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Seasonal testosterone pattern in Hawaiian monk seals (*Monachus schauinslandi*)

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Summary. Blood samples from four captive male Hawaiian monk seals were collected at intervals of one month for one year for testosterone assay. Plasma testosterone concentrations, measured by radioimmunoassay, revealed a clear seasonal pattern. The lowest mean testosterone concentration ($0{\cdot}09 \pm 0{\cdot}04$ ng ml^{-1}) occurred in January, and the highest ($1{\cdot}78 \pm 0{\cdot}40$ ng ml^{-1}) in June. The seasonal occurrence of births and of injuries related to mating in wild populations of Hawaiian monk seals showed a distinct association with the period of high testosterone. This study supports other data that indicate that the Hawaiian monk seal is a seasonal breeder and is reproductively active for longer than monachine seals that live in higher latitudes.

Keywords: Hawaiian monk seal; testosterone; seasonal breeding

Introduction

Ecological factors have significantly influenced the evolution of breeding patterns in most animals, including pinnipeds. Two fundamental characteristics of pinniped life history are related to the evolution of their sociosexual behaviour: they are marine feeders and parturition and postnatal pup care must occur on land (Bartholomew, 1970). In addition, reproduction in most pinnipeds is characterized by highly synchronized pupping and mating (Stirling, 1983), as well as delayed implantation of the blastocyst (Fisher, 1954). Most pinnipeds inhabit temperate and polar regions where the combination of delayed implantation and synchronized mating and pupping ensures that the maximum number of offspring are produced at the optimal time of year. Monk seals (*Monachus* spp.) are an exception to this high degree of synchrony.

Monk seals include three species: the Hawaiian monk seal (*Monachus schauinslandi*), the Mediterranean monk seal (*M. monachus*), both of which are endangered, and the extinct Caribbean monk seal (*M. tropicalis*). Monk seals are considered to have evolved from temperate monachine seals of the North Atlantic, with *M. schauinslandi* or its ancestor moving into the Pacific tropics through the then submerged region of Panama during the mid-Miocene (Ray, 1976). The North Atlantic ancestral monachine stock of the monk seal probably exhibited highly synchronized pupping and mating (Stirling, 1983), but millions of years of reduced critical environmental constraints related to reproductive success in the tropical ecosystem appear to have diminished the requirement for this characteristic.

Studies on other phocid seals suggest that seasonal changes in reproductive physiology are directly related to the breeding season. For example, the Southern elephant seal demonstrates seasonal changes in testis weight and spermatogenesis, with the highest testicular weights occurring during the breeding season and the lowest in mid-winter (Griffiths, 1984). Concentrations of plasma testosterone in these seals was increased only during the first month of breeding (Griffiths, 1984).

Captive hooded seals also showed seasonally increased concentrations of plasma testosterone one month prior to the onset of breeding behaviour (Noonan *et al.*, 1991).

Hawaiian monk seals were listed as endangered under the US Endangered Species Act following a 50% reduction in beach counts (Johnson *et al.*, 1982).

With only rare exceptions, the distribution of the Hawaiian monk seal is limited to the Hawaiian Archipelago, and almost all breeding is restricted to the Northwestern Hawaiian Islands. Kenyon & Rice (1959) reported that Hawaiian monk seal pups were born from January until at least the end of June, with most births from mid-March to May. Intensive population monitoring at Laysan Island, one of the primary breeding sites of the Hawaiian monk seal in the northwestern Hawaiian Islands, supports the earlier observation of seasonal breeding (Alcorn, 1984; Johanos *et al.*, 1987; Johanos & Austin, 1988; Alcorn & Buelna, 1989). Monk seal mating is rarely observed because it occurs in the water, probably some distance from shore. However, injuries related to mating (primarily superficial bite wounds and scratches on the dorsal surface of females) are seen seasonally, from April to July at Laysan Island (B. Becker, pers. commun.), and can be used as an indicator of mating activity. This study was based on the hypothesis that, if female Hawaiian monk seals demonstrate seasonal pupping characteristics, a parallel pattern in males should be apparent in concentrations of testosterone in plasma.

Materials and Methods

Animals and blood sampling

Four adult male Hawaiian monk seals were collected as adults at Laysan Island in the Northwestern Hawaiian Islands in May 1987 and housed in a large naturalistic enclosure at Sea Life Park on Oahu, Hawaii (21°N, 158°W). Blood samples were collected at monthly intervals from June 1989 to June 1990, and the animals were observed for signs of moulting. The animals were fed 120–140 mg of the tranquillizer diazepam in their foodfish 2 h before blood collection. Blood was collected from the extradural vein while the seals were physically restrained with netting. Blood samples for testosterone assay were also collected from two wild adult male monk seals at Kure Atoll in the northwestern Hawaiian Islands in June 1990. These two seals were physically restrained and given an intravenous injection of 20 mg diazepam before blood sampling.

Testosterone assay

Testosterone was measured in the plasma using a single antibody radioimmunoassay (Atkinson *et al.*, 1986). The plasma was extracted in 1:10 (v/v) diethyl ether. Extraction recovery was 91·4%. The sensitivity of the assay was 0·05 ng testosterone per tube, and nonspecific binding was 5·5%. A pool of monk seal plasma containing $2{\cdot}06 \pm 0{\cdot}18$ ng testosterone ml^{-1} was included in each assay to determine interassay variation, which was 11·1%. Log–logit transformations were applied to the standard curve of the assay (Rodbard, 1974).

Data analysis

Data on number of births per month for Hawaiian monk seals from Laysan Island, from 1982 to 1990 (Alcorn, 1984; Johanos *et al.*, 1987; Johanos & Austin, 1988; Alcorn & Buelna, 1989; Johanos *et al.*, 1990), were averaged over 9 years to obtain the mean number of births per month. Data on injuries resulting from mating for Laysan Island, 1988–1990 (Johanos *et al.*, 1990 and B. Becker, pers. commun.), were similarly averaged over 3 years to obtain the mean number of injured animals per month.

Results

A distinct seasonal pattern of circulating concentrations of testosterone was apparent in all four captive seals (Fig. 1). Testosterone concentrations were lowest in January with a mean concentration of $0{\cdot}09 \pm 0{\cdot}04$ ng ml^{-1}, and highest in June with a mean concentration of $1{\cdot}78 \pm 0{\cdot}40$ ng ml^{-1}. Variation among animals increased as they approached the nadir (October–December) and apex (May–June) of the seasonal cycle. All captive seals moulted between mid-October and mid-December. Variation in the daily circulating concentration of plasma testosterone from two seals sampled at 3 h intervals from 08:00 to 17:30 h, was less than 15%, with the highest

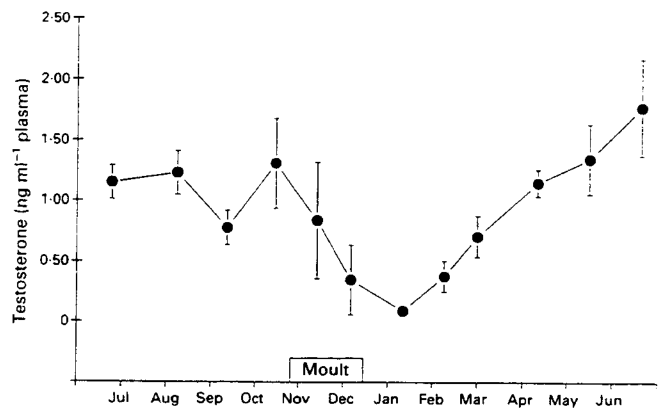

Fig. 1. Plasma concentrations of testosterone (mean ± SEM) in four adult male Hawaiian monk seals, July 1989 – June 1990.

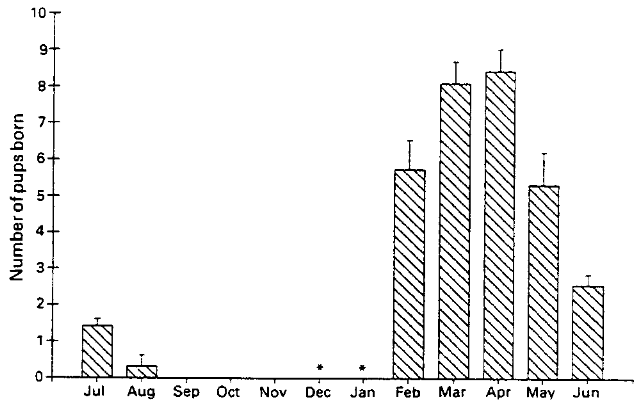

Fig. 2. Numbers (mean ± SEM) of Hawaiian monk seal pups born per month, 1982–1990, at Laysan Island in the northwestern Hawaiian Islands (data from Alcorn, 1984; Johanos *et al.*, 1987; Johanos & Austin, 1988; Alcorn & Buelna, 1989; Johanos *et al.*, 1990; National Marine Fisheries Service, unpublished). Asterisk indicates a few early-season births that were excluded because the month could not be established with certainty.

concentrations occurring in the afternoon. The plasma testosterone values from the two wild adult males sampled at Kure Atoll in June were 2·70 and 1·59 ng ml^{-1}.

The mean number of births per month at Laysan Island from 1982 through 1990 shows that most pups were born between February and June (Fig. 2). Mating injuries occurred primarily from April to June (Fig. 3).

Discussion

This study demonstrated that the male Hawaiian monk seal has maintained a seasonal, albeit lengthy, breeding trait in a tropical environment (Fig. 1). The nadir of the testosterone concentrations

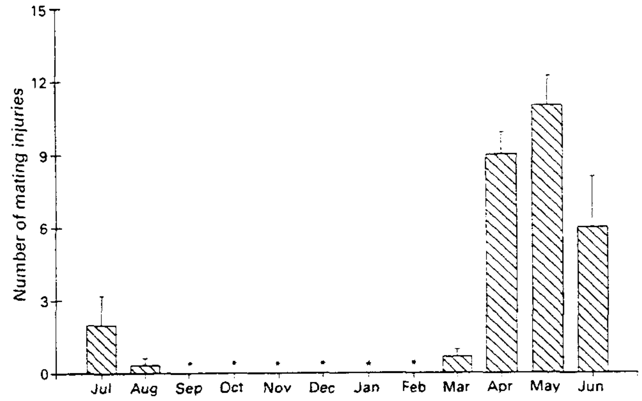

Fig. 3. Numbers (mean ± SEM) of female Hawaiian monk seals exhibiting mating injuries (scratches and lacerations on mid-back apparently from bites) by month from Laysan Island, 1988–1990 (B. Becker, NMFS Honolulu Laboratory, unpublished). Asterisks indicate that observations were not performed during these months.

occurred in January in all four seals and then steadily increased over the next few months as the seals entered the breeding season. Plasma testosterone remained high through midsummer to autumn and began to decline only after the October sampling, concurrent with the onset of moult. We do not understand why there was a slight depression in the curve in September, which was experienced by all four males, although it is possible that it may be related to pre-moult hormonal changes. This pattern of testosterone concentrations is typically the result of changes in hypothalamic drive that determine pituitary and gonadal function. The higher testosterone concentrations during the breeding season (March–August) reflect increased hypothalamic drive and active spermatogenesis. Low seasonal testosterone concentrations usually indicate reduced, but not necessarily absent, spermatogenesis during the nonbreeding season (October–February). Spermatogenesis usually lags behind testosterone production, as production of testosterone by Leydig cells is necessary for germ-cell differentiation in the tubules of the testes (Turner & Bagnara, 1976). This time lag varies with species but is generally of the order of 3–8 weeks. If this time lag is applied to the testosterone concentrations (Fig. 1), the spermatogenic cycle could be estimated for this species.

The seasonal cycle of testosterone secretion found in these captive males is also consistent with the observed pattern of births and breeding-induced wounds observed in the wild. Pupping occurs over 6 months with the greatest percentage being born from March through May. The seasonal pattern of mating is more difficult to define, as it is not possible to identify accurately when copulation takes place. However, high testosterone concentrations occurred during the time that mating-induced wounds were observed on mature females. When these patterns are compared with other monachine seals, it appears that, although the Hawaiian monk seal has maintained a seasonal pattern of breeding, the length of the breeding season has been extended compared with related species in higher latitudes. The monachine subfamily of seals is found in a broad range of habitat types and its breeding seasons are similarly varied. The high-latitude Antarctic crabeater seal (*Lobodon carcinophagus*) and Weddell seal (*Leptonychotes weddelli*) give birth and mate within 3–4 weeks; the more temperate climate monachines (*Mirounga* spp.) show a less temporally confined pattern of 4–8 weeks, and the monk seals (*Monachus* spp.) in their low-latitude habitats have a broad temporal distribution of pupping and breeding over at least 6 months (Stirling, 1983). The prolonged pattern of high testosterone concentrations is consistent with the extended pupping season, as it allows for females that may give birth late to be bred in the same season.

A major concern in using captive individuals in this study was whether the endocrine system may have been suppressed in the captive environment. The testosterone values from two wild adult male seals sampled at Kure Atoll in June are within the measured range of the captive monk seals at the same time of year. This indicates that the high testosterone concentrations in the cycle exhibited by the captive monk seals is probably representative of circulating testosterone concentrations in wild adult male monk seals.

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