Aquatic Mammals 1991, **17.3**, 152–155

Testosterone profiles in male grey seals (*Halichoerus grypus*)

Alison J. Seely and Keith Ronald[1]

Department of Zoology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1
[1]The Institute of Environmental Policy and Stewardship, The Arboretum, University of Guelph, Ontario, Canada, N1G 2W1

Summary

Peripheral plasma samples of eight male grey seals (*Halichoerus grypus*) were analysed for testosterone concentration by radioimmunoassay. Levels in six males captured from Amet Island, Nova Scotia, at the beginning of the sampling period were contrasted with levels measured in two males raised in captivity. The male seals were housed in pairs in Ontario, Canada, and were exposed to female seals during the breeding season or during the entire season. Both groups of males underwent reproductive cycles of testosterone levels in synchrony with the cycle of wild captured females. The two males raised in captivity had lower levels of testosterone for significantly longer periods than their wild conspecifics, but the timing was similar and baseline levels did not differ in non-breeding months. Captive rearing in an outdoor enclosure in Ontario did not prevent a cyclic pattern of testosterone secretion.

Key words: testosterone; grey seal; *Halichoerus grypus*

Introduction

Interest in the management of seal species has grown in recent years, particularly in two groups with opposing idealogies. Real or imagined competition with fisheries has promoted demands for population control of 'pest' species such as the grey seal (Royal Commission, 1986). In contrast, greatly reduced stocks of other phocine species survive in small mainly undefined numbers in the wild and in controlled zoos and aquaria. Information on the breeding biology of seals is needed both to implement successful breeding programmes for endangered groups while offering guidelines to evaluate the repercussions of population control.

The class Phocidae share similar breeding patterns, with all those examined being seasonal breeders and exhibiting a period of delayed implantation (King, 1964). Most information on the grey seal (*Halichoerus grypus*), has been furnished through lethal histological studies during the short breeding season lasting roughly one month (Backhouse, 1964; Boyd, 1983; Hobson and Boyd, 1984; Mansfield, 1973; Ronald *et al.*, 1982), a pattern repeated in studies of other phocid species (e.g. Amoroso *et al.*, 1965, Craig, 1963; Daniel, 1981). Non invasive methods of evaluating reproductive condition are needed to supplement this anatomical detail for maintenance of captive populations.

Mating in grey seals follows the events of pupping and three weeks of lactation (Backhouse and Hewer, 1964). The breeding season varies considerably between the three grey seal populations. In British waters, the breeding season ranges from September to November, in Eastern Canada the season occurs later in late December through February, and the Baltic population breeds in March (King, 1964). Individuals in each of these groups must be in synchrony with its conspecifics in order to successfully reproduce.

Histological studies demonstrate that males cycle annually, with testes regressing to half breeding size during most of the non-breeding year. In the British Isles, meiosis begins in May with spermatocytes becoming plentiful in the seminiferous tubules by July (Backhouse, 1964). In the Canadian population, sperm production is only evident by October (Mansfield, 1973). Androgens supporting spermatogenesis can be used as a non-intrusive method of evaluating reproductive condition. Sangalang and Freeman (1976) reported on levels of androgens in 3 male grey seal samples and found that testosterone may be elevated in January relative to July. Noonan (1989) found evidence of cycling testosterone levels in male hooded seals which corresponded to oestrus behaviour but reported no evidence of successful breeding in the captive animals.

Synchrony of male and female cycles is critical in seasonal breeders. Backhouse (1964) reports that males are incapable of breeding out of season. Of key concern to captive breeding of phocids is whether the animals remain fertile and cyclic in an artificial environment. This study examines whether male grey seals captured from the wild at the onset of the study and males reared in captivity undergo reproductive cycles of testosterone levels in synchrony with the reproductive cycle of wild captured females.

Materials and Methods

Experimental animals

Nineteen grey seals, six males and 13 females, were collected from the breeding ice surrounding Amet Island on the north-western shore of Nova Scotia (45°50'N; 63°10'W) between January 11–13, 1989. The ages of the seals were not known except that all were reproductively mature in the 1989 breeding season as the cows had all pupped and the bulls defended harems (3+ for females, 10+ for males). The males were numbered 89-1 through 89-6. These animals were transported to the Guelph holding facility within three days of capture.

An additional two males donated by the Rockton Lion Safari, Ontario, Canada, were also made available. The two males had been captive since birth and were aged 8 (89-22) and 9 years (89-23).

The animals were maintained in four cement outdoor tanks (10 × 7 × 1.5 deep) with haul out ledges. Three tanks each housed two male and four-five female seals, while the last tank held 89-22 and 89-23. The tank members varied through the year and all male seals were exposed to female seals during the breeding season. The seals were fed twice daily a diet of Atlantic herring (*Clupea harengus*) supplemented with Seatab® multi-vitamins and NaCl. Each animal received approximately 6–7 kg of fish per day.

Blood samples were taken weekly during November through May, and biweekly during the remainder of the year. The bulls were sampled between January 1989 and February 1990. Blood was collected by vacutainer method from the hind flipper plexus into heparinized tubes (Geraci, 1971) while the seal was restrained in a net. Samples were centrifuged at 3000 RPM for 10 minutes. The plasma was stored at −20°C until assayed.

Steroid radioimmunoassay

The concentrations of testosterone were determined by radioimmunassay as described for hooded seals by Noonan (1989). Sensitivity of the antibody P4311 for testosterone is 10–25 pg and cross reacts (61.5% and 15.7%) with dihydrotestosterone and androstenedione respectively (Noonan, 1989). Intrassay variation was 4.6% (n = 10) and interassay variation was 15.0% (n = 10).

Statistical analysis

Data are expressed as mean ± standard error of the mean (s.e.m.) and n is the number of seals. The values for each month were computed as means of the weekly measurements in each seal. Statistical differences between monthly means were examined using one-tailed Student's t-test with Bonferroni's adjustment for repeated variables (Trippel and Hubert, 1990) to ensure that overall alpha level was not greater than 0.05. The corrected value for detection of significant difference was $p < 0.008$ when six months were considered (Fig. 2).

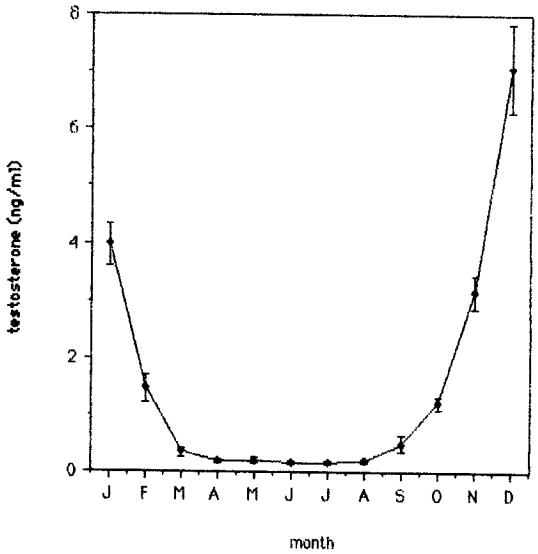

Figure 1. Monthly mean concentrations of plasma testosterone (±S.E.M.) in male grey seals. n = 8 male seals: January 1989–February 1990.

Results

Grey seals exhibited a definite seasonal pattern of androgen secretion. Mean monthly concentrations of testosterone ± s.e.m. in peripheral plasma of male seals were plotted (Fig. 1). Values from the eight male seals were pooled for both years for each month in the calculation of monthly means. The plasma mean testosterone concentration was at baseline levels (< 1 ng/ml) during March to September and then rose from October to January. The pattern was similar in all animals sampled.

The average monthly values of plasma testosterone ± s.e.m. in the two captive-reared seals (89-22 and 89-23) and the six wild-captured males (89-1, -2, -3, -4, -5, and -6) during the breeding seasons in 1989 and 1990 were contrasted (Fig. 2). Only those six months where values rose above baseline were included. Mean plasma testosterone was higher in the wild seals than in the captive reared males in October to January, but the difference was not significant. The levels also declined more gradually in the latter group with significantly higher levels recorded in March in 89-22 and -23 than in the other 6 male grey seals.

Behavioural data parallels the testosterone level trend. Males became increasingly territorial and engaged in aggressive encounters with conspecific males in November and December. Copulations

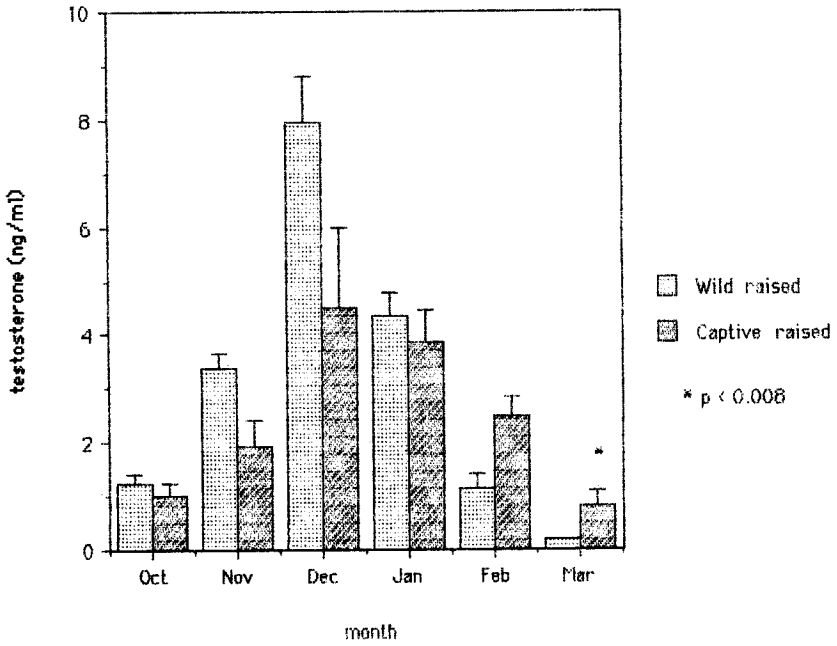

Figure 2. Monthly mean concentrations of plasma testosterone (±S.E.M.) in grey seals. Wild raised n = 6 male seals; captive raised n = 2 male seals: January–March from 1989 + 1990; October–December from 1990 only.

were observed in January. Aggression subsided in February and the males were passive in March.

Discussion

Testosterone levels in male grey seals showed a pattern of activity typical of seasonal breeders. Levels of testosterone rose above baseline in those months when breeding and antagonistic behaviour were noted, namely November to February (Fig. 1). Levels of the androgen remained high for a minimum of three months and fell after the cessation of breeding activity.

A period of male reproductive activity longer than the breeding season is advantageous to seasonal breeders. The mating season in grey seals does not exceed one month and follows parturition and three weeks of lactation (Backhouse and Hewer, 1964). The time of breeding varies considerably between the three main grey seal populations. In British waters, the breeding season ranges from September to November, in Eastern Canada from December through February, and the Baltic population breeds in March (King, 1983).

The data presented here corresponded with histological studies which suggested that reproductively, males cycle annually, with testes regressing to half the breeding size for most of the non-breeding period of the year. In British waters, meiosis began in May with spermatocytes becoming plentiful in the seminiferous tubules by July (Backhouse, 1964). In the Canadian population, sperm production was only evident by October (Mansfield, 1973). Measurement of androgens supporting spermatogenesis can be used as a non-lethal method of evaluating reproductive condition. Sangalang and Freeman (1976) reported on levels of androgens in three male grey seal serum samples and found that testosterone may be elevated in January relative to July. The measurement of testosterone in the present captive study with a larger number of samples and animals detailed more exactly those initial observations on wild grey seals. Noonan (1989) found evidence of cycling testosterone levels in male hooded seals which coincided to oestrous behaviour but reported no evidence of successful breeding in the young captive animals.

Synchrony of male and female cycles is critical in seasonal breeders. Backhouse (1964) reported that males are incapable of breeding out of season. Photoperiod has been implicated as the most likely environmental cue entraining the reproductive cycle of both males and females (Bigg and Fisher, 1972) although water temperature (Coulson, 1981) and body condition (Boyd, 1984) have also been suggested. Of key concern to captive breeding of phocids is whether the animals remain fertile and cycle in an artificial environment.

In this study, male grey seals captured from the wild at the onset of the study and males reared in captivity underwent reproductive cycles of testosterone levels in synchrony with the cycle of wild captured females. The two males raised in captivity had lower levels of testosterone for significantly longer periods than their wild conspecifics (Fig. 2), but the timing was similar and baseline levels did not differ in non-breeding months. Captive rearing in an outdoor enclosure in Ontario did not prevent cyclic pattern of testosterone secretion, and these male seals were able to breed successfully with wild females. It is possible that studies at different latitudes and in warmer conditions may reveal a somewhat different pattern. Bigg and Fisher (1972) showed that it is possible to disrupt the reproductive cycle of female harbour seals by changes in experimental photoperiod and temperature.

Acknowledgement

We thank H. Pedersen and P. Mavronicolas for the care of the animals and technical assistance; L. Noonan for RIA assistance; and Drs G. Van Der Kraak, R. Liptrap, and J. Raeside for advice and critique. Financial support was provided by an N.S.E.R.C. graduate scholarship to A.J.S., an N.S.E.R.C. operating grant, and a D.S.S. contract (n. FP 802-7-2771/01-OCS) to K.R.

References

Amoroso, E. C., Bonner, G. H., Harrison, R. J., Matthews, L. H., Fowlands, I. W. & Sloper, J. C. (1965). Reproductive and endocrine organs in foetal, newborn, and adult seals. *J. Zool.* **147,** 430–486.

Backhouse, K. M. (1964) The male reproductive cycle in the grey seal. In: A Seals Symposium, Dep. Zool. Univ. Cambridge. (E. A. Smith, ed.). pp. 52–55. Edinburgh: Nat. Comserv.

Backhouse, K. M. & Hewer, H. R. (1964) Features of reproduction in the grey seal. *Med. and Biol. Illust.* **14,** 144–150.

Bigg, M. A. & Fisher, H. D. (1975) Effect of photoperiod on annual reproduction in female harbour seals. In: Biology of the Seal, (K. Roland and A. W. Mansfield, eds), pp. 169. Int. Counc. Explor. Sea (I.C.E.S.), Rapp. Reun, Copenhagen.

Boyd, I. L. (1983) Luteal regression, follicle growth, and the concentration of some plasma steroids during lactation in grey seals. *J. Reprod. Fert.* **69,** 157–164.

Boyd, I. L. (1984) The relationship between body condition and the timing of implantation in pregnant grey seals (*Halichoerus grypus*). *J. Zool. Lond.* **203,** 113–123.

Coulson, J. C. (1981) A study of the factors influencing the timing of breeding in the grey seal (*Halichoerus grypus*). *J. Zool. Lond.* **194,** 533–571.

Craig, A. M. (1963) Key to the reproductive condition of female fur seals (*Callorhinus ursinus*) and the reproductive cycle of mature female fur seals. *Fish. Res. Bd. Can.*, Nanaimo, B. C., Manuscript.

Daniel, J. C. Jr. (1981) Delayed implantation in the northern fur seal (*Callorhinus ursinus*) and other pinnipeds. *J. Reprod. Fert.* **29** (Suppl), 35–50.

Geraci, J. R. (1971) Functional hematology of the harp seal *Pagophilus groenlandicus*. *Physiol. Zool.* **44,** 164–170.

Hobson, B. M. & Boyd, I. L. (1984) Gonadotrophin and progesterone concentrations in placentae of grey seals (*Halichoerus grypus*) *J. Reprod. Fert.* **72,** 521–528.

King, J. E. (1983) Seals of the World. Cornell University Press, Ithaca. pp. 240.

Mansfield, A. W. (1971) The distribution and population status of the grey seal (*Halichoerus grypus*) in eastern Canadian waters. Int. Counc. Explor. Sea (I.C.E.S). Marine Mammal Comm., Counc., Meet. Copenhagen, N, 7.

Noonan, L. M. (1989) Determination of plasma estrone sulfate and progesterone for female hooded seals and plasma testosterone for male hooded seal, *Cystophora cristata*. Masters thesis. Department of Zoology, Unviersity of Guelph, Ontario.

Ronald, K., Selley, L. J. & Healey, P. J. (1982) In wild Mammals of North America (J. A. Chapman and G. A. Feldhamar, eds.). John Hopkins University Press, Baltimore. pp. 769–822.

Royal Commission on Seals and the Sealing Industry in Canada (1976) Chair. A. H. Malouf. Ottawa. pp. 525–585.

Sangalang, G. B. & Freeman, H. C. (1976) Notes: Steroids in the plasma of the gray seal, *Halichoerus grypus*. *Gen. Comp. Endocrin.* **20,** 419–422.

Trippel, E. A. & Hubert, J. J. (1990) Common statistical errors in fishery research. Writing for Fisheries Journals, American Fisheries Society, 93–102.