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Plasma testosterone concentrations in captive male harp seals (*Pagophilus groenlandicus*)

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Abstract

The main objective of this study was to describe plasma testosterone in male harp seals (*Pagophilus groenlandicus*) before, during, and after the breeding season. In addition, the relationship between testosterone concentration and vocal activity was examined. Levels of plasma testosterone were investigated in four captive harp seals of different ages. Concentrations varied from a minimum of ca. 0.01 ng/ml in April and May to a maximum of 9.4 ng/ml in March. Peaks in concentration occurred at the time when testes were largest in mature wild males, which is several weeks before wild females enter oestrus. Also, peaks in concentration were correlated with high rates of vocalisations. Peak levels of testosterone were lowest in the young and socially subordinate seals.

Key words: harp seals, *Pagophilus groenlandicus*, testosterone, hormone concentration, seals, underwater vocalizations.

Introduction

Pinnipeds have diverse social and reproductive behaviour, including extreme aggression among males and high levels of polygyny (Bartholomew, 1970; Le Boeuf, 1991; Atkinson, 1997) in some species. This diversity has led to numerous studies on the social behaviour of pinnipeds, but surprisingly few investigations on the physiological basis of male behaviour. For example, endocrinology has been investigated in only five species (Boyd, 1991; Atkinson, 1997). Harbor seal (*Phoca vitulina*) testosterone levels were studied for 8 months in a captive seal, which exhibited seasonal changes (Kirby, 1990). Testosterone concentration changes in two hooded seals (*Cystophora cristata*) were monitored by Noonan *et al.* (1991), suggested that the breeding season extends over several months. Hawaiian monk seals (*Monachus schauinslandi*) testosterone levels were investigated by Atkinson & Gilmartin (1992), found a long period of activity. Territorial behaviour and breeding frequency of Weddell seals (*Leptonychotes weddellii*) were studied in relation to testosterone and cortisol concentrations, age and size, by Bartsh *et al.* (1992). They proposed that territorial Weddell seal males have the highest testosterone concentrations and are the biggest males. The sub-antarctic fur seal (*Arctocephalus tropicalis*) presents two peaks in plasma testosterone concentration and mean testicular weight during the summer (Bester, 1990), the first peak occurred during the breeding season and the second occurs during moult. These few studies suggested that testosterone levels might be correlated with reproductive behaviour of seasonal breeders.

Some aspects of harp seal biology, such as migration patterns, feeding habits, and reproductive physiology, have been studied due to the importance of this species to commercial fisheries. Harp seals are seasonal breeders with precisely timed reproduction each year with births occurring only over about three weeks (Lavigne & Kovacs, 1988; Sergeant, 1991; Atkinson, 1997). In this paper, I describe seasonal changes in testosterone levels of harp seal males and attempt to correlate these with one aspect of reproductive behavior, underwater vocalisation rates.

Materials and Methods

Study animals and facility

Four adult male harp seals of different ages were studied at the Ocean Sciences Centre (Memorial University of Newfoundland) at Logy Bay, Newfoundland (47°38′N 52°40′W). Seals were maintained in two wooden outdoor tanks, each measuring 12.3 m in diameter and 2.5 m in depth. The tanks were bordered by approximately 190 m^2 of wooden decking to which the seals had free

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Table 1. Composition of harp seal groups during 1996[1].

Seals in Tank 1	Seals in Tank 2	Dates
F4, M1, M2, M5, M6	F1 to F3, M3, M4	2–23 February
M1 to M3, M5, M6	F1 to F4, M4	23–28 February
M1, M3 to M6	F1 to F4, M2	28 February–5 March
F2, F3, M1, M4	F1, F4, M2, M3, M5, M6	6–8 March
F1 to F4	M1 to M6	8–12 March
F1 to F4, M3	M1, M2, M4 to M6	13–14 March
F1 to F4, M3, M4	M1, M2, M5, M6	14–15 March
F1, F3, M3, M4	F2, F4, M1, M5, M6	15–21 March
F1, F3, M3, M4	F2, F4, M1, M2, M5, M6	21–23 March
F1 to F3, F4, M1	M2 to M6	23–27 March
F1 to F4, M1 to M6	None	27–31 March

[1]F1-F4 refer to females 1 through 4; M1-M6 refers to males 1 through 6.

access. The tanks were supplied with fresh seawater pumped from Logy Bay and were drained and cleaned at least weekly. The seals were usually fed twice a day, around 1000h and 1500h. Moulton *et al.* (1999) provided further information on these animals.

During the 1996 breeding season, seals were placed in the tanks in several combinations (Table 1): Treatment 1 (T1), one adult male with all four females; T2, one adult female with all adult males; T3, all six males only; T4, all four females only; and T5, all seals combined. It was not possible to repeat this design in 1997 due to conflicts with other studies. However, having all adult females and adult males in the same tank for most 1997 was possible.

Eqipment and data collection

Blood samples were collected approximately monthly from January 1996 to April 1997, except during February and March (breeding season), when they were collected approximately weekly. Blood was taken from the hind flipper plexus and placed into heparinized tubes before seals received their daily feed. The sample was centrifuged at 5000 rpm for 20 min, and the serum frozen at −70°C for analysis. The University of Prince Edward Island veterinary laboratory analyzed the samples. Determinations of plasma testosterone concentration were done by radioimmunoassay (RIA), using the Coat-a-count procedure (Atkinson *et al.*, 1986). Body mass, was determined approximately weekly from March 1996 to March 1997 (Table 2).

Underwater vocalizations usually were recorded twice per week outside the breeding season, and daily in the breeding seasons of 1996 and 1997. Recordings were made for 3–4 h in mornings (0530–0930h) and 5–6 h in evenings (1700–2300h or 1800–2400h). These sampling times were chosen because of low human disturbance and because harp seals are considered to be most active then (Terhune & Ronald, 1976). Recording equipment consisted of: a Sony stereo cassette recorder model TC-D5PROII, with a recording response of 0.04–14.0 kHz (± 3 dB); and an omnidirectional hydrophone with a recording response of 0.006 to 40.0 kHz (at 4 dB down) (Marine Mammal Research Unit, Fisheries Centre, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4).

A one-way ANOVA was used to determine if there was a significant difference in testosterone concentration among seals within seasons and throughout the year, and to relate testosterone levels to vocal activity.

Table 2. Summary of body mass (kg) of the four, captive adult male harp seals during March 1996 and 1997.

Date	Male 1	Male 2	Male 3	Male 4
1996				
7 March	158.2	176.0	174.4	128.0
14 March	152.6	176.6	174.0	132.6
20 March	145.6	178.0	169.0	131.4
27 March	140.4	174.2	160.2	132.8
Mean	149.2	176.2	169.4	131.2
1997				
5 March	149.4	156.2	198.6	147.8
18 March	145.6	151.0	201.6	142.0
26 March	139.2	149.8	191.8	141.8
Mean	144.73	152.33	197.3	143.86

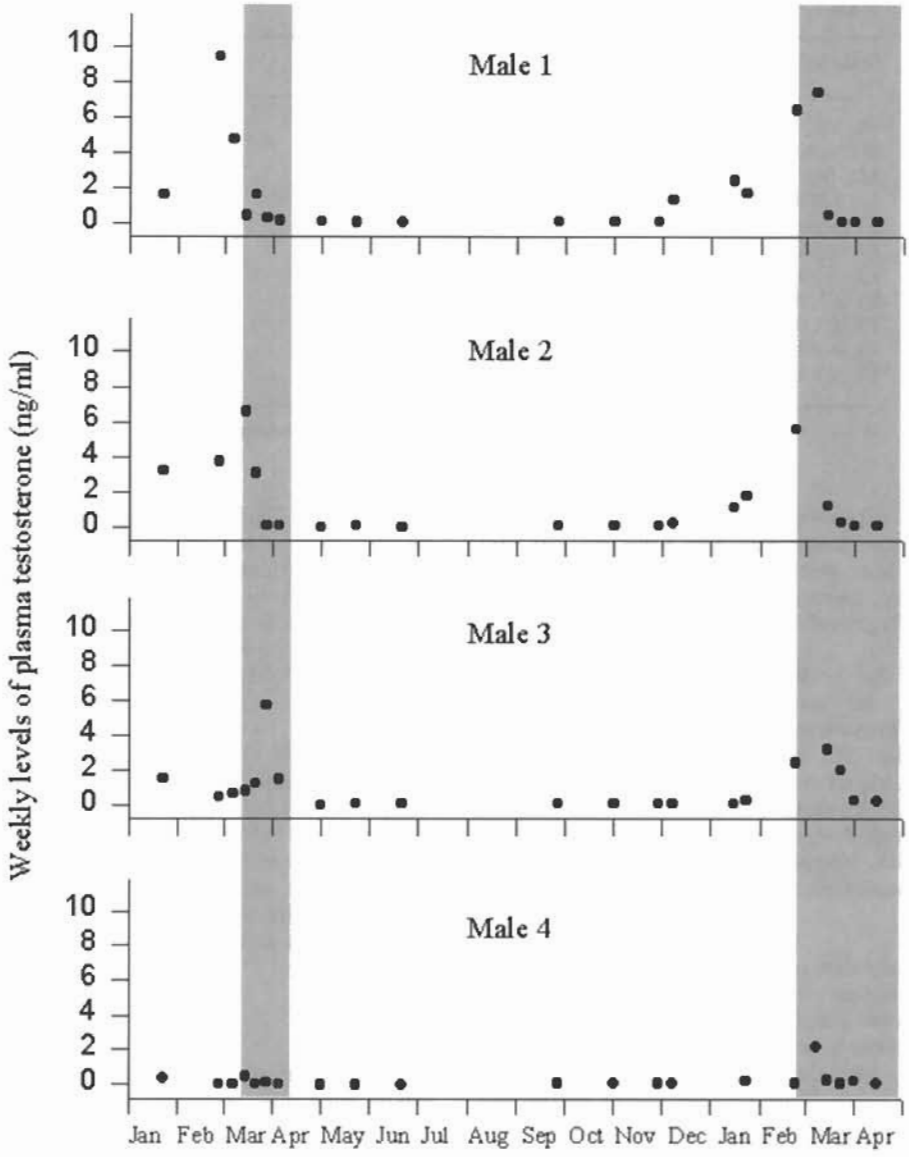

Figure 1. Seasonal trends in levels of harp seals plasma testosterone. Shaded area indicates the breeding season (1996 and 1997).

Results

Testosterone concentration exhibited a clear seasonal pattern, though differences among animals were not significant in either year. High levels of testosterone were present for only a brief period (*ca.* 8 days). During 1996, the baseline level of testosterone was *ca.* 0.01 ng/ml; this occurred from the end of April to June (the moulting season). The highest level of testosterone was 9.43 ng/ml, which was noted in male 1 in March and the beginning of April (Fig. 1). Male 1 was the first seal to reach his hormonal peak (27 February), followed by male 2 and 4 (both on 14 March); male 3 was last, reaching his peak on 27 March (Fig. 1). One week after their peaks, testosterone concentration decreased by 49% in male 1, by 53% in male 2, 74% in male 3, and 93% in male 4.

Males 3 and 4 were the first to decline to baseline levels (late April), followed by male 1 on 22 May,

and male 2 on 20 June (Fig. 1). Plasma testosterone concentration did not differ significantly among males in 1996 ($P=0.230$).

In 1997, the lowest testosterone concentration was 0.10 ng/ml (mid April) and the highest was 7.40 ng/ml (again for male 1). Male 2 was the first to reach his hormonal peak (20 February), followed by males 1 and 4 (11 March), and lastly by male 3 (18 March; Fig. 1). In 1997, one week after peaking, testosterone levels decreased in male 1 by 94%, 78% in male 2, 37% in male 3 and 88% in male 4. In 1997, hormone concentrations were generally lower than in 1996, except in male 4 (Fig. 1). As in 1996, in 1997 there was not a significant difference within males in the breeding season or non-breeding season ($P=0.348$).

Plasma testosterone concentration and underwater vocalising rates tended to increase and decrease in tandem (Fig. 2), although these was no statistically or significant correlation between them.

Discussion

The timing of peaks in plasma testosterone of the captive seals is consistent with the observed timing of births and copulations in the wild: births in this population occur around late February and the beginning of March, with copulations presumably 10–14 days later (Sergeant, 1991). High levels of testosterone were present for only a brief period (*ca.* 8 days), and began to decline (along with testicular size [Miller *et al.*, 1998]) during the mating period. This pattern is consistent with results reported for other seasonally breeding mammals (Gustafson & Shemesh, 1976; Griffiths, 1984; Bubenik & Schams, 1986; Schroeder & Keller, 1989; Bartsh *et al.*, 1992). In other words, testosterone levels are elevated before the onset of the mating season, but are declining or have reached basal levels before the mating season is over. In 1996, two copulations were observed (in the water): on 14 March male 3 copulated with an adult female, and on 21 March male 1 copulated with a different adult female. In 1997, no copulations were observed; however, on 5 March one of the seals gave birth to a pup.

Three of the four seals exhibited sexual behaviour, such as chasing females, attempting copulation, and aggressive behaviour toward other males. The seal with the lowest testosterone concentration did not exhibit such behaviours. Instead, it was behaviourally subordinate to the other seals in moving away from other animals, deferring to them at feeding time, etc. Sergeant (1991) suggested that dominance is related to size and mating experience in male harp seals. When investigating influences of testosterone levels and behaviour, Bartsh *et al.* (1992) grouped Weddell seal males into 3 categories: territorial, non-territorial, and transitional. They found that each group had different testosterone levels, and that the levels declined at different rates in each seal. Territorial males had the highest testosterone levels and their levels declined more slowly than in non-territorial males. Bartsh *et al.* (1992) also suggested that territorial males had the highest body mass among the three categories studied. Our data suggested that dominance is related to testosterone levels and how rapidly it declines.

In 1996, the dominant male was male 1. This male was very aggressive towards the other males, which generally avoided him. No males displayed aggressive behaviour towards male 1. Male 1 had the highest testosterone levels, and these levels decreased more slowly than in the other males. In 1997, male 3 was the heaviest (Table 2); however, it never showed dominance over male 1 and 2, only over male 4. If males 1 or 2 displayed aggressively towards male 3, he usually swam away. However, his aggressive behaviour towards male 4 caused this male to swim away. In 1997, it is likely that male 2 was dominant, because, was very aggressive towards the other males, which generally avoided him. No males displayed aggressive behaviour towards him. Male 2 did not have the highest testosterone levels, but his levels decreased only by 78% one week after it peaked. In contrast, male 1 had the highest concentration of testosterone, but it decreased by 94% one week after it peaked. Male 3 was the heaviest, but he only showed dominance over male 4. Male 4 was subordinate to all other males in 1996 and 1997 and had the lowest concentration of testosterone and the lowest body mass in both years. This male was never observed displaying aggressive behaviour, and always swam away from the other males as soon as they were aggressive towards him. Even in the absence of the other males, male 4 never attempted copulation. These data suggested that testosterone levels, and how rapidly it declines after peaking, are the main factors underlying social dominance in harp seals. In future studies, sampling from animals of known age will be important to determine how important experience is to social dominance.

Testosterone concentration might have an influence on the vocal behaviour of harp seals, but we do not know how testosterone affects this behaviour. Even though there was not a statistically significant correlation between testosterone levels and underwater vocal activity, from Figure 2 it is obvious that there is a biologically significant correlation. These results are in accordance with suggestions made by Terhune and Ronald (1986) were they suggest that vocal activity in harp seals peaks in March and that vocalizations mainly are used in the breeding season.

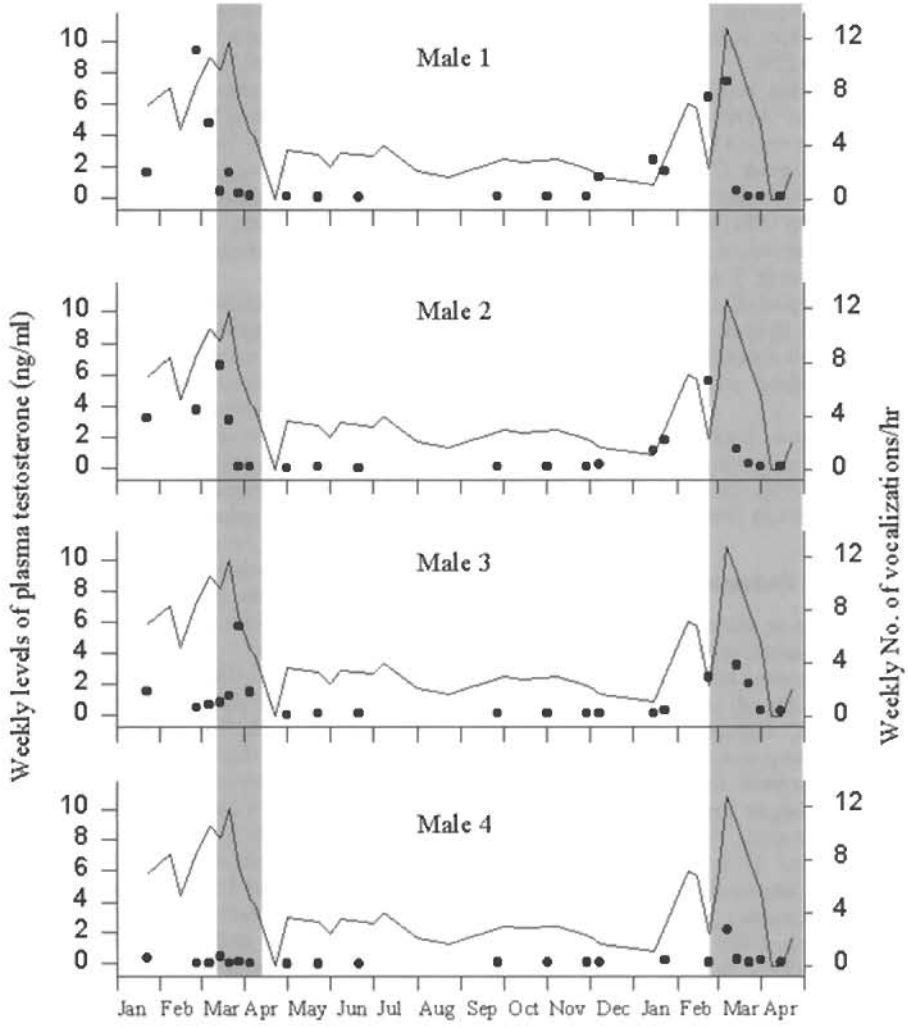

Figure 2. Seasonal trends in calling rates and levels of plasma testosterone of harp seals. Shaded area indicates the breeding season (1996 and 1997). The calling rates represent number of vocalizations/hour for all the seals in a tank, and do to represent the calling rates of a single individual.

Seasonal hormone patterns were significant vocal activity, though suggestive, could not be correlated. Coordinated field and captive reproductive physiology studies are needed to clarify the behavioural and ecological significance of these observations.

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