Seasonal and age-related changes in the micro-anatomy of the prostate gland of the Subantarctic fur seal, *Arctocephalus tropicalis*

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The prostate glands of male Subantarctic fur seals *Arctocephalus tropicalis*, taken at Gough Island (40°20'S, 09°54'W) between November 1977 and October 1978, were examined. Significant changes in the mass of the prostate, the diameter of the alveoli and height of the secretory epithelium precede the breeding season and support the fact that *A. tropicalis* is a seasonal breeder. Peak activity of the prostate is reached during November-January. We propose that seasonal changes in the prostate gland, and in male reproductive activity in general, may be cued by changing photoperiod. Micro-anatomical features of the prostate of the male Subantarctic fur seal differ between different age groups. Peak prostate mass, alveoli diameter and secretory cell height indicate that Subantarctic fur seal males reach full adulthood at 10–11 years of age.

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Introduction

An understanding of the salient features of the reproductive cycle in seals is of interest not only to the specialist, but also to workers in other fields, and for comparative purposes. The complex social behaviour shown by seals is interpretable only in the light of the reproductive status of the animals concerned. An understanding of seal population dynamics can only be achieved if the reproductive success of the members of the population is known. Furthermore, observing changes in the mean age of puberty can be an indicator of changes in the population dynamics (Bester 1988).

Studies on the reproduction of pinnipeds have concentrated mainly on females and have described anatomical and histological characteristics of the female reproductive tract (Scheffer 1950; Rand 1955; Laws 1956; Craig 1964; Boyd 1991; Tedman 1991; Bester 1995). Studies on the male reproductive system in pinnipeds have concentrated on phocids (Harrison 1969; Laws & Sinha 1994). These studies dealt with the histology of the testis and epididymis (Laws 1956), endocrine regulation of seasonal breeding (Griffiths 1985), fine structure of seminiferous tubules and Leydig cells (Sinha, Erickson & Seal 1977) and the annual cycle in the male (Laws 1956; Griffiths 1984a,b; Bartsh, Johnston & Siniff 1992). Recently, attention was accorded to the seasonal reproductive cycle of male fur seals (Bester 1990; Stewardson, Bester & Oosthuizen 1998). However, published accounts of the histology and annual cycle of the prostate of seals are limited (Bester 1990; Laws & Sinha 1994).

Subantarctic fur seals show a regular annual cycle imposed by the seasonality of the subpolar environment. Male Subantarctic fur seals converge on Gough Island (40°20'S, 09°54'W) during the austral summer, and disperse in winter (Bester 1981). They breed on land, in large aggregations, on rocky coasts from November to January. During winter males are present in low numbers when they haul out occasionally. Bester (1990) gave good evidence of seasonal activity in the male Subantarctic fur seal reproduction based on examination of the testis and plasma testosterone concentrations, and mass changes of the prostate gland.

The present study deals with the fine anatomy of the prostate of the Subantarctic fur seal at Gough Island. The aims are to determine if there are any changes in the microanatomy of the male fur seal prostate over the study period, which might correlate with the reproductive state, and how the size and histology of the prostate change with increasing age. The prostate glands used in this study came from animals that had been collected as part of a comprehensive study of the reproduction of the Subantarctic fur seal.

Materials and methods

Male Subantarctic fur seals were shot with a 0.22 rifle from close range on breeding, nonbreeding and idle colony sites at Gough Island (40°20'S, 09° 545'W). Samples were taken from November 1977 to October 1978 during monthly sampling periods. Each sampling period started on the 22nd day of a month and ended on the 21st day of the following month to coincide with the summer and winter solstice. The sampling procedure was selective to include all age groups. Body size and external morphological characteristics were used to judge age at the time of shooting (Bester 1990). The age at puberty was taken as the age at which reproduction first becomes possible, and sexual or social maturity as the age when the seal reaches its full reproductive capacity (Laws & Sinha 1994). Males were considered adult, that is fully grown, and displaying all the secondary sexual characteristics, when ≥ 10 years of age, following Bester (1990).

Tissue processing

The reproductive tracts of all males were dissected out and the prostate gland was weighed to the nearest 0.1 g using Pesola spring balances. A transverse slice of tissue was removed, fixed in Bouin's fluid for 24 h, and stored in 70% ethanol. The segments were processed using standard histological procedures. Sections were stained with haematoxylin and counterstained with eosin. The outside diameters [(width +depth)÷2] of 10 round alveoli viewed in cross-section were

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measured using a calibrated micrometer eyepiece and a 10 x objective. This measure was taken as an indication of the level of development of the prostate. For each of the 10 alveoli the height of 10 secretory cells was measured using the above procedure, and the mean ± SD calculated. The height of the cells was taken as evidence of the level of activity of the prostate gland (Johnson & Everitt 1991).

Age determination

Ages were estimated by examining the incremental lines in dentine of tooth sections (Bester 1990). Sections, 0.2 mm (200 μm) thick, were obtained from the upper right canine using a low-speed saw fitted with diamond wafering blades, and viewed under a binocular microscope. Ages were assigned in years rounded off to the closest birthday, assumed to lie between 9 and 13 December (Bester 1987). Pulp cavities usually closed at about 13 years, and most older animals were assigned to the 13+ age category (Bester 1987).

Statistical analysis

All means are followed by one standard deviation of the mean. Because of the small sample sizes, data sets for spring/summer (22 September to 21 March) and autumn/winter (22 March to 21 September) have been pooled and compared using a student's *t*-test, with significance set at the 1% level. For graphical representation and more detailed analysis, data have been grouped together into four seasons. Simple linear regressions were used to measure the bivariate normal-distribution data and correlation coefficients were considered significant at the 5% level.

Results

Seasonality

In adult males, mass of the prostate, height of the luminal secretory epithelium and diameter of the alveoli differed significantly between spring/summer and autumn/winter seasons (Table 1). Mean prostate mass (n = 56 inclusive of the 13+ age category) increased from August to October to reach a peak in the November to January sampling period, and decreased through February to April to a low in May to July (Figure 1a). The mean diameter of alveoli increased to a peak value in November to January, declining thereafter to a low during May to July (Figure 1b). This pattern is less evident in the height of the secretory cells which was greater in summer than winter (Figure 1c). We interpret these changes as indicating that the prostate is inactive in winter (May to July) and becomes secretorily active in summer.

Testis mass (data from Bester 1990) correlated significantly with prostate mass ($r = 0.679$, $d.f. = 38$, $p < 0.01$) during the summer months. Similarly, secretory cell activity correlated weakly with plasma testosterone concentration (data from

a)

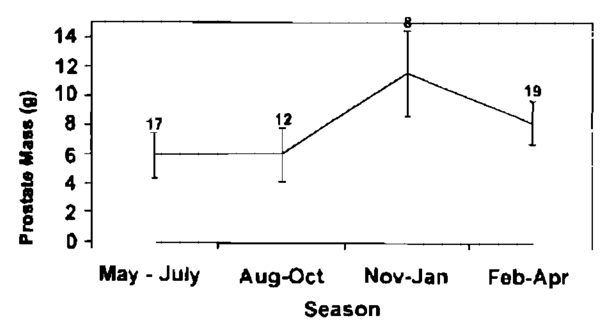

Figure 1a Seasonal variation in prostate mass in adult male *Arctocephalus tropicalis*. Sample sizes are given above the means and vertical lines represent one standard deviation

b)

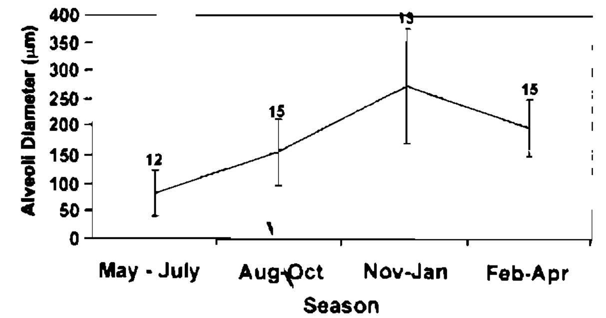

Figure 1b Seasonal variation in alveoli diameter in adult male *Arctocephalus tropicalis* prostates. Sample sizes are given above the means and vertical lines represent one standard deviation

c)

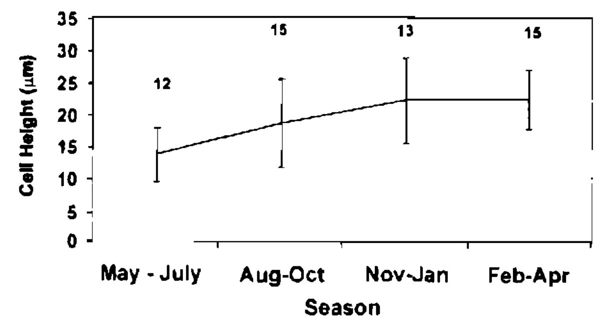

Figure 1c Seasonal variation in the height of luminal secretory epithelium in adult male *Arctocephalus tropicalis* prostates. Sample sizes are given above the means and vertical lines represent one standard deviation

Table 1 Seasonal changes in the size (mean ± 1sd) and micro-anatomy of the prostate of adult male *A. tropicalis*

	Seasonal average			
	22 Sept – 21 March	22 March – 21 Sept	*t*-values	Significance
Prostate weight (g)	8.678 ± 2.895	6.031 ± 1.706	0.00003	p < 0.001
Cell height (μm)	24.008 ± 6.343	15.035 ± 5.064	0.00001	p < 0.001
Alveolar diameter (μm)	213.899±86.87	109.921±68.85	0.00004	p < 0.001

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Bester 1990) during the summer months, although the correlation was significant ($r = 0.357$, $d.f. = 38$, $p < 0.05$).

Age-related changes to the size and histology of the prostate

Using only material which could be accurately aged and which was collected during the September/October to January/February period when pubertal males would be in reproductive condition (n = 53), prostate mass increased from the age of one year to peak at about 11 years. In the small sample of males older than 11 years, the mass of the prostate was highly variable but always greater than 7.8 g (Figure 2a). Mean diameter of the alveoli increased over the period of one to 11 years of age, with individual diameters varying between 100 and 350 µm in males older than 11 years (Figure 2b). Mean secretory cell height showed two periods of increase, a relatively rapid increase (7–21 µm) from the age of one to five, and a slower increase (20-30 µm) from about ten to fourteen years of age (Figure 2c).

Discussion

Seasonality

Otariid seals have a summer breeding (pupping and mating) season varying in length, dependant on the species and latitude (Bester 1981). Adult male Subantarctic fur seals breed (mate) from late November until mid-February at Gough Island (Bester 1981), with a peak in numbers ashore during December. Subsequent to the decline in numbers to end January, adult males start hauling out and numbers show a second peak in March during the moulting season (end-December to May) (Bester 1981). Most of the males are at sea during winter. The onset of reproductive activity of the males (Bester 1990) coincides with that of the females (Bester 1995), although the females arrive later (Bester 1981).

In many mammals, the sets of glands that open into the urethra, such as the prostate, enlarge dramatically just before the mating season. This phenomenon was first studied by Hunter (1935) and numerous studies on this phenomenon followed (e.g. Laws 1956; Racey 1978; Griffiths 1984a,b; Bester 1990; Stewardson *et al.* 1998). Studies on the seasonality of reproduction of seals indicate that there are significant increases in testis and epididymis mass (Scheffer 1950; Griffiths 1984a,b; Stewardson *et al.* 1998), plasma concentrations of testosterone (Bester 1990; Bartsh *et al.* 1992) and prostate mass (Bester 1990) preceding the breeding season.

Bester (1990) and Stewardson *et al.* (1998) found significant increases in mean seminiferous and epididymal tubule diameters during the spring and summer season, and recorded the absence of spermatozoa in the corpus epididymis in winter for Subantarctic and Cape fur seal males respectively. Seasonal infertility started in February/March and ended with fertility onset in August/September (Bester 1990; Stewardson *et al.* 1998). The present study shows similar significant responses in the micro-anatomy of the prostate preceding the breeding season. These findings support the fact that the Subantarctic fur seal is a strictly seasonal breeder.

Griffiths (1985) believes that there is a correlation between rate of change in day length and the activation of reproductive organs and accessory reproductive glands during the breeding season in the elephant seal. This would be mediated through the pineal gland-pituitary axis and gonadotrophic action on these tissues. Bester (1990) and Stewardson *et al* (1998) also suggested a photoperiodic influence on the reproductive cycle in the male Subantarctic and Cape fur seal respectively. When calculating mean monthly day length at 40 degrees South where Subantarctic fur seals breed at Gough Island, it is apparent that reactivation of the prostate coincides with the initial increase in day length (July = 09 h 35 min) following the winter solstice (21–22 June), and pupping/mating coincides with the longest days (November – 14 h 12 min; December = 14 h 57 min; January = 14 h 47 min). Seasonal

a)

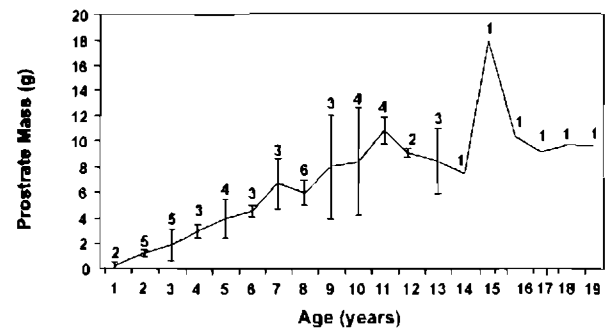

Figure 2a Increase in prostate mass with age in male *Arctocephalus tropicalis* collected during the September/October to January/February period. Sample sizes are given above the means and vertical lines represent one standard deviation

b)

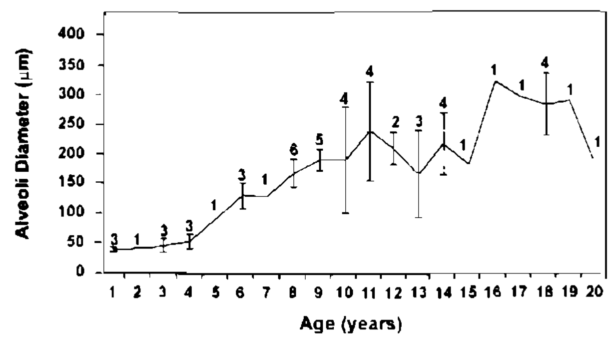

Figure 2b Increase in the alveoli diameter of the prostate with age in male *Arctocephalus tropicalis*. Sample sizes are given above the means and vertical lines represent one standard deviation

c)

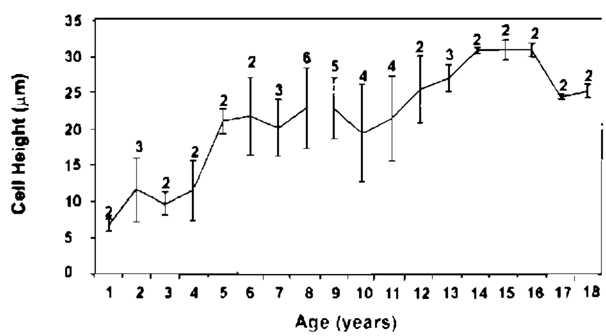

Figure 2c Increase in the height of the luminal secretory epithelium of the prostate with age in male *Arctocephalus tropicalis*. Sample sizes are given above the means and vertical lines represent one standard deviation

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micro-anatomical changes in the prostate of male Subantarctic fur seals may therefore also be an indirect response to seasonal changes in photoperiod.

Age-related changes

The presence of spermatozoa in the epididymides of male fur seals during the breeding season shows that puberty is reached at the age of 3–4 years (McCann & Doidge 1987; Bester 1990; Stewardson *et al.* 1998). A rapid increase in testis mass from one to five years of age, slowing perceptibly thereafter to peak at 11 years, indicates that sexual maturity has unequivocally been reached from 9 years of age (Bester 1990). All Subantarctic fur seal males have reached full adulthood at 10–11 years of age based on peak testis mass and mean baculum length. Similarly males in this study show a peak in mean prostate mass and diameter of the alveoli at 10–11 years of age and thus support previous findings of age at sexual maturity and the attainment of full adulthood. The first increase in height of the secretory cells coincides with the phase during which the males reach puberty while the second occurs at about the time, and just after males reach full sexual maturity.

It can be concluded that the onset of the breeding season coincides with significant changes in the micro-anatomy of the prostate gland of the Subantarctic fur seal, *A. tropicalis*. This variation in micro-anatomy of the prostate includes cell proliferation, variation in the diameter of the alveoli and changes in epithelial cell height. Using these variations as reproductive parameters, it can be concluded that all male Subantarctic fur seals breed seasonally and reach full adulthood at 10–11 years of age.

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