Reproductive morphology and status of female Hawaiian monk seals (Monachus schauinslandi) fatally injured by adult male seals

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Female Hawaiian monk seals at Laysan Island in the Northwestern Hawaiian Islands seasonally risk aggressive mating attempts by groups of adult male monk seals. These attacks, which also target immature female and male seals at a lower frequency, result in injuries that are often fatal and are termed mobbings. This study was undertaken to assess the reproductive status of nine female seals that died after mobbing attacks and to obtain basic morphological data of reproductive tracts from ten females. Reproductive morphology of the seals indicated that the lengths of the uterine body and both uterine horns were significantly shorter in nulliparous than in parous seals. Seven of the nine seals were periovulatory, on the basis of gross morphology of the ovaries at death. The ovaries of the other two seals possessed immature follicles. Histological studies of the vagina and uterus confirmed the reproductive status of the seals. When the reproductive status at the time of first injury was estimated, all seals were in the follicular phase of the oestrous cycle. At least four of these seals were estimated to be in oestrus at the time of their first injury, and seven of the seals sustained at least one injury during the estimated period of oestrus (2–6 days). These results support the hypothesis that most adult female Hawaiian monk seals that die following an attack by male monk seals are periovulatory, and that the majority of the attacks occur during oestrus.

Introduction

The Hawaiian monk seal (Monachus schauinslandi) was listed as endangered in 1976 following a 50% reduction in the number of seals counted on their breeding beaches in the Northwestern Hawaiian Islands over 20 years (Johnson et al., 1982). The most recent estimate of the population was approximately 1750 animals in 1988 (Gilmartin et al., in press). Unfortunately, adult females, the portion of the population that is most crucial to the recovery of the species (Eberhardt and Sinf, 1977), are experiencing high mortality at several locations. For example, 47% of all documented deaths (excluding nursing pups) from 1987 to 1991 at Laysan Island were adult females (National Marine Fisheries Service (NMFS), unpublished data).

A major factor that contributes to adult female mortality is aggressive attacks, or mobbings, by adult male seals. During a mobbing, a group of male seals attempt to mount a single victim, causing distinctive dorsal injuries and often death (Johanos and Kam, 1986). Although immature seals of both sexes receive mounting attacks, more than 70% of these victims are adult females (NMFS, unpublished data). Furthermore, 93% of the documented deaths of adult females from 1987 to 1991 at Laysan Island were seals that had been severely injured by adult males. Most females that were fatally injured were between 4 and 8 years old. Immature seals in this age group are being recruited to the reproductive population (NMFS, unpublished data).

Mobbings are rarely observed; however, distinctive new injuries occur predominantly from April to July, during the Hawaiian monk seal pupping and mating season (Atkinson and Gilmartin, 1992; Atkinson et al., 1993; Hiruki et al., 1993a). Because severe mounting injuries increase female mortality and thus impair potential population growth (Hiruki et al., 1993b), it is important to examine factors that may precipitate mobbing behaviour, such as the reproductive status of female seals involved in mobbing incidents.

The hypothesis for this study was that the timing of mobbings was influenced by the oestrous cycle of a female seal, and that the majority of mature, female seals that died after mobbing incidents were periovulatory. This hypothesis was tested by examination of reproductive tracts from female, Hawaiian monk seals that died following severe mobbing attacks by adult male monk seals. In addition, the basic morphology of the reproductive tracts of female monk seals is described.
Materials and Methods

Reproductive tracts from ten female Hawaiian monk seals were collected during field necropsies from 1987 to 1991 at Laysan Island in the Northwestern Hawaiian Islands. Histories of these seals include their age, parity and the dates and times when they were first observed with fresh injuries, last seen alive, and found dead. Age determinations of seals without recorded ages, from tagging data, were based on dental band analyses (NMFS, unpublished data). Each seal was necropsied as soon as the carcass was found, usually within 24 h of death. The reproductive tracts were excised and preserved in a 10% buffered formalin solution.

During dissection of the fixed specimens, measurements were taken of the lengths of the cervix, cervical os to the uterine bifurcation, and both uterine horns. Although ten tracts were collected and fixed, only nine tracts were analysed for reproductive status and histological sectioning. Ovaries were weighed and examined for follicles or corpora lutea. Tissue sections from the vagina and uterine horns were collected for histological sectioning. The sections were stained with haematoxylin and eosin, and examined under a stereo-microscope (×100) to determine the thickness of the vaginal epithelial cell layer and the presence of uterine secretory glands and mucus within these glands.

Data analysis

The reproductive status of each seal was determined on the basis of the most prevalent ovarian structure. A seal was classified as periovulatory, when a dominant ovarian follicle or a corpus haemorrhagicum was present in one of the ovaries, or anovulatory, when neither ovary had a dominant structure. A periovulatory seal was further classified as preovulatory, when a dominant ovarian follicle, or postovulatory, when a corpus haemorrhagicum was present in one of the ovaries. A dominant ovarian follicle was defined as a healthy follicle that was destined to ovulate (Hodgen, 1982), characterized by apparent thinning of the follicle wall on the exterior surface of the ovary. A corpus haemorrhagicum was defined as a recently ruptured follicle that had not yet formed a corpus luteum (McDonald, 1980), characterized by an opening on the exterior surface of the ovary that may or may not possess signs of haemorrhage. Thus, it may have looked like a bruise with an opening, or like a ruptured follicle. In the case of anovulatory seals, follicles may have been present in the ovaries; however, no follicle appeared dominant or destined to ovulate.

Histological examination of vaginal and uterine sections was used to evaluate the reproductive status of the seal. Specifically, each vaginal epithelial cell layer was measured, and uterine sections were examined for the presence of basal vacuolation, secretory gland formation and the presence of mucus within those glands, which would confirm that the seal was periovulatory. From the status of the ovaries at death, the reproductive state of the seal was examined at the time of each mobbing injury. The first injury date was primarily used in the analysis because four females were injured two or three times within several days, and the causes of these injuries may not have been independent.

Four assumptions were made to estimate the reproductive status at the time of the mobbing injuries: (1) oestrus is a 2–6 day period within the periovulatory phase (J. R. Pietrzesk and S. Atkinson, unpublished data), occurring throughout the preovulatory phase and ending shortly after ovulation; (2) a dominant follicle will be functional and persist throughout the preovulatory phase (Hodgen, 1982) and is the prevalent ovarian structure for 2–4 days (J. R. Pietrzesk and S. Atkinson, unpublished data); (3) a corpus haemorrhagicum will fully luteinize and become a corpus luteum within 2–3 days after ovulation (Yoshida, 1982), the end of the periovulatory phase; and (4) the timing of ovarian events in the oestrous cycle is not altered by injury. On the basis of these assumptions, the age of the ovarian structure at injury was estimated by subtracting the interval between injury and death from the estimated age of the structure at death. The duration of the oestrous cycle of a captive female Hawaiian monk seal is approximately 35 days (Pietrzesk, 1992).

For a basic morphological description, the data from all ten seals were separated into three groups on the basis of parity of the seal: (1) parous seals known to have given birth within the breeding season of their death; (2) parous seals known to have given birth in an earlier season, but not in the year of their death, and (3) nulliparous seals. The groups were compared using a one-way ANOVA, with P < 0.10 as the level of significance due to the small sample size. One seal was eliminated from this part of the study because her parity was unknown. Her parity was estimated from the group means.

Results

Seven of the nine seals examined were periovulatory at the time of death. Three seals possessed a dominant follicle on one ovary and four seals possessed corpora haemorrhagica (Table 1). Of the two seals that were anovulatory, one was a nulliparous 3-year-old seal that had three antral follicles approximately 2–4 mm in diameter. The other anovulatory seal was a parous 14-year-old seal with two follicles, approximately 4 mm in diameter, present on one ovary.

The vaginal and uterine histology confirmed the reproductive status of each seal. Although the thickness of the vaginal epithelial cell layer did not differ significantly with reproductive status, sealed classified as preovulatory tended to have a thicker vaginal epithelial cell layer than did postovulatory or anovulatory seals (Table 1). Pre- and postovulatory seals also had prolific, tortuous, uterine glands and basal vacuolation present (Fig. 1a), whereas the anovulatory seals possessed very few secretory glands and no signs of basal vacuolation (Fig. 1b).

The reproductive status at the time of injury was estimated for nine seals from the state of the ovaries at death (Fig. 2). For all seals, the first injury occurred when the animals were in the follicular phase of the oestrous cycle. Only one seal was injured after she had ovulated, but her first two injuries were during oestrus (Fig. 2). When the mean time of the first mobbing injury before death was compared between groups, the postovulatory seals had been injured 2–3 days earlier than had the preovulatory seals (Table 1). A range of 2–6 days was assumed for the period of oestrus. If the minimum period of oestrus is 2 days, four of the nine seals were estimated to be at oestrus at...
Table 1. Structural and cellular differences at death in the reproductive tracts of female Hawaiian monk seals fatally injured by adult male seals

<table>
<thead>
<tr>
<th>Reproductive status at death</th>
<th>Prevalent ovarian structure</th>
<th>Height of vaginal epithelium (μm)</th>
<th>Uterine gland formation and basal vacuolation</th>
<th>Time of first injury before death (days)</th>
<th>Estimated reproductive status at time of first injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preovulatory (n = 3)</td>
<td>Dominant follicle</td>
<td>7.0 ± 0.9</td>
<td>Yes</td>
<td>3.3 ± 1.9</td>
<td>Preovulatory (2 in oestrus)</td>
</tr>
<tr>
<td>Postovulatory (n = 4)</td>
<td>Corpus haemorrhagicum</td>
<td>4.5 ± 1.1</td>
<td>Yes</td>
<td>5.8 ± 1.8</td>
<td>Preovulatory (2 or 3 in oestrus)</td>
</tr>
<tr>
<td>Anovulatory (n = 2)</td>
<td>None</td>
<td>5.7 ± 0.9</td>
<td>No</td>
<td>1.0 ± 0.0</td>
<td>Anovulatory, but follicular phase</td>
</tr>
</tbody>
</table>

Values are means ± SEM. No significant differences between groups were found for any of the mean values presented.

Reproductive status was estimated by subtracting the interval between injury and death from the estimated age of the structure at death.

Number in oestrus depends on the estimated duration of oestrus (2–6 days).

Each seal had at least two antral follicles (≤ 4 mm diameter) that were not large enough to classify as preovulatory.

Fig. 1. Histological section of the mucosal layer of the uterine horns from female Hawaiian monk seals fatally injured by adult male seals. (a) This seal was 11–12 years old and had a dominant follicle on her left ovary. There are proliferative uterine secretary glands connecting to the uterine lumen and significant basal vacuolation. (b) This seal was 14 years old and had two small follicles on her left ovary. There is no basal vacuolation and minimal connection of the glands with the uterine lumen. Bar represents 500 μm. L: uterine lumen; BV: basal vacuolation; UG: uterine secretary gland; M: mucus within the secretory gland.

The reproductive morphology of parous seals that gave birth in the same breeding season in which they died were compared with parous seals that gave birth before the year in which they died. There were no differences between the groups in terms of mean age, length of the cervix, distance between the cervical os and the uterine bifurcation, length of either uterine horn, or the ovarian weight. The measurements of the two groups were, therefore, combined and classified as parous.
Fig. 2. Temporal relationship between the prevalent ovarian structure and the oestrous cycle from nine female Hawaiian monk seals injured by adult male monk seals. The age of the ovarian structure at the time of injury (○) was estimated by subtracting the interval between injury and death from the estimated age of the structure at death (●). Seals 1–4 were classified as postovulatory at death. Seals 5–7 were classified as prevulatory at death. Seals 8 and 9 were classified as anovulatory but possessed antral follicle development indicative of the follicular phase (the placement within the follicular phase could occur anywhere between days 7 and 14). The x-axis was taken from the oestrous cycle (as determined by hormonal analysis) from a captive, adult female Hawaiian monk seal (Pietraszek, 1992). Day 0 is the day of ovulation.

seals for the morphological data (Table 2). The active ovaries from the parous seals were significantly heavier \((P = 0.053, F = 4.811)\) than the inactive ovaries from these seals.

Several aspects of the reproductive morphology of the nulliparous seals differed from that of the parous seals. The nulliparous seals were younger \((P = 0.074, F = 4.392)\) than the parous seals (Table 2). The uterine body (cervical os to uterine bifurcation; \(P = 0.017, F = 9.579\)) and both uterine horns of the nulliparous seals were significantly shorter \((P = 0.062, F = 4.879\) and \(P = 0.047, F = 5.819\) for right and left horns, respectively) compared with those of the parous seals. Although the nulliparous animals were significantly younger than the parous seals, differences in reproductive tract measurements between these two groups did not reflect a correlation with age. All other reproductive tract parameters were similar between groups. The uterine horns of the seal of unknown parity were longer than all other seals measured.

Discussion

The results of this study support the hypothesis that the timing of mobbing attacks is influenced by the oestrous cycle of female Hawaiian monk seals. Indeed, seven of nine mobbing victims sustained at least one injury during oestrus. Our results corroborate the evidence presented by Banish and Gilmartin (1992); they examined the ovaries of two adult Hawaiian monk seals that were fatally injured by adult male seals, and both had recently ovulated. Previous evidence indicated that the reproductive cycle influenced the timing of mobbing events, as most mobbings occurred to adult females during the pupping and mating season, when circulating concentrations of testosterone are increasing in adult male seals (Atkinson and Gilmartin, 1992). However, the study reported here provides the first definitive evidence that mobbing events are stimulated by ovarian activity in female seals.

Although two of the seals in the study reported here were classified as anovulatory, they exhibited signs of ovarian activity (antral follicle development) and could have been early preovulatory. In addition, Banish and Gilmartin (1992) examined the ovaries of a 2-year-old mobbing victim and reported histological evidence of pubertal follicular development. The earliest known age at which female seals give birth is 5 years (Johanos et al., 1990, after an estimated 11 month gestation (NMFS, unpublished data). The 3-year-old in the present study and the 2-year-old in the study of Banish and Gilmartin (1992) were considered immature on the basis of size, and were younger than any Hawaiian monk seals known to conceive. This finding indicates that puberty in Hawaiian monk seals may occur well before young seals can sustain a pregnancy.

The periovulatory phase of the oestrous cycle can be separated into pre- and postovulatory phases. During both of these phases, biosynthesis of steroid hormones is very dynamic. The oestrous cycle of a captive adult Hawaiian monk seal was 35 ± 3 days, and the preovulatory phase coincided with several days of increasing concentrations of circulating oestrogen, which reach a maximum at about the time of ovulation (Pietraszek, 1992). After ovulation, follicular granulosa cells of the ruptured follicle change to luteinized cells and begin to secrete progesterone (Yoshida, 1982). Circulating concentrations of progesterone in a captive female monk seal persisted for 17–20 days (J. R. Pietraszek and S. Atkinson, unpublished data). When these findings were applied to the data from the study reported here, the frequency of mobbings were clustered over two weeks, just before ovulation. The exception was a single seal that was injured approximately 24 h after ovulation but received two injuries just before this while in oestrus. Thus, all of the seals would have been under, or very recently had been under, the influence of increased oestrogen concentrations.

Steroid hormones and their metabolites are the structural basis for pheromones in many mammals. Pheromones are known to extend anoestrus, initiate, accelerate and synchronize oestrous cycles, block pregnancy, accelerate puberty, and act as sex attractants (Silverman, 1977). The behavioural ecology of Hawaiian monk seals suggests that there is olfactory communication: seals are seen nuzzling and possibly sniffing each other upon approach. Although Ceruti et al. (1985), suggested that pheromones are present in marine mammals, none have been confirmed or isolated. Ceruti et al. (1985) analysed various body fluids for pheromonal-type compounds in cetaceans. Although they did find several compounds, including steroids, in most of the body fluids, the pheromonal nature of these compounds was not confirmed. There are no other reports relating pheromones to the behavioural ecology of pinnipeds or other marine mammals.

As most of the seals in this study would have been in oestrus, or approaching ovulation, when they were injured by
adult male seals, it is likely that high concentrations of oestrogen were present in these females. These oestrogens could be acting as pheromonal attractants to the male seals. This form of olfactory communication, along with behavioural cues, may be one mode of detection used by males of this non-gregarious species to locate, court and mate female seals. Females with increased concentrations of oestrogen may be at higher risk of mobbing by adult males. For example, if a female seal was in oestrus, she may attract more than one male, which could lead to a mobbing incident, whereas a non-oestrous female would not solicit males, decreasing the likelihood of being a victim of a mobbing event. Yet, non-oestrous seals, including males and immature females, are also victims of mobbings. In these situations, and possibly cases where a seal is injured more than once over several days, behavioural cues may be the primary catalyst for mobbings. Behavioural and social interactions that precipitate mobbings may include lethargic or submissive behaviour by potential victims and vigorous seal interactions nearby that attract potential mobbers.

Morphometric analysis of the reproductive tract can be used to answer basic physiological questions about a particular species. Mammalian species differ in the location of fetal attachment, some species holding the fetus in the body of the uterus, for example primates, while others, for example sheep and dogs, hold the fetus in the uterine horns (McDonald, 1980). From our observations of the reproductive tracts of Hawaiian monk seals, we conclude that the fetus is carried in one of the uterine horns, although the site of attachment is unknown. Reports of fetal attachment in a variety of phocid and otariid seals, including the related Northern elephant seal, indicate that fetal attachment occurs in the uterine horn (Craig, 1964; Boshier, 1981). However, there are no reports for any of the species of monk seal.

Morphometric analyses may also be used to establish the reproductive status of a dead seal. The measurements of the body of the uterus (length of cervical os to uterine bifurcation) and each uterine horn of the seal whose parity was unknown indicated that she had recently given birth. She was 5 years old, the earliest known age of first reproduction in Hawaiian monk seals (Johanos et al., 1990). The uterine horns of this seal were larger than any others measured in the study. It is possible that this seal was still undergoing postpartum uterine involution when injured, although she also had a dominant ovarian follicle.

In conclusion, this study has provided basic information on the reproductive tract of female Hawaiian monk seals, which may be useful in differentiating between parous and nulliparous seals. This study also demonstrated that most reproductive tracts from mature female Hawaiian monk seals, fatally injured by adult males, were periovulatory at the time of injury. This finding indicates that the timing of mobbing events involving female seals are influenced by the oestrous cycle. We conclude that the reason the majority of mobbing victims are female is because mobbing is a sociosexual behaviour in which male seals are seeking and attempting to mate with a female seal in oestrus. We suggest that olfactory cues of a pheromonal nature may communicate the reproductive status of a female seal.

The authors thank W. G. Gilmartin, L. M. Hiruki and T. J. Ragen for stimulating discussions and review of this manuscript; NMFS staff for assisting with the field necropsies; L. M. Hiruki for performing the dental band analyses to age the seals; and J. Palmer for helping with the histology. This work was supported by the National Marine Fisheries Service and Sea Grant College Program of the University of Hawaii. B. Kuhn was an NSF-REU student at the University of Hawaii; School of Ocean and Earth Science and Technology.

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