PART 4

GENERAL BIOLOGY
REPRODUCTION

CHAPTER 8

Reproduction in the male Cape fur seal *Arctocephalus pusillus pusillus:* age at puberty and annual cycle of the testis

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ABSTRACT

Seasonal changes in the reproductive anatomy and histology of the male Cape fur seal *Arctocephalus pusillus pusillus* were examined. Studies were based on 99 specimens collected between Algoa Bay on the south-east coast of South Africa, and Cape Frio, Namibia, during 1974 to 1990. Reproductive organs are briefly described. The presence of sperm in the seminiferous and epididymal tubules indicates that males attain puberty between 3 and 4 years of age. Quantitative measurements of testis weight, testis volume and the diameter of the seminiferous and epididymal tubules were analysed on a monthly basis and spermatogenesis documented. Although some males may remain in breeding condition until March, the absence of spermatozoa in the epididymis during February to June, when mean testis mass and mean tubule diameter reached a minimum, clearly showed reproductive quiescence following the rut. Four stages of spermatogenesis were observed:(1) inactive (February/March–June); (2)early spermatogenesis (July); (3) late spermatogenesis (July/August–December/January); and (4) epithelial regression (February–June). Individual variation between males, possibly differences in social status and body condition, may influence the duration of spermatogenesis, hence the overlap in duration between epithelial regression and inactivity. It appears that photoperiod may act as an obligatory proximate factor initiating spermatogenesis 3–4 months before the relatively short breeding season from November to December.

Key words: Cape fur seal, *Arctocephalus pusillus pusillus*, reproduction, testis, histology, spermatogenesis, annual cycle

INTRODUCTION

Following some earlier anatomical studies reviewed by Harrison (1969) and Laws & Sinha (1993), there has been little concerted effort to document the seasonal cycle or physiology of reproduction in male pinnipeds (Boyd, 1991). Detailed anatomical and histological descriptions of the annual cycle of the gonads (Laws, 1956; Griffiths, 1984*a*, *b*), age at puberty (Laws, 1956), and endocrine regulation of seasonal breeding (Griffiths & Bryden, 1981; Griffiths, 1985) have been compiled for the southern elephant seal *Mirounga leonina*. Other pinniped species have been less well studied (Boyd, 1991; Laws & Sinha, 1993). With the exception of the sub-Antarctic fur seal *Arctocephalus tropicalis* (Bester, 1990) and the northern fur seal *Callorhinus ursinus* (Kenyon *et al.,* 1954; Ashchepkova & Fedoseev, 1988), our understanding of the seasonal cycle of reproduction in male otariids is rudimentary.

It is thought that males of all seasonally breeding pinnipeds experience periods of fertility and infertility (Boyd, 1991). However, difficulties in obtaining reproductive material outside the breeding season have generally restricted longitudinal seasonal studies that might confirm this theory (see Hamilton, 1939; Bertram, 1940; Harrison *et al.,* 1952; Harrison, 1960; Bigg, 1969). Seasonal breeding, in which periods of sexual quiescence alternate with periods of sexual activity, has been documented in several species of phocids (Laws, 1956; McLaren, 1958; Carrick, Csordas & Ingham, 1962, Carrick, Csordas, Ingham & Keith, 1962, Boulva & McLaren, 1979; Griffiths, 1984*a*, *b*; Ryg, Smith & Oritsland, 1991), and two species of otariids – *C. ursinus* (Kenyon *et al.*, 1954; Ashchepkova & Fedoseev, 1988) and *A. tropicalis* (Bester, 1990). Preliminary findings suggest that the gonads of the Antarctic fur seal *Arctocephalus gazella* are also seasonally active (Laws & Sinha, 1993). These studies indicate that mean testis mass and seminiferous tubule diameter increase before and during the breeding season. The testes then regress and the epididymis is aspermatic. Testicular changes coincide with the onset and cessation of spermatogenesis.

The present paper provides a monthly description of the annual cycle of the testis and epididymis in the male Cape fur seal *Arctocephalus pusillus pusillus* with quantitative measurements of testis weight, testis volume, and the diameter of the seminiferous and epididymal tubules. A photographic record of the spermatogenic cycle is presented and age at puberty determined. A brief description of reproductive anatomy in an 8-year old male is also provided.

Study animal

Arctocephalus p. pusillus breeds at 25 colonies distributed from Algoa Bay (lat. 34˚S, long. 26˚E) on the south-east coast of South Africa, to Cape Cross (lat. 21˚46'S, long. 13˚57'E), Namibia. Preferred habitat and associated climatic conditions have been described by Rand (1967) and Warneke & Shaughnessy (1985). As with other otariids, annual reproduction is characterized by synchronous breeding, embryonic diapause, a polygynous breeding system, pronounced sexual dimorphism, copulation soon after parturition, protracted lactation periods and, a comparatively slow pup growth rate. The breeding (pupping and mating) season extends from November to late December (Shaughnessy & Best, unpubl. report). Reproduction in the female has been described (Rand, 1955; Warneke & Shaughnessy, 1985; David & Rand, 1986); however, little is known of the male cycle.

MATERIALS AND METHODS

Cape fur seals were collected at random during Sea Fisheries Research Institute research cruises and routine field trips to breeding rookeries between 34˚S, 26˚E and 18˚S, 12˚E from 1974 to 1990. Most animals were shot at sea with a 12-bore shotgun for dietary studies; collection details are provided by David (1987*a*).

In the field, routine necropsies were performed and biological parameters recorded, based on recommendations of the Committee on Marine Mammals, American Society of Mammalogists (1967). The right testis with attached epididymis was measured (length × width × height) to the nearest 1.0 mm, frozen, and brought back to the laboratory to be weighed on an electronic balance. An equatorial segment of each left testis with attached epididymis was stored in 10% phosphate buffered formalin for subsequent histological analysis. Upper canines were collected for age determination. Specimens were accessioned at the Sea Fisheries Research Institute, Cape Town. From this collection, 99 males – 10 months to 13^{+} years of age – were selected for reproductive studies: 40 males (< 5 years) were examined to determine age at puberty and 59 males (≥ 4 years of age) were used to determine the yearly cycle of the testis and epididymis.

The gonads of males < 5 years of age (n = 40 seals) were examined to determine the age at puberty; the age at which sperm first accumulates within the seminiferous and epididymal tubules in quantity. These animals were collected during September to January when pubertal males should be in reproductive condition (see Results). Testes were considered pre-pubertal when the tubules were completely aspermatic and the epididymis was devoid of sperm. All males < 4 years of age (n = 21) had been tagged within 6 weeks of birth and were therefore of known-age.

The gonads of post-pubertal males ≥ 4 years of age (n = 59 seals) were examined to determine the annual cycle of the testis and epididymis (i.e., 4–5 seals for each calendar month). Preliminary age determination suggested males 4 years of age were at least 145 cm long (SBL); therefore, seals exceeding this length were selected from the collection and then aged at a later date. Seasonal studies were limited to 4–5 adult

males per month because few specimens were collected during the summer period and accessioned material was not always suitable. Although socially mature specimens were included in this study, territorial status was unknown because animals were shot at sea. Males in prime 'breeding condition' were not collected from territories.

The body of the left testis, and the attached epididymis of each specimen were sliced into thin cross-sections, dehydrated, and embedded in paraplast-wax using standard histological procedures (Drury & Wallington, 1967). Equatorial segments were sectioned at 5 µm and stained with Ehrlich's haematoxylin and counter-stained in eosin. An image analysing computer (Quantimet 520, Cambridge Instruments) was used to measure the diameter of both the seminiferous and epididymal tubules. Specimens (n = 59) were examined under × 10 objective and diameters were calculated as the mean of 10 measurements of circular profile. The nucleus diameter of 20 randomly selected Leydig cells from 4 males collected in June (gonads inactive) and 4 males collected during August (gonads active) were measured to 0.5 µm using an eyepiece micrometer.

Histological sections of the testis and epididymis were categorized into one of 4 stages: (1) inactive (spermatogonia and Sertoli cells present; no sperm in the epididymis); (2) early spermatogenesis (spermatogonia and spermatocytes present; no sperm in the epididymis); (3) late spermatogenesis (spermatogonia, spermatocytes, spermatids and spermatozoa present; sperm in epididymis) and (4) epithelial regression (reduced number of epithelial cells with evidence of degeneration; sperm absent from the epididymis). Specimens were assigned to a given category if > 50% of the tubules examined (i.e., 50 seminiferous/10 epididymal tubules per animal) showed features characteristic of that condition (modified from Bernard, Cotterill & Fergusson, 1996).

An additional tissue processing technique was incorporated into this study in order to document the finer detail of the spermatogenic cycle. The left testis was sliced into 8 × 2 mm sections and rinsed 3 times in 0.075 M sodium phosphate. Tissues were dehydrated in 70% ethanol (15 min), 100% ethanol (15 min), 100% n-propanol (1 h) and 100% n-butanol (1 day). Dehydrated tissues were placed in gelatin capsules filled with complete monomer solution (dissolve 180 ml purified glycol methacrylate; 12 ml PEG/Carbowax 200^T and 1 g benzoyl peroxide using an ultra sound) and infiltrated at room temperature for 12 h, 12 h, and then 3 days, following changes in solution. Tissues were then placed in an oven (24 h at 60˚C) for polymerization, sectioned at 2 µm using an ultra-microtome and stained with 0.5% toluidine blue borax buffer for light microscopical observations (modified from Feder & O'Brien, 1968).

The age of animals was estimated from counts of incremental lines observed in the dentine of tooth sections. Upper canines were sectioned longitudinally using a circular diamond saw. Sections were ground down to 280–320 µm, dehydrated, embedded in resin and viewed under a stereomicroscope in polarized light (Oosthuizen, 1997). Each section was counted 5 times by an individual reader and ages were rounded off to the nearest birth date. The median date of birth was assumed to be 1 December (Shaughnessy & Best, unpubl. report). Closure of the pulp cavity prevented precise age determination of older animals (i.e., > 13 years) (Oosthuizen, 1997).

Mean testis volume was calculated as follows: (testis width + testis height)/2) × π × testis length (Graham Ross, pers. comm.). Monthly and seasonal reproductive parameters were compared using 1-way analysis of variance (ANOVA), Genstat 5 Release 3 (Genstat 5 Committee, 1993). Mean values are given as ± 1 standard deviation unless otherwise stated.

RESULTS

Morphology

The otariid testes are said to be scrotal (Laws & Sinha, 1993). In the adult male Cape fur seal, the scrotum appears to be fully pendulous only when the animal is in prime 'breeding condition', actively defending territory. However, for animals shot at sea and not actively defending territory, the testes are usually withdrawn into the groin, positioned obliquely either side of the penis, external to the muscle of the abdominal wall (Fig. 8.1). The left testis is more caudal than the right. The testis and epididymis are enclosed in the tunica albuginea. The epididymis is a narrow, elongated structure (with little differentiation into caput, cauda and corpus), attached to the lateral surface of the testis. The head of the epididymis is located at the cranial extremity of the testis; the epididymal tail is at the caudal end of the testis, and differentiates into the ductus deferens which extends into the peritoneal cavity through the processus vaginalis. The ductus deferens loops over the ureter and extends posteriorly to the prostate gland at the base of the bladder.

The mean dimensions of the right testis, with attached epididymis, were length (68 ± 5.6 mm) × height (22 ± 3.5 mm) × width (31 ± 3.1 mm), and mean weight was 24 ± 4.7 g (17 post-pubertal males collected between July/August–January/Febuary). Gonads from harem bulls were not available for analyses.

Age at puberty

The epididymal tubules of seals < 2 years of age were aspermatic. The youngest male in which sperm was observed in both the seminiferous and epididymal tubules was 2 years 10 months old. All males 3 years of age had attained puberty (Fig. 8.2).

Testes weight and volume of post-pubertal males

Growth in body weight with age is presented in Table 8.1. Body weight of 59 post-pubertal males ranged from 59 to 289 kg. Therefore, it was necessary to adjust for body weight when examining seasonal fluctuations

in mean testis weight and testis volume (i.e., log body weight was treated as a covariate of log testis weight and log testis volume). It is important to note that because males of the same age may be of different sizes, and individual weights may vary seasonally (see Schusterman & Gentry, 1971), the calculation of seasonal fluctuations in mean testis weight and volume is more complex than is presented in this study.

Mean testis weight differed significantly between the spring/summer (22 September–21 March) and autumn/winter (22 March–21 September) seasons ($F_{1,46}$ = 9.9; P = 0.003). Testis weights increased in July, remained high throughout the pupping/mating season (November–December), declined in February (post-breeding), and remained low until the following June (Fig. 8.3a). A second, insignificant increase in mean testis weight was observed in March. When younger animals (5–7 years) were excluded from the analysis, observed seasonal trends remained significantly different ($F_{1,23}$ = 14.7; P < 0.001).

This seasonal trend was also evident in mean testis volume ($F_{1,47}$ = 2.5; P = 0.123). Although testis volumes were also low from February to June, they increased continually until October, but then declined towards the end of the pupping/mating season (Fig. 8.3b). Change in mean testis volume was primarily attributed to fluctuation in testis length ($F_{1,49}$ = 10.5; P = 0.002) and width ($F_{1,49}$ = 8.2; P = 0.006) which increased with the onset of spermatogenesis. Seasonal fluctuation in testis height was minimal.

The annual cycle of the seminiferous and epididymal tubules

Mean diameter of the seminiferous ($F_{1,56}$ = 12.0; P = 0.001) and epididymal tubules ($F_{1,56}$ = 28.3; P < 0.001) differed significantly between the spring/summer and autumn/winter seasons. Seminiferous tubule diameters peaked in October (arrival of males at the rookeries), remained high throughout the pupping/mating season (November/December), declined in January/February (post-breeding), and remained low until the following June (Fig. 8.4a). Epididymal tubule diameters were high from September to December, declined in January, and remained low until August (Fig. 8.4b).

The spermatogenic cycle

Inactive

After breeding, from February to the end of June, the testes of all males had regressed, with the exception of one 9-year old male collected in March. Six (24%) of the testes of reproductively regressed males (n = 24) were inactive. Seminiferous tubules of inactive testes contained only spermatogonia and Sertoli cells, although a limited number of round primary spermatocytes were present in some tubules (Fig. 8.5a). Strands of Sertoli cell cytoplasm and cellular debris filled the lumen. Tubule diameter was minimal. A considerable amount of connective tissue surrounded individual tubules and the nuclei of Leydig cells were small. Epididymal tubules were devoid of sperm (Fig. 8.6e).

Early spermatogenesis

The first signs of spermatogenic activity were observed in July. Tubules contained several rows of round spermatids (Fig. 8.5b) and sperm was absent from the epididymis. Seminiferous and epididymal tubule diameters, and testis weight and volume, increased during this period (Figs 8.3 & 8.4). Sperm had already accumulated in the epididymes of two 6-year old animals (i.e., late spermatogenesis). By August, sperm were present in the epididymis of 4 of the 5 males examined (Fig. 8.6f). The nuclei of hormone-producing Leydig cells had increased significantly in size from 6.3 ± 0.9 µm (June) to 8.1 ± 1.7 µm (August) ($F_{1,7}$ = 58.8; P < 0.001).

Late spermatogenesis

From July/August to December/January, all stages of germ cell development were observed (Figs 8.5c–f & 8.6a). Sertoli cells were elongated, extending from the basal lamina to the base of maturing spermatids. From July to the onset of the breeding season testis weight, testis volume and seminiferous tubule diameter increased steadily (Fig. 8.3 & 3.4). The seminiferous tubules were closely packed (little connective tissue) and Leydig cells were enlarged. However, towards the end of the breeding season spermatogenic cells began to show varying degrees of epithelial regression and a slight decrease in testis weights.

Epithelial regression

From February to June, the testes of all males had regressed (Fig. 8.6b–c) with the exception of one 9-year old male collected in March. The diameter of the seminiferous tubules was minimal and there was a corresponding decrease in mean testis weight and volume. Epithelial height was greatly reduced. Spermatids and spermatocytes were eliminated into the lumen and often fused to form multinucleated giant cells comprising 8–31 nuclei (Fig. 8.6d). Giant cells were observed from December to June, but were not evident in all sections. Sperm was absent from the epididymis during this period. The transition phase between epithelial regression and inactivity was not established, yet started as early as February in one 10-year old male.

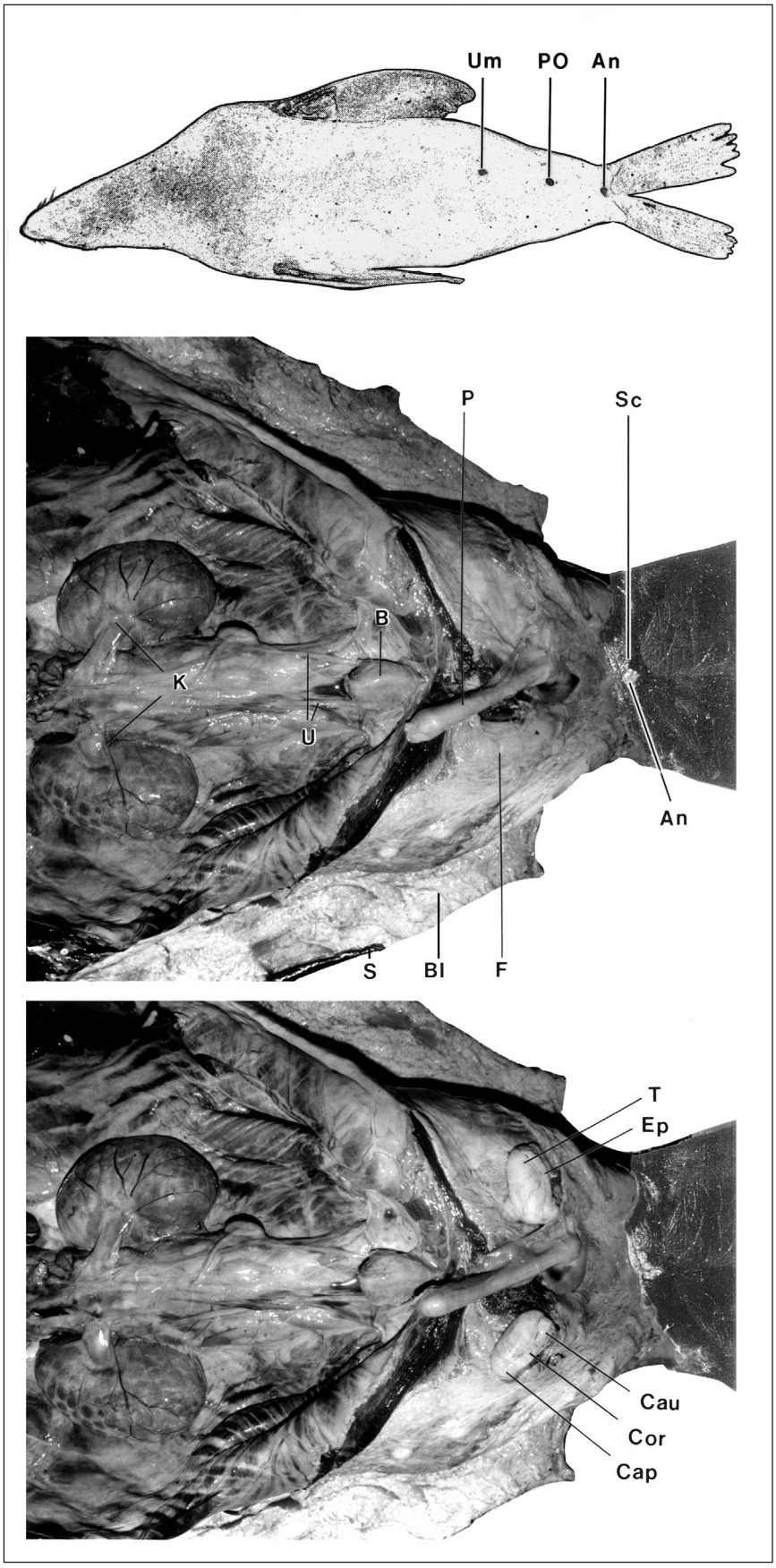

Fig. 8.1 Reproductive tract of an 8-year old male *A. p. pusillus* collected in July showing appearance of genital opening and orientation of the testes.

Anus (An); bladder (B); blubber (Bl); epididymis (Ep): Cap, Caput; Cor, Corpus; Cau, Cauda; fascia (F); kidney (K); penis (P); penial opening (PO); skin (S); scrotum (Sc); testis (T); ureter (U); umbilicus (Um).

Table 8.1 ***Growth in body weight and testis weight with age in male A. p. pusillus (n = 99 seals)***

Mean age[a] (years)	No. of males	Body weight[b] (kg) ± S.D.	Right testis weight[c] (g) ± S.D.
1	3	12 ± 5	1.2 ± 0.2
2	5	20 (2)	2.2 (2)
3	19	52 ± 13 (7)	14.1 ± 0.3 (7)
4	15	59 ± 10 (12)	18.9 ± 0.5 (13)
5	4	75 (2)	24.5 ± 11.3 (3)
6	12	84 ± 12 (11)	18.4 ± 12.5 (11)
7	6	100 ± 24	19.9 ± 5.1 (5)
8	9	116 ± 35	21.0 ± 4.0
9	7	119 ± 18 (6)	20.0 ± 4.6
10	4	125 ± 30	19.6 ± 1.3 (3)
11	4	139 ± 48	24.0 ± 8.3
12	4	154 ± 41	25.0 ± 5.8 (3)
13+	3	212 (2)	26.4 (1)
Total[d]	**95**		

[a] Age was estimated from tooth sections; all males < 4 years of age (*n* = 21) had been tagged within 6 weeks of birth and were therefore of known-age.

[b] Body weights and testes weights were not available for all males. Sample sizes are indicated in parentheses.

[c] Testis weights = each right testis with attached epididymis.

[d] No upper canines for 4 post-pubertal males (i.e., total = 99 males of which 95 were aged).

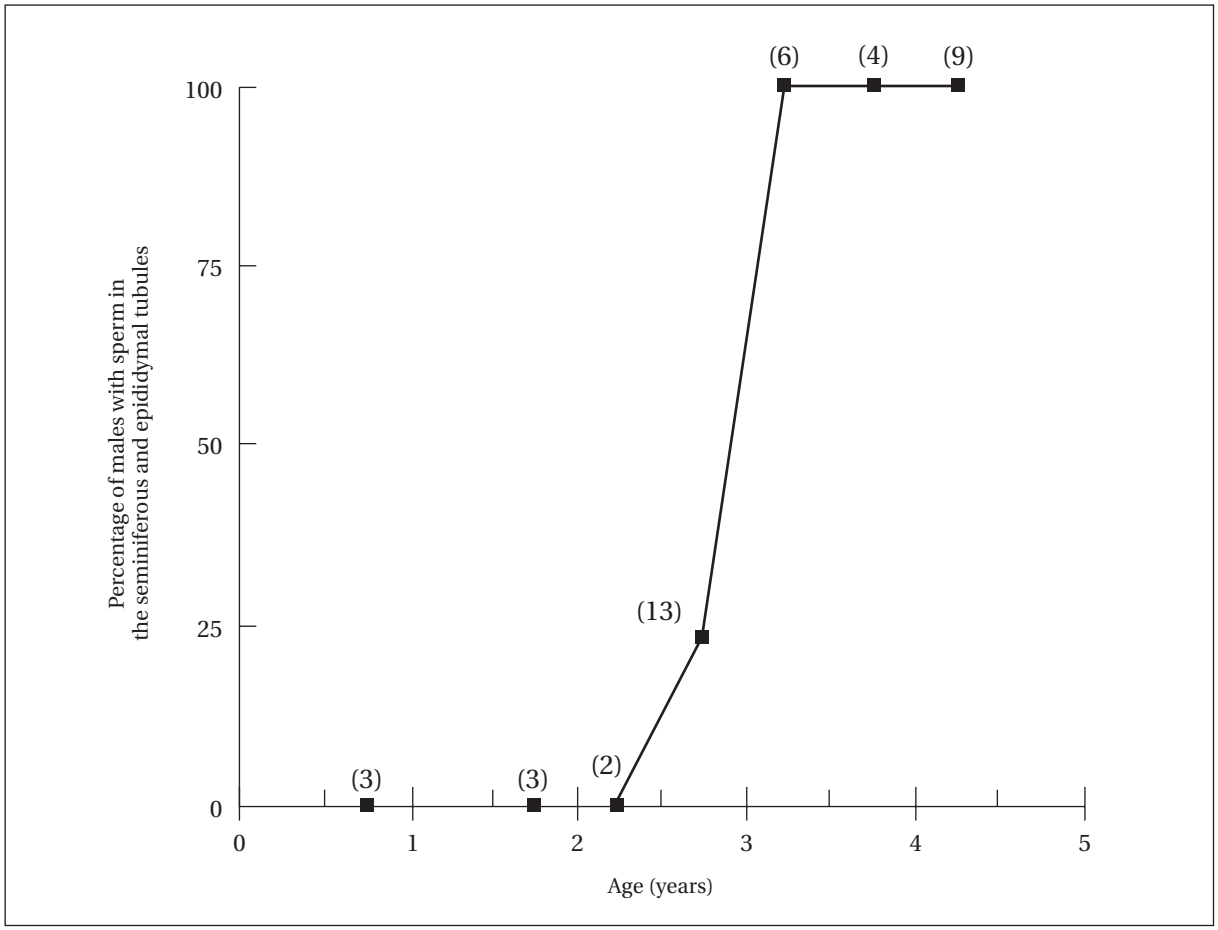

Fig. 8.2 Age at puberty determined by the presence of sperm in both the seminiferous and epididymal tubules of *A. p. pusillus* (*n* = 40 males) collected between September and January.

All males < 4 years of age (*n* = 21) had been tagged within 6 weeks of birth and were therefore of known-age. Sample size in parentheses.

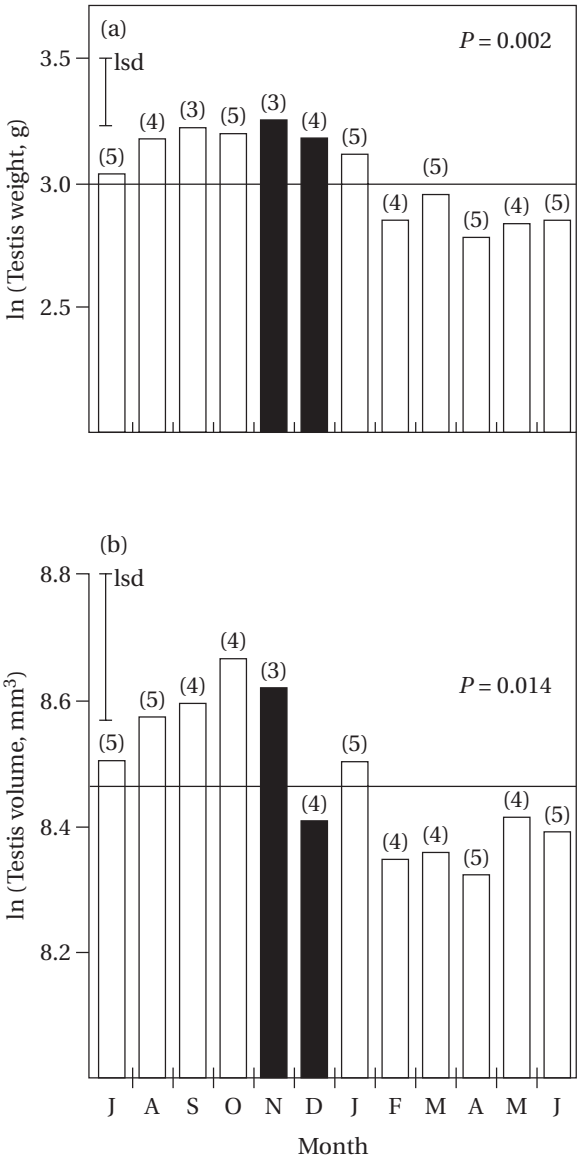

Fig. 8.3 Mean monthly (a) testis weight and (b) testis volume in post-pubertal *A. p. pusillus*.

Mean values were obtained from the right testis with attached epididymis. Body weight of seals ranged from 59 to 289 kg, therefore it was necessary to adjust for body weight (i.e., log body weight was treated as a covariate of log testis weight and log testis volume). Sample sizes in parentheses; solid bars, pupping/mating season; horizontal line, grand means.

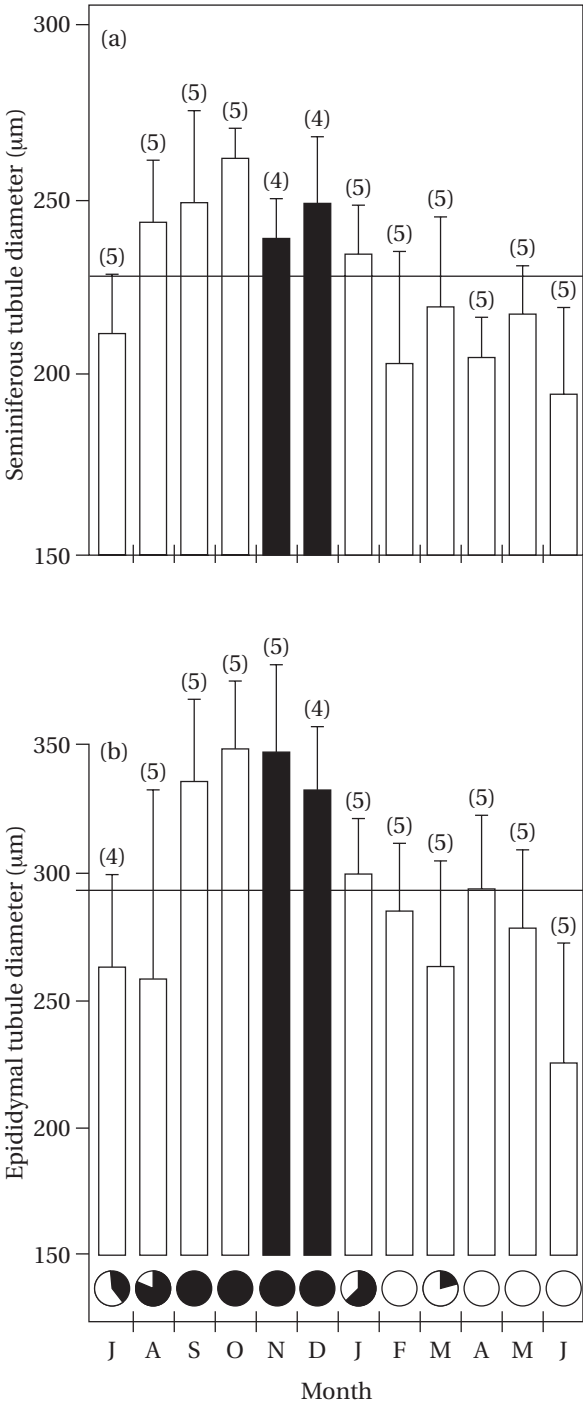

Fig. 8.4 Mean monthly diameter of (a) seminiferous tubules and (b) epididymal tubules in post-pubertal *A. p. pusillus*.

Vertical bars are 1 sample standard deviation. Sample sizes in parentheses; solid bars, pupping/mating season; horizontal line, grand means. The presence of sperm in the epididymis is indicated in fifths: (●) Spermatozoa present in the seminiferous and epididymal tubules of all males; (○) No spermatozoa.

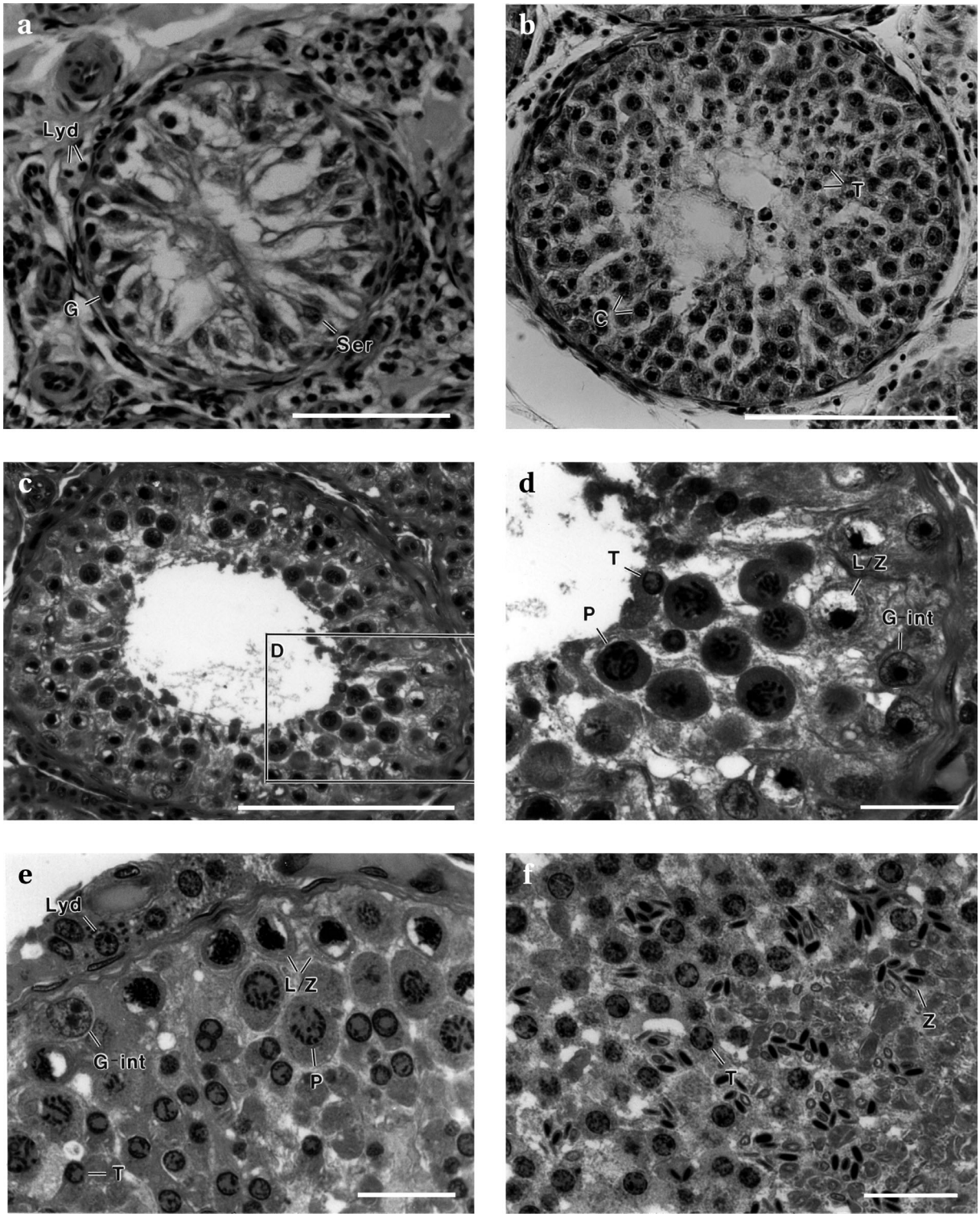

Fig. 8.5 Histological sections of the testis in post-pubertal *A. p. pusillus* collected during the breeding and non-breeding season.

(a) Inactive: seminiferous tubule collected in April showing Sertoli cells (Ser), type Ad spermatogonia (G) and strands of Sertoli cell cytoplasm within the lumen; Leydig cells (Lyd) small.

(b) Early spermatogenesis: seminiferous tubule (July) showing primary spermatocytes (C) with condensed nuclear chromatin and several rows of young spermatids (T).

(c–f) Active spermatogenesis: (c–d) seminiferous tubule showing spermatogonia in interphase (G-int); leptotene/zygotene (L/Z) and pachytene (P) division of primary spermatocytes, and young spermatids (T); (e) differentiation of primary spermatocytes; spermatogonia in interphase (G-int); leptotene/zygotene (L/Z) and pachytene (P) stages of primary spermatocytes and a large number of young spermatids (T); Leydig cells (Lyd) enlarged; (f) spermiogenesis showing spherical nuclei of young spermatids (T) and elongated nuclei of maturing spermatids (Z).

Scale bars: a–c = 50 µm; d–f = 10 µm. H & E = a–b; Toluidine = c–f.

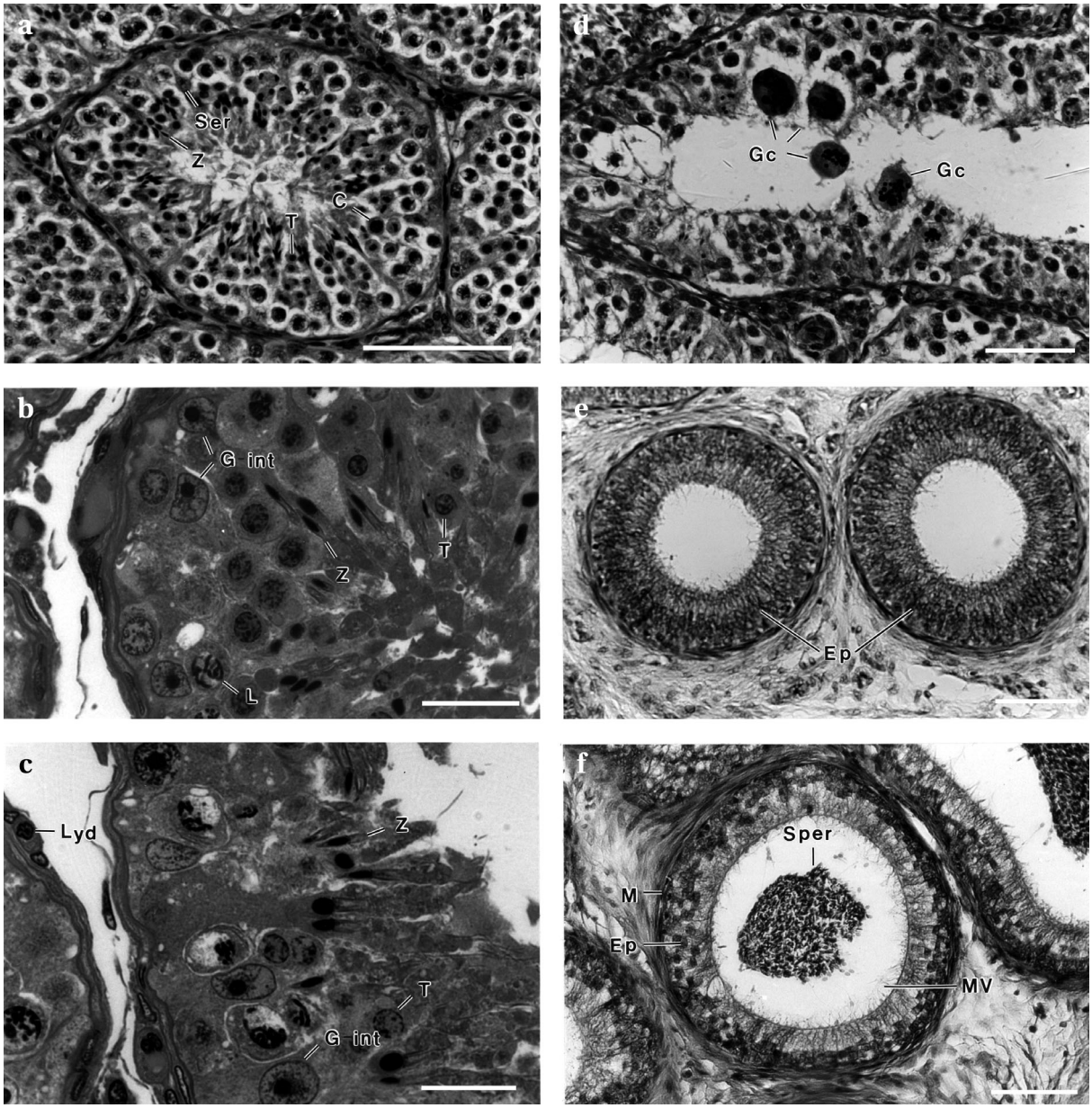

Fig. 8.6 Histological sections of the testis and epididymis in post-pubertal *A. p. pusillus* collected during the breeding and non-breeding season.

(a) Late spermatogenesis: seminiferous tubule (November) showing maturing spermatids (Z); individual Sertoli cells (Ser) extend from the basement membrane to the heads of elongating spermatids and are surrounded by irregular columns of spermatocytes (C) and round spermatids (T); scale bar = 50 µm.

(b–d) Epithelial regression: (b–c) seminiferous tubule (February) showing spermatogonia in interphase (G-int); leptotene (L) stages of primary spermatocytes and mature spermatids (Z); spermatogenic cells have decreased in number and the lumen is filled with Sertoli cytoplasm; Leydig cells (Lyd) are small and irregular in shape; scale bar = 20 µm; (d) regressed seminiferous tubule (February) showing depletion of the germinal epithelium and the formation of giant cells (Gc); scale bar = 80 µm.

(e–f) Epididymis: (e) Epididymis (June); tubule diameter small, lumen area reduced and columnar epithelium height at a maximum. Note the absence of sperm; scale bar = 50 µm; (f) Epididymis (November); tubule diameter large, lumen wide and columnar epithelium height reduced; note the accumulation of sperm (Sper) within the lumen, tall microvilli (MV) and narrow band of circular muscle fibre (M); scale bar = 50 µm.

H & E = a & d–f; Toluidine = b–c.

DISCUSSION

The gross anatomy of the male reproductive tract conforms with the standard otariid pattern described by Laws & Sinha (1993); however, the position of the testes is variable. The position of the testes is a characteristic feature used to distinguish otariids (i.e., testes scrotal) and phocids (i.e., testes inguinal). The testes of the Cape fur seal are generally withdrawn into the groin and covered by a layer of blubber and fascia, protected within the cremasteric pouch, the anterior section of the scrotum. The scrotum appears to be fully pendulous only when the testes descend from the pouch, when the seal is in prime 'breeding condition', actively defending territory; or when the seal is suffering heat stress (see Laws & Sinha, 1993). Within the scrotum, the testes are at least 6˚C below body temperature (Bartholomew & Wilke, 1956). Thus, externalization of the testes may optimize sperm production during long periods of territorial tenure, when fighting and sunny conditions elevate body temperature.

It has now been established that male *A. p. pusillus* reach puberty between 3 and 4 years of age. By comparison, male *A. gazella* attain puberty at 3–4 years of age (McCann & Doidge, 1987), *A. tropicalis* at ≥ 4 years (Bester, 1990) and *Arctocephalus pusillus doriferus* at 4–5 years (Warneke & Shaughnessy, 1985). In captive populations, male Cape fur seals are capable of fertilising receptive females at 4 years of age (Linda Clokie-Van Zyl, pers. comm.), which supports the estimated age at puberty arrived at in the present study.

In wild populations, male Cape fur seals do not have the ability to acquire and maintain a harem until they are approximately 10–14 years of age (David, 1989). Large body size and the ability to gain high social rank may contribute to the lifetime reproductive success of this polygynous breeding species (see Bartholomew, 1952, 1970; Le Boeuf & Peterson, 1969; Le Boeuf *et al.,* 1972; Le Boeuf, 1974, 1981; Miller, 1975; Cox & Le Boeuf, 1977; Le Boeuf & Briggs, 1977; McCann, 1980; Anderson & Fedak, 1985; Le Boeuf & Reiter, 1988; Deutsch *et al.,* 1990; Andersson, 1994). The estimated maximum longevity of the male Cape fur seal is at least 20 years of age (Wickens, 1993). During this time, a male may mate multiple times over a 2 to 3 year period (Gentry & Kooyman, 1986), or longer (Oosthuizen, pers. obs). Although little is known of changes in fertility with age, it is likely that males die before reaching reproductive senescence (e.g., Laws, 1956). Although few males ≥ 12 years of age (n = 7) were collected in this study, histological examination of the testes did not suggest a decline in sexual activity with age.

The seasonal cycle of the testis in adult *A. p. pusillus* is similar to many other temperate, seasonal breeding mammals (Sadleir, 1969). A significant increase in both testicular mass and tubule dimensions reflects a fertile period extending from July/August to December/January. Comparison of quiescent and active testes suggested that Leydig cell activity increased with the onset of spermatogenesis. Although some males may remain active until March (e.g., one 9-year old male), the absence of spermatozoa in the epididymis during February to June, when mean testis mass and mean tubule diameter reached a minimum, clearly showed that *A. p. pusillus* males were quiescent shortly after the rut. Similar findings were reported in *A. tropicalis*; fertile periods extended from September/October to January/Febuary, with some individuals also remaining active until March (Bester, 1990). Although species at higher latitudes generally have shorter breeding seasons (Gentry *et al.*, 1986), *A. tropicalis* (38–48˚ lat.) in fact has a slightly longer breeding season (i.e., November to early January) than *A. p. pusillus* (18–34˚ lat.); therefore, it is not unexpected that the duration of male fertility is similar in the two species.

Four stages of spermatogenesis were observed: (1) inactive (February/March–June); (2) early spermatogenesis (July); (3) late spermatogenesis (July/August–December/January) and (4) epithelial regression (February–June). Germ cell proliferation appeared to follow the normal mammalian pattern (Guraya, 1987), but the transition stage between epithelial regression and inactivity was not as well defined as for Antarctic phocids (Laws, 1956). These two stages, although distinctly different, overlapped in time (i.e., February to June). Only six of the 24 reproductively regressed males collected between February and June were inactive; one 9-year old was active (n = 25). Individual variation between males, particularly differences in social status and body condition, may influence the interval between reproductive regression and inactivity (e.g., Laws, 1956; Vivier & Van der Merwe, 1996). Harem bulls may fast for up to 40 days, and then abandon their territories when they have exhausted their physiological reserves; if they cannot replenish them quickly, they will die (Wartzok, 1991). In contrast, younger males or subordinates do not fast so rigorously; therefore, may remain in breeding condition longer than harem bulls.

Large breeding bulls (up to 350 kg) arrive at the rookeries late October/early November (Rand, 1967) and are therefore in breeding condition (this study). Pregnant females gather in high densities shortly afterwards to find suitable pupping sites. Mean harem size is variable and ranges from approximately 3–28 females (Rand, 1972). Females give birth to a single black pup within 1–2 days of coming ashore. Ninety percent of pups are born over a 34-day period and mating occurs 6 days post-partum (Shaughnessy, 1979). David & Rand (1986) reported 90% of births in a 26-day period in Namibia. Spatial separation of food resources from the breeding rookeries requires males to fast in order to ensure access to receptive females (Boness, 1991). Bulls may stay ashore without

feeding for up to 40 days (Rand, 1967). Large body size enables breeding bulls to remain on territory for extended periods (i.e., metabolize extensive blubber stores), and also provides a direct advantage in competitive interactions (i.e., greater strength) (Rand, 1967; Bartholomew, 1970; Wartzok, 1991). The territorial system gradually breaks down in late December/early January as dominant bulls return to sea to replenish their physiological reserves (Rand, 1967). This period marks the onset of epithelial regression and finally a decline in testes volume and weight (this study).

Although no harem bulls were collected in this study, socially mature males (≥ 12 years of age) have larger gonads, in relation to body weight, than younger males (Table 8.1). Large gonad size would facilitate the production and storage of large quantities of sperm, enabling harem bulls to service many females within a short time (i.e., within 26–34 days) (Rand, 1967; David & Rand, 1986). The adaptations allowing for production of large quantities of sperm over a short period have not been investigated in pinnipeds (Wartzok, 1991); however, Bartholomew & Hoel (1953) found that harem bulls are able to produce sufficient sperm to maintain high levels of conception in females (e.g., 161 northern fur seal females were mated by a single male). It is possible that Cape fur seals achieve a similar high level of polygyny in some colonies (David, 1987*b*; Boness, 1991).

It is generally believed that photoperiod is a proximate control factor in the annual cycle of pinniped reproduction (Boyd, 1991; 1996). Synchronized breeding is mediated through the pineal-pituitary axis and gonadotrophic action which varies according to species and latitude (Daniel, 1981; Griffiths & Bryden, 1981). When calculating mean monthly day length between 32° S and 34° S (the latitudinal band in which the majority of *A. p. pusillus* were collected) it is apparent that early spermatogenesis coincides with the initial increase in day length (July = 10 h 9 min) following the winter solstice (21–22 June), and pupping/mating coincides with the longest days (November = 13 h 52 min; December = 14 h 21 min). A second (non-significant) peak in testis weight was recorded in March (autumnal equinox), when the majority of males come ashore to moult. In *A. p. pusillus*, the adult moult extends from late January to mid-April, and peaks in early March (Warneke & Shaughnessy, 1985). Photoperiodic cueing is also thought to explain seasonal reproductive trends in male *M. leonina* (Griffiths, 1984*a*) and *A. tropicalis* (Bester, 1990).

Cape fur seals inhabit a temperate, moderately seasonal clime, dominated by areas of coastal upwelling and cold oceanic currents (Shannon, 1985). Their breeding cycle is shaped by reduced seasonality and unpredictable, yearly fluctuations in marine productivity (e.g., El Niño events/ Southern Oscillations) (Cane, 1983). Inshore on the west and south coasts, primary productivity is maximal in spring (September-November) and summer (December-February), when wind-induced upwelling is intense (Azam *et al.*, 1983; Shannon, 1985; Brown, 1992). It is during this period that Cape fur seals give birth (summer) and generally wean their pups (spring). Females suckle their young for 9–11 months, during which time they make many foraging trips to sea of short duration (David & Rand, 1986). If food resources are abundant, close to the rookery, lactating females would spend less time away from their pups, increasing the probability of reproductive success (Majluf, 1992). Thus, the need for abundant food resources after the perinatal fast and during 'early' foraging trips to sea may determine the timing of birth (Majluf, 1992). This is in agreement with Boyd (1996) who suggested that photoperiod is responsible for inducing implantation in Antarctic fur seals, and that the duration of pregnancy is increased in years associated with low availability of food (i.e., later births). Considering that 90% of Cape fur seal pups are born over a 34-day period from November to late December, and mating occurs 6 days post-partum (Shaughnessy, 1979), reproduction in the male is thus geared to coincide with the short summer oestrus. Spermatogenesis begins 3–4 months before the breeding season (this study) to enable complete maturation of the testis (Setchell, 1978) and to cover any small temporal shifts in the receptiveness of females during the brief breeding season.

In conclusion, we have established that male Cape fur seals reach puberty at between 3 and 4 years of age and that bulls are seasonally active, with spermatogenesis ending shortly after the rut, and it is suggested that the regular cue of photoperiod entrains reproduction. Further studies addressing: (i) the relationship between growth in body weight and sperm accumulation in the epididymis; (ii) seasonal changes in reproductive endocrinology; and (iii) age at reproductive senescence, would greatly improve our understanding of reproduction in this species. Long-term studies documenting the seasonal distribution and abundance of prey species in relation to the energetics of lactation are required to test the hypothesis that reproductive synchrony may be largely determined by food availability in the summer months.

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