+	-
SZ-017				2	4	13	13		1		8		1	4	+	+	-	-	-
MFB-130		1			1	6	2								++	++	+-	-	-
MFB-430		1			2	1						8	5	3	+++	++	-	-	-
JCR-1815			1		2										++++	+++	+-	+-	-
JAS-050		1			6	14	6								+	+	-	-	-
MFB-718		1		1	8	1	2								+++	+-	-	-	-
MFB-168		1			9										++	++	+	+-	-
RJD-004		1		1	8										++	+	-	-	-
KVW-1931		1			1		4								+++	+-	-	+-	-
JCR-1485		1		3	3	1	1								++	++	-	-	-
MFB-457				3	4	17	2				1	1			+-	+	-	-	-
*AGG-090		1			5	2	4								+++	++	+	+++	-
JAS-033		1			6	3	29								+-	+-	+-	-	-
JCR-1628					7	6							7	2	+	+	+	-	-
JCR-1830		1			8	2	5								+++	+-	+	-	-
KVW-1958			1	1	5		4								+-	+	-	-	-
JAS-048		1			1										+++	++	+	+	-
*AGG-755	1				1	9	3								++	+-	-	-	-

Among 21 pregnant females, 20 presented a single *corpus luteum* and one (JCR-1472, with 23 cm foetus) did not. However, a *corpus luteum* was likely present in its missing right ovary. The presence of only three *corpora albicantia* in the left ovary indicated low activity. Maximum dimensions of the *corpora lutea* ranged 28.20 x 15.20 mm to 33.2 x 25.2 mm. The largest *corpus luteum* belonged to a 160 cm porpoise (MFB-162), pregnant with a 191 mm male foetus, collected in April.

The left ovary of female KOS-270 had two *corpora lutea* in involution (Table 3). They measured 4.70 x 3.95 mm and 8.50 x 7.20 mm and showed a wide whitish center composed of hyaline material. A large, young *corpus albicans* was present in the right ovary. Individual KOS-270 was neither pregnant nor lactating.

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 (Figs 2 and 3). 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 (Figs 2A and B) but some right ovaries also had *corpora albicantia* (Fig. 3, Table 3).

Young *corpora albicantia* slightly protruded from the ovarian *tunica albuginea*, leaving a visible scar. They had 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 3). Porpoise SZ-017 showed a total of

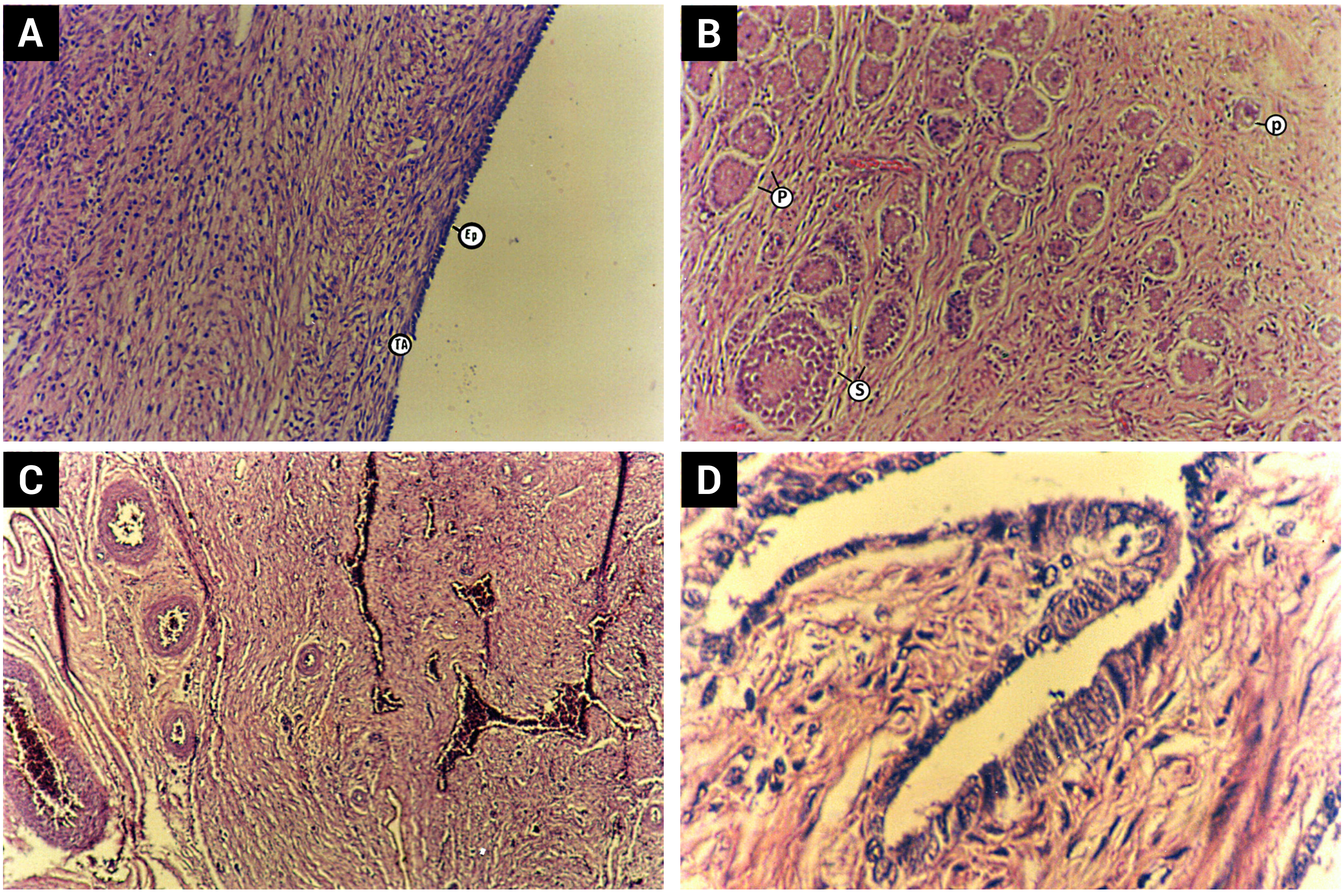

Figure 4. Photomicrographs of ovarian morphology in Burmeister's porpoise *Phocoena spinipinnis*. A) The epithelium of the ovary (Ep) and the *tunica albuginea* (TA) (JAS-050, left ovary) appear as a capsule of dense connective tissue, whose collagen fibers are oriented parallel to the surface of the ovary. This tissue is poorly vascularized. 100 x magnification. B) Ovarian stroma (MFB-167, right ovary), where the primordial follicles (p), primary follicles (P) and secondary follicles (S) can be observed. 100 x magnification. C) Ovarian medulla (JCR-1472, left ovary). 40 x 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. 400 x magnification.

28 CA in both ovaries (Fig. 3C). The highest number of *corpora albicantia* in the left ovary was 24 (MFB-457; 173.5 cm) (Fig. 3A) and 13 in the right ovary (MFB-430; 165.5 cm) (Fig. 3B). Five of the 27 females presented *corpora albicantia* also in the right ovary and one animal (MFB-474) presented two *corpora albicantia* only in the right ovary (Fig. 2C).

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 (Table 3), 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 with only n = 11 *corpora* (KOS-270, n = 36 *corpora*; SZ-017, n = 46; JAS-050, n = 27; MFB-457, n = 28; JAS-033, n = 39; JCR-1628, n = 29). 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*.

1.2.2. Microscopic characteristics

Ovaries generally presented numerous primordial follicles (Table 3) and maturing follicle atresia. The histological appearance of atresia is highly variable depending on the stage of development. Atresia could only be evidenced by microscopic analysis. In one individual (MFB-474) collected in mid-January, the right ovary (Fig. 2C) presented one follicle that had recently ovulated (5.75 x 8.30 mm) and whose walls were folded, with clusters of small capillaries and scattered blood (*corpus hemorrhagic*) (Fig. 5A). There were also five 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, slightly separated from each other (dimensions, in mm, of five 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). The left ovary of another individual (JCR-1815) presented 11 large atretic follicles that macroscopically resembled maturing follicles, with 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).

Residues of a collapsed *zona pellucida* were seen inside fibro-collagenous connective tissue of some *corpora fibrosa*. These

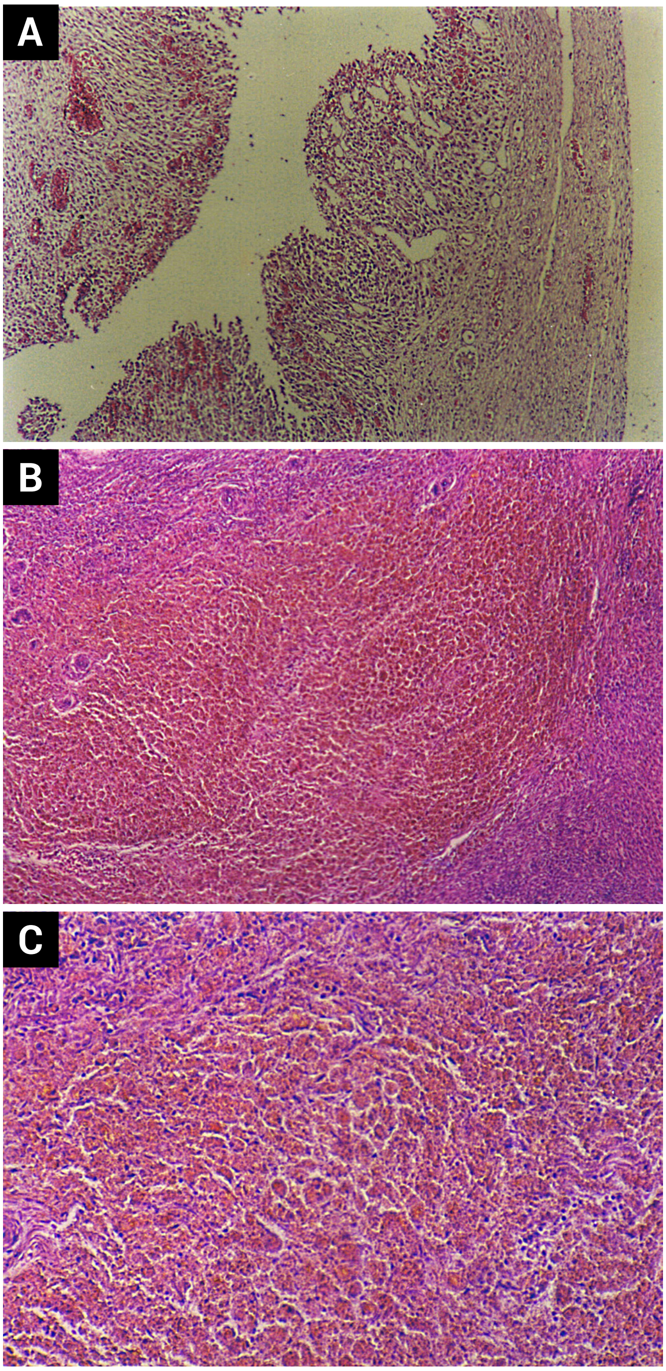

Figure 5. Microphotographs of ovaries in Burmeister's porpoise *P. spinipinnis*. A) Recently ruptured follicle (5.75 x 8.30 mm) (MFB-474, right ovary). Its walls are folded and clusters of small capillaries and scattered blood can be observed towards the center. 40 x magnification. B) Luteinized *corpus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

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, with 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, histologically showed fibrous connective tissue and groups of cells similar to the luteal cells of the theca, some still conserved and others already degenerated with vacuolated cytoplasm and pyknosis (Figs 5B and 5C). In some, mostly round, *corpora atretica,* there were less fibrous connective tissue and granulosa cells with luteinization. The latter were heterogeneously arranged towards the center of the *corpus*, enclosed by the still visible basal membrane, and also near the luteinized cells of the *theca interna*. Some of the cells of these corpora showed signs of atresia. None of the *corpora atretica* with luteinization were vascularized.

Large (36.5 to 56.7 µm in diameter), swollen, pale staining luteal cells with one or two round nuclei (averaging 16.2 µm in diameter) were observed in the granulosa of the *corpus luteum* cells (Figs 6A and 6C). Characterized by a small size and a dark staining, the thecal luteal cells were detected on the periphery of the *corpus*, mainly along the fibrous septa (Fig. 6C). In the well-formed, mature *corpora lutea*, a small central area was occupied by collagen fibers and surrounded by a larger 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 center was observed with clusters of red blood cells (evidence of recent bleeding) mixed with polymorphs. The walls of the *corpora* were slightly folded and formed by a thick layer of granulosa lutein cells, and by theca lutein cells at the periphery.

The absence of fibrous septa was also noted (Fig. 6C). The involute *corpus luteum* had a fibrous center 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).

In the young *corpus albicans* the trabecular arrangement of the anterior *corpus luteum* was still recognizable. Clusters of scattered fibres were seen close to the center of the *corpora* and the luteal cells of the granulosa were replaced by fibrous cellular tissue, with numerous fibroblasts. Some degenerating luteal and thecal cells were still visible. The peripheral limits of the *corpus* were clearly visible, with vascularization mainly in this area (Fig. 7A).

The connective tissue of the mature *corpus albicans* was less abundant than in the young *corpora albicantia,* with clusters of blood vessels of different sizes scattered throughout. Remains of luteal cells were still present at the periphery of a few mature *corpora*. Though less visible, the peripheral limits of the trabeculae were still discernible (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 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.

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.

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, seven were immature, five pubescent and 24 mature. Their lengths are presented in Table 4. The mean length of sexually mature males was 166.39 cm (n = 24; SD = 10.40).

Table 4. Collection data, field observation of maturity stage, gonadal metric data and histological maturity for male Burmeister's porpoises *P. spinipinnis* from Peruvian waters. Sidedness of testes generally was not registered (codes without superindex). The codes with superindex *a* and *b* have sidedness data (Testis 1 = left; Testis 2 = right), however for *b* specimens the mean diameter of tubules (DT) and the thickness of *tunica albuginea* (TA) were taken from an unmarked testis sample, either left or right. Other abbreviations used: Weight (W), Length (L), Width (Wd), Thickness (Th).

Code	Total length (cm)	Date	Maturity stage (field observation)	Testis 1						Testis 2						Maturity stage by histology
				W	L	Wd	Th	DT	TA	W	L	Wd	Th	DT	TA	
				g	mm	mm	mm	µm	µm	g	mm	mm	mm	µm	µm	
AGG-090	64.5	21 Aug 1991	Immature	0.5	18.0	6.5	7.8	68.1	164.0	0.4	16.9	6.7	7.0	71.0	164.0	Immature
AGG-033	122.0	12 Mar 1991	Immature	4.9	36.9	19.0	10.8	60.6	180.0	4.4	37.4	16.2	12.8	56.8	204.0	Immature
AGG-135	129.0	24 Aug 1991	Immature	4.2	37.9	15.0	12.7	56.0	155.0	3.4	38.1	11.9	12.2	56.1	168.0	Immature
JAS-046	130.0	09 Mar 1995	Immature	3.1	29.6	13.3	12.2	54.2	189.0	3.0	29.6	13.2	12.0	51.0	198.0	Immature
AGG-034	130.5	12 Mar 1991	Immature	6.4	38.9	19.0	14.4	51.7	150.0	5.8	39.1	17.5	15.3	50.6	194.0	Immature
JCR - 903[a]	139.0	16 Jan 1987	Probably Immature	8.7	44.2	20.6	17.2	70.5	267.0	-	-	-	-	-	-	Immature
JAS-145[a]	142.0	17 Jun 1999	Probably Immature	13.1	50.3	21.3	20.9	82.5	240.0	12.6	51.1	20.90	19.9	76.9	198.0	Pubescent
AGG-037[a]	143.5	13 Mar 1991	Not determined	13.2	53.1	22.2	15.5	73.6	214.0	-	-	-	-	-	-	Mature
AGG-129	144.0	23 Aug 1991	Immature	6.5	45.0	17.4	17.5	61.8	188.0	6.4	42.2	17.5	15.5	53.8	180.0	Immature
AGG-726	150.0	02 Feb 1993	Probably Immature	-	-	-	-	67.9	306.0	-	-	-	-	-	-	Pubescent
MFB-528	151.5	26 May 1994	Immature	78.5	96.0	44.0	22.0	158.8	730.0	85.5	95.0	49.0	30.0	-	-	Mature
AGG-031[a]	152.0	12 Mar 1991	Not determined	10.5	46.5	20.0	18.7	60.7	299.0	10.3	50.0	17.6	18.6	63.4	290.0	Pubescent
AGG-049	152.0	22 Mar 1991	Not determined	-	-	-	-	200.5	-	-	-	-	-	-	-	Mature
AGG-081	153.0	21 Aug 1991	Immature	7.9	42.5	16.5	18.2	70.5	270.0	8.3	43.5	18.0	19.6	68.8	269.0	Pubescent
JCR-1587	154.0	16 Sep 1989	Immature	-	-	-	-	71.9	298.0	-	-	-	-	-	-	Pubescent
JCR-1932	155.0	20 Mar 1991	Probably mature	10.4	50.6	21.5	17.8	81.8	219.0	-	-	-	-	-	-	Mature
MFB-495[b]	155.5	19 Feb 1994	Probably mature	109	100.0	49.0	32.0	178.9	742.5	119	101.0	48.0	34.0	-	-	Mature
AGG-055	157.0	24 Jun 1991	Probably mature	-	-	-	-	212.9	-	-	-	-	-	-	-	Mature
MFB-181	159.1	29 Apr 1993	Mature	-	-	-	-	195.4	-	-	-	-	-	-	-	Mature
JCR-1630	160.0	25 Apr 1990	Mature	-	-	-	-	203.9	-	-	-	-	-	-	-	Mature
DMI-019[a]	160.0	24 Mar 1994	Not determined	-	-	-	-	177.6	580.0	-	-	-	-	176.5	582.5	Mature
MFB-526[b]	164.0	22 May 1994	Mature	250	135.0	65.0	39.0	214.7	-	217	132.0	63.0	41.0	-	-	Mature
AGG-609	164.5	12 Sep 1992	Probably Immature	-	-	-	-	154.9	-	-	-	-	-	-	-	Mature
AGG-134	171.0	24 Aug 1991	Mature	-	-	-	-	348.6	-	-	-	-	-	-	-	Mature
JAM-002[a]	171.0	05 Nov 1997	Probably mature	230	130.0	70.0	40.0	212.9	682.5	230	137.0	71.0	37.0	199.3	750.0	Mature
MFB-672[b]	171.5	29 Jun 1994	Mature	205	122.0	68.0	40.0	223.9	-	170	122.0	60.0	35.0	-	-	Mature
JCR-1792	172.0	23 Aug 1990	Not determined	-	-	-	-	251.8	-	-	-	-	-	-	-	Mature
MFB-161[b]	172.6	21 Apr 1993	Mature	-	144.0	-	-	265.5	785.0	-	144.0	-	-	-	-	Mature
MFB-145	173.1	16 Apr 1993	Mature	350	153.0	64.0	-	220.5	772.5	350	146.0	67.0	-	-	-	Mature
MFB-461[b]	173.5	08 Dec 1993	Mature	235	132.0	63.0	45.0	220.9	815.0	198	125.0	59.0	36.0	-	-	Mature
KVW-2438[a]	174.0	25 Jan 1998	Mature	108	93.4	38.0	53.7	159.2	515.0	111	96.5	47.0	46.6	163.7	612.5	Mature
JCR-1627	175.0	22 Apr 1990	Mature	-	-	-	-	251.5	-	-	-	-	-	-	-	Mature
KVW2427[b]	177.0	08 Nov 1995	Mature	-	-	-	-	200.8	610.0	-	-	-	-	-	-	Mature
MFB-459	178.5	08 Dec 1993	Mature	321	152.0	68.0	45.0	227.1	782.5	316	146.0	65.0	50.0	-	-	Mature
JCR-1814[b]	180.0	20 Sep 1990	Mature	231	130.0	65.0	38.0	238.5	-	231	130.0	67.0	38.0	-	-	Mature
JCR-1578	182.0	26 Aug 1989	Mature	-	-	-	-	215.4	662.5	-	-	-	-	-	-	Mature

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 with 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 4. 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 tubules which in the mediastinum testis form the *rete testis.*

2.1. Immature males

Of the 36 males examined, seven 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.

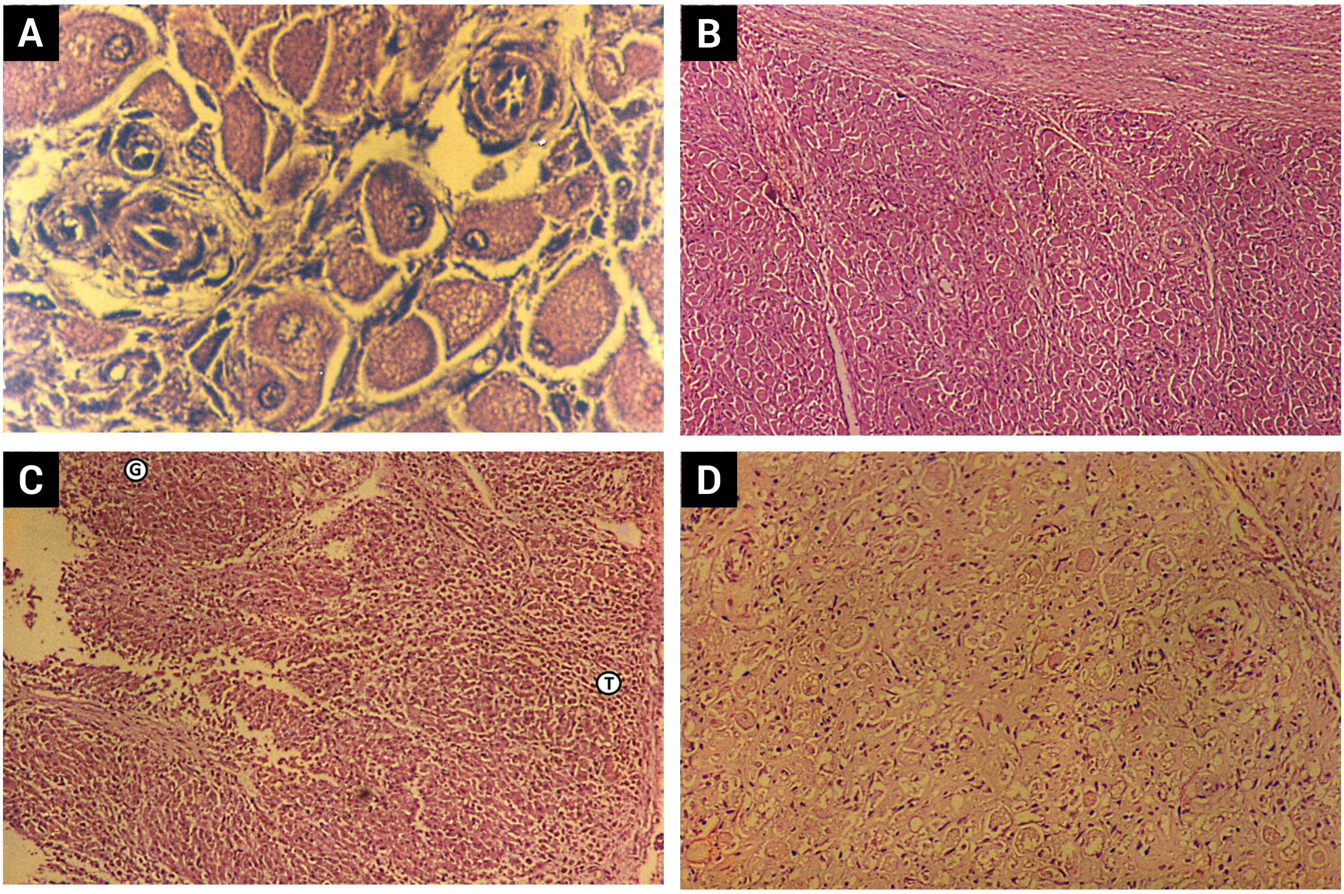

Figure 6. Microphotographs of *corpora lutea* in Burmeister's porpoise *P. spinipinnis*. A) Granulosa luteal cells (JAS-048, left ovary). 400 x magnification. B) Mature *corpus luteum* (MFB-482, left ovary). 40 x magnification. C) Young *corpus luteum* (AGG-755, left ovary). Granulosa lutein cells (G) and small theca lutein cells (T) can be seen. 40 x magnification. D) Involuting *corpus luteum* (JCR-1815, left ovary). 100 x magnification.

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 29.6 to 45.0 mm long; 11.9 to 20.6 mm broad and 10.8 to 17.5 mm thick (Table 4, Fig. 8A). The testes of a 64.5 cm foetus 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.

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 = 56.6 μm; SD = 5.8; n = 11; range 50.6 - 70.4 μm), embedded in the abundant interstitial tissue. A regular layer of Sertoli cells lining the tubules and a few centrally located spermatogonia or interspersed with Sertoli cells were found. 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 69.5 μm and the other histological characteristics were similar to those described in the six immature males.

2.2. Pubescent males

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).

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 five pubescent porpoises were elongated and cylindrical like the immature ones. The length of the testes ranged from 42.5 to 51.1 mm, the width from 16.5 to 21.3 mm and the thickness from 18.2 to 20.9 mm (Table 4). The testes weighed without epididymis 7.9 to 13.1 g ($\bar{x}$ = 10.4 g; SD = 2.1; n = 6).

2.2.2. Microscopic characteristics

The thickness of the *tunica albuginea* ranged from 198.0 to 306.0 μm ($\bar{x}$ = 271.2 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 ($\bar{x}$ = 70.3 μm; SD = 6.9; n = 8; range = 60.7 to 82.5 μm) (Table 4) and were immersed in moderate to little interstitial tissue.

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.

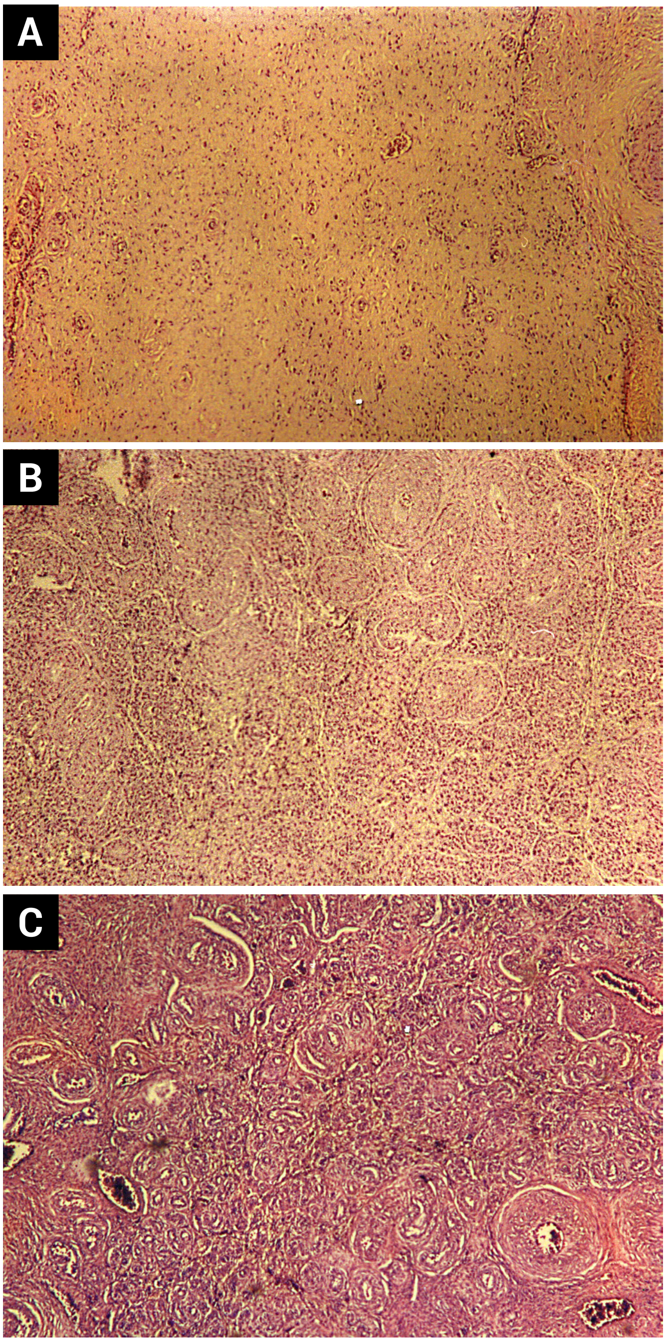

Figure 7. Microphotographs of *corpora albicantia* in Burmeister's porpoise *P. spinipinnis*. A) Young *corpus albicans* (AGG-755, left ovary). 40 x magnification. B) Mature *corpus albicans* (MFB-718, left ovary). 40 x magnification. C) Old *corpus albicans* (JCR-1472, left ovary). 40 x magnification.

In a 150 cm male (AGG-726), only a few Sertoli cells, abundant spermatogonia, a regular number of primary and secondary spermatocytes, few spermatids, and four spermatozoa were observed in a peripheral tubule. This was still considered pubertal because all the other tubules presented the histological characteristics of puberty (Collet & Saint Girons, 1984; Hohn et al., 1985; Sorensen & Kinze, 1994).

2.3. Mature males

The sample included 24 mature specimens with SBL varying between 143.5 and 182.0 cm. ($\bar{x}$ = 166.4 cm; SD = 10.4).

2.3.1. Macroscopic characteristics

The adult testes weighed without epididymis from as low as 10.4 g (JCR-1932, 155 cm, little semen) to 350.0 g ($\bar{x}$ = 189.5 g; SD = 99.8; n = 22). Their length ranged from 50.6 to 350.0 mm ($\bar{x}$ = 119.4 mm; SD = 28.2; n = 24), the width from 21.5 to 146.0 mm ($\bar{x}$= 56.1 mm; SD = 14.5; n = 22) and thickness from 15.5 to 71.0 mm ($\bar{x}$ = 36.8 mm; SD = 9.9; n = 20) (Table 4).

2.3.2. Microscopic characteristics

The thickness of the *tunica albuginea* ranged from 214.0 to 815.0 µm ($\bar{x}$ = 628.5 µm; SD = 183.2; n = 27). Most of the tubules presented 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 73.7 to 348.6 µm ($\bar{x}$ = 201.1 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.

The determination of the sexual maturity status in males at the macroscopic level (presence of a perceptible quantity of

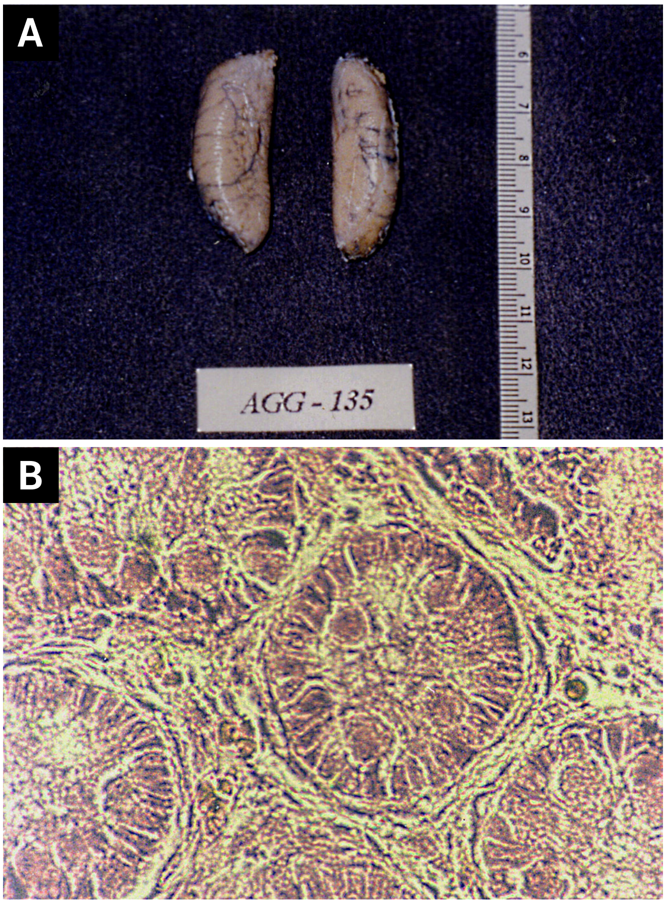

Figure 8. Immature testes of Burmeister' porpoise *Phocoena spinipinnis*. A) Testes (AGG-135) are elongated and cylindrical, with an external smooth, shiny and cream-colored surface. B) Testicular tissue microphotograph of AGG-135, testis 2. 400 x magnification.

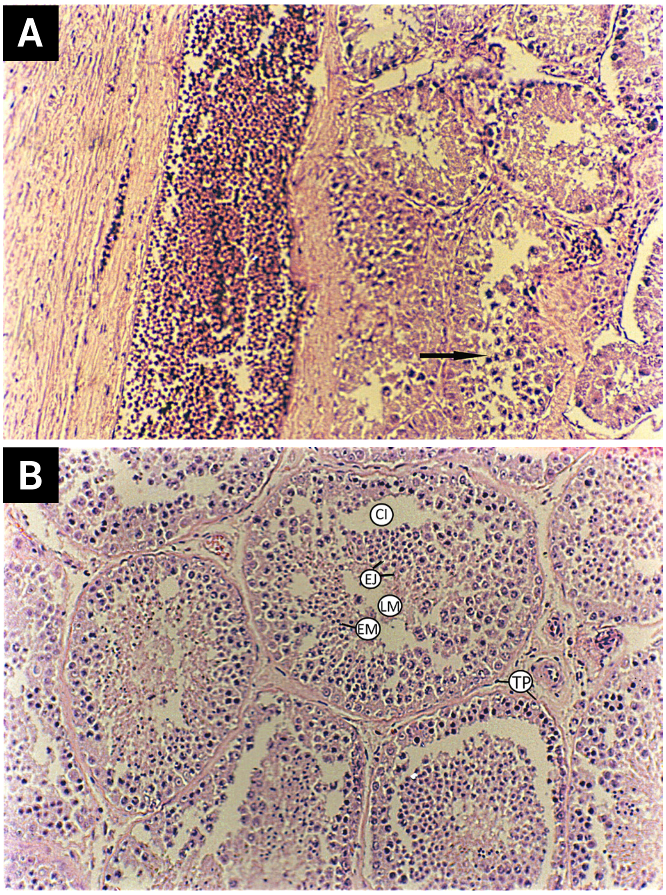

Figure 9. Microphotographs of testes of Burmeister's porpoise *P. spinipinnis*. A) Testicular tissue (from the periphery) of pubescent (AGG-726) specimen, where groups of primary spermatocytes can be observed [arrow]. 100 x magnification. B) Testicular tissue of mature male (MFB-161). The seminiferous 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). 100 x magnification.

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. Body length at sexual maturity

In our 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.

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.

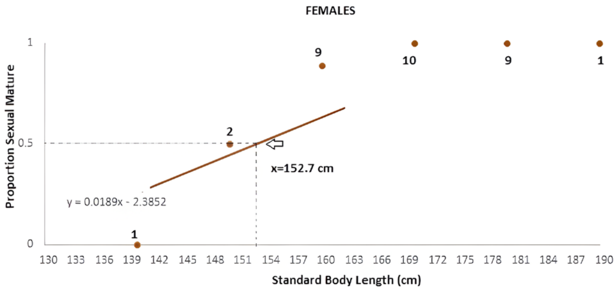

Figure 10. Proportion of sexually mature Burmeister's porpoise *Phocoena spinipinnis* females according to body length and determination of the LSM *P*(0.50) by linear adjustment, showing trend line and equation.

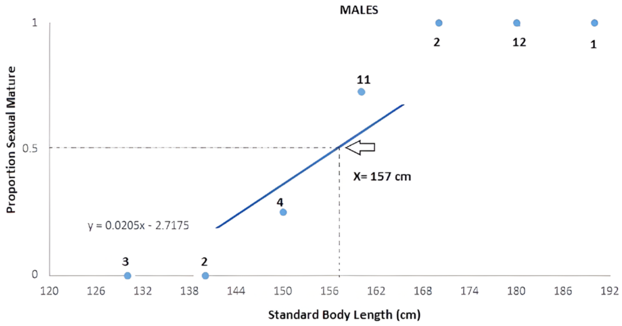

Figure 11. Proportion of sexually mature Burmeister's porpoise *Phocoena spinipinnis* males according to body length and determination of the LSM *P*(0.50) by linear adjustment, showing trend line and equation.

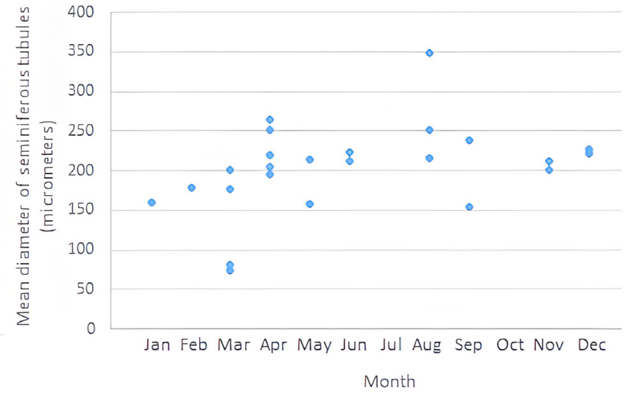

Figure 12. Mean diameter of seminiferous tubules versus collection month for 24 mature Burmeister's porpoise *Phocoena spinipinnis*. Though the highest tubule diameters occurred in April and August, no clear seasonality was perceptible.

Discussion

In 26/31 of Burmeister's porpoise females (83.9%) there was 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. However, remarkably, in 16.1% of mature females both ovaries were active, mainly in those specimens with numerous *corpora*. This phenomenon may be related to the increased reproductive need of a species that lives in the highly unstable ENSO (El Niño-Southern Oscillation) habitat (Fiedler, 2018) and/or to the adaptation to an unusually high mortality rate in Peruvian coastal fisheries for decades (Read et al., 1988; Van Waerebeek & Reyes, 1990, 1994a, b).

High activity in both ovaries ensures a high pregnancy rate, estimated at 60% during a previous study (Reyes & Van Waerebeek, 1995). Thus, in Burmeister's porpoise ovarian activity (oocyte release) is not limited to the left ovary, in contrast to Dall's porpoise *Phocoenoides dalli* from Japanese waters (Kasuya, 1978). For harbor porpoise *P. phocoena*, Fisher and Harrison (1970) reported only one specimen with a *corpus albicans* in the right ovary, while Sorensen and Kinze (1994) in Denmark found a single female with four *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 a 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 female 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), on average seven years earlier, *i.e.* an estimated two generations (3-4 yrs/generation) with harbour porpoise as proxy (Read & Gaskin, 1990). No estimates are available for other populations of Burmeister's porpoise to compare with, but these values are surprisingly similar to the estimated LSM *P*(0.50) = 154.4 cm reported for female harbor porpoise from Japanese waters (Matsui et al., 2021). Larger female asymptotic lengths were recorded for harbour porpoise 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).

Porpoises exhibit early maturation, relatively short gestation/lactation periods and a faster rate of reproduction as compared to other cetaceans (Read & Gaskin, 1990; Murphy et al., 2020). Although our samples were relatively small, it is possible that the body length (and age) at first ovulation in Burmeister's porpoise have effectively decreased since the first sampling period (Reyes & Van Waerebeek, 1995) or even earlier, as porpoise captures have gradually increased since the 1960 - 1970s (Clarke, 1962; Clarke et al., 1978). This would be consistent with a working hypothesis of a density-dependent response effect as a result of the very intense, continuous exploitation in the period 1984 - 1999 (Van Waerebeek & Reyes, 1994a, b; Van Waerebeek et al., 1999) and the suspected reduction in the size of the population of Burmeister's porpoise in Peru (Reyes, 2018). In addition, the fact that simultaneously lactating and pregnant females were encountered since the 1980s (Reyes & Van Waerebeek, 1995; this paper), is equally coherent with a density-dependent response. Such an effect has been well-documented in harbour porpoise populations that suffer high bycatch rates (Read & Gaskin, 1990; Read, 2001; Murphy et al., 2020) and in commercially hunted whale populations (*e.g.*, Fowler, 1984; Wade, 2018).

Although several studies have been published on overexploitation in small cetaceans, only a few report the occurrence of variation in the age and length at sexual maturity (Chávez, 1998). In Eastern Tropical Pacific populations of *Stenella longirostris* (spinner dolphin) and *S. attenuata* (Pantropical spotted dolphin) that suffered high levels of mortality in tuna fisheries, several authors (Kasuya, 1976; Perrin & Henderson, 1984; Chivers & 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 & Reyes, 1994a, b), is lower than that estimated for other populations of this species (Chávez, 1998; Van Waerebeek & Read, 1994). A gradual long-term decline of the prevalence of dusky dolphins in bycatches 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. However, in the present study, there was no histological evidence to support this hypothesis. Two *corpora albicantia* of the same status (young, mature, old) often differed by the color tone (orange to yellowish) or intensity (pale to more intense) of their pigmentation, as well as their dimensions, but otherwise did not vary in histology. This variability in pigmentation was observed between individuals, but often also between *corpora albicantia* within the same ovary. We suggest it may have the same origin as the variation observed in *corpora atretica* with luteinization, due to a differential 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 harbor porpoise.

The size of the *corpora albicantia* is not indicative of its status, *i.e.* it is independent of whether it is a young, a mature, or an old *corpus albicans*. 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.

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 center 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 status within an ovary had little difference in size, which could indicate parallel or close ovulations. Similarly, Harrison and McBrearty (1973-1974) 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 Burmeister's porpoise than in any other phocoenid, such as harbour porpoise (maximum 16) (Sorensen & Kinze, 1994) and Indo-Pacific finless porpoise *Neophocaena phocaenoides* (maximum 11) (Harrison & McBrearty, 1973-1974), which is consistent with the higher ovulation activity in both ovaries of Burmeister's porpoise 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 (Table 3), 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 were more numerous in these seven mature females, and were 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 showed stochastic monthly variation. This result is consistent with Reyes and Van Waerebeek (1995) who did not observe significant seasonal variation in the mean testis weight of Burmeister's porpoises, while admitting that sample size was small. Thus, in contrast with other porpoises (Robeck & O'Brien, 2018) which exhibit a discrete male reproductive seasonality (whereby sperm production is limited to a 2 - 3 month annual period), in Burmeister's porpoise from Peruvian waters 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.

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 & 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. Our results can help future field researchers to classify small male porpoises with small testes and low quantities of semen in epididymides as pubescent.

Based on histological analysis, Learmonth et al. (2014) classified the males of harbor porpoise 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 immature (84 - 130 cm), pubescent (119 - 153 cm), active mature (135 - 157 cm) and resting mature (116 - 160 cm) males, however these ranges were wide and greatly overlapping. We did not find similar histological characteristics as those reported in the study by Learmonth 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 *P. spinipinnis* from Peru.

Conclusions

Our study is important in being the first to yield essential knowledge of the normal, healthy gonad morphology and maturation process as a comparative base for future cross-sectional studies of reproductive biology of Burmeister's porpoise, to recognize pathologic cases, as well as provide a practical diagnostic tool to determine sexual maturity status. It provides significant new information on the natural history of *P. spinipinnis* including parameters (*e.g.*, mean body length at maturation, reproductive seasonality) highly relevant to its conservation.

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