Aquatic Mammals 2005, *31*(1), 52-61, DOI 10.1578/AM.31.1.2005.52

Use of Faecal Testosterone Concentrations to Monitor Male Florida Manatee (*Trichechus manatus latirostris*) Reproductive Status

Iskande L. V. Larkin,[1] Timothy S. Gross,[1,2] and Roger L. Reep[1]

[1]*Department of Physiological Sciences, Box 100144 HSC, 1600 SW Archer Road, University of Florida, Gainesville, FL 32610, USA*
[2]*U.S. Geological Survey, Florida Caribbean Science Center, Gainesville, FL 32653, USA*

Abstract

The Florida manatee is an aquatic herbivore found in tropical, coastal waterways. This endangered species becomes sexually mature at ~2 to 5 years of age. Reproductively active adults often form mating herds, consisting of one focal female pursued by several males. Understanding Florida manatee (*Trichechus manatus latirostris*) reproductive biology is important for establishing population models, making management decisions, and recognizing differences between healthy and unhealthy states. Field and necropsy data indicate that manatees do not have well-defined breeding seasons and are diffuse in their breeding patterns. They exhibit reproductive activity throughout the warm months, having peaks during spring and lulls during winter months. Monitoring male testosterone concentrations in both wild and captive populations can provide direct comparisons of physiological data on the reproductive status of these animals. The objectives were to (1) validate the use of a commercial testosterone radioimmunoassay kit for measuring faecal concentrations of the parent steroid in male Florida manatees, (2) identify biologically meaningful distinctions between gender and levels of maturation measured with faecal testosterone concentrations, and (3) identify seasonal hormone fluctuations. Of individual adult mean values, 62% of males had higher testosterone concentrations than all adult females measured. The total range of male faecal testosterone concentrations measured was 120.8–36,240 ng/g dry weight; whereas, females ranged 120.8–5,919 ng/g. Seasonal fluctuations in hormone concentrations were observed in captive manatees with peaks during spring and/or fall, supporting the hypothesis that Florida manatees are a diffusely seasonal breeding species. Results indicate that radioimmunoassays of faecal hormones can be a useful, non-invasive tool for monitoring testosterone concentrations in Florida manatees, enhancing the accuracy of current monitoring methods of using behavior or morphometrics. This can be particularly helpful in field sites where animals are not captured for health assessments and water clarity limits observations of the animals, which is typical of most water bodies manatees inhabit.

Key Words: radioimmunoassay, faecal steroids, testosterone, Sirenia, Florida manatee, reproduction, endocrinology, *Trichechus manatus latirostris*

Introduction

The Florida manatee (*Trichechus manatus latirostris*) is a member of the unique mammalian order Sirenia, which evolved a number of specialized adaptations for a life of aquatic herbivory. Studies of this endangered species' breeding patterns indicate that manatees are a semi-social species, interacting with conspecifics without forming long-term bonds, except in the case of a mother and her calf (Hartman, 1979; Reynolds, 1981). Gestation length is estimated at 12 to 14 months (Odell et al., 1995; Rathbun et al., 1995; Reid et al., 1995) and manatees reach sexual maturity at approximately 2 to 5 years of age (Marmontel, 1995; Odell et al., 1995; Rathbun et al., 1995). Reproductively active adults often form mating herds, which consist of one focal female pursued by several males. Individual male members participating in the herd are transitory, try relentlessly to hold on to the female, and roll over in attempts to gain access to her ventrum for mating (Bengtson, 1981). Males can pursue a focal female for two to four weeks (Hartman, 1979; Rathbun et al., 1995); however, physiological estrus may not necessarily occur during the entire period of pursuit but, instead, could last for only a brief period during the whole mating herd scenario. Observations of captive breeding suggest mating behaviour is not an accurate indicator of female reproductive status (Odell et al., 1995), but unpublished hormone data indicate physiological estrus may range from one to six days (Larkin, 2000; Odell et al., 1995). It is possible that the majority of the two to four weeks of male pursuit comprises the establishment of dominance among males or relates to a strategy of sperm

competition, with males breeding as frequently as possible while the female is receptive (Gomendio et al., 1998; Reynolds et al., 2004). Female manatees exhibit promiscuous breeding behaviour, mating with several males in the herd (sensu, Wilson, 1975; Wittenberger, 1978). As a breeding strategy, this may be more specifically described as "scramble competition polygyny" (Alcock, 1983). This reproductive strategy was similarly described for humpback whales (*Megaptera novaeangliae*) (Tyack & Whitehead, 1982).

Hartman (1979) conducted the first major comprehensive study of Florida manatee behaviour and indicated that manatee breeding occurred throughout the year without seasonal trends. He also noted some evidence suggesting an increase in the natality of calves during the spring. Since then, many studies have provided further evidence of some reproduction throughout the year, but with seasonal peaks and lulls in different aspects of reproduction. These aspects include changes in male and female gonad activity; periods of increased calf mortality; and field observations of mating herds, pregnancy, and lactation (Boyd et al., 1999; Hernandez et al., 1995; Marmontel, 1995; Odell et al., 1981; O'Shea & Hartley, 1995; O'Shea & Langtimm, 1995; O'Shea et al., 1985; Rathbun et al., 1995). Hernandez et al. (1995) observed regressed seminiferous tubules with immature cell stages and reduced diameters during the winter months (December to February), which corroborate with changes in testicular weight and appearance noted by Odell et al. (1981). Of males measuring 241 to 280 cm and greater than 280 cm in length, 13% and 25%, respectively, had sperm present in either the testes or epididymides in winter, but none were fully spermatogenic. In non-winter months (March through November), mature sperm were found in 75% and 93% of the 241-280 cm and the > 280 cm groups. These data suggest that male manatees exhibit decreased reproductive activity during colder months of the year, consistent with a diffuse seasonal reproductive pattern. A diffuse breeding pattern is illustrated by species that breed throughout the year, with peaks and lulls in reproductive activity that occur during energy rich or poor periods, as exemplified by another sirenian, the dugong (*Dugong dugon*) (Marsh et al., 1984c). The fact that fewer manatee mating herds are seen during the winter months, when individuals are in closer proximity because they congregate in warm water refuges, further supports the hypothesis that reproduction is suppressed during the winter.

This research is the first to characterize reproductive hormone concentrations over time in the Florida manatee. The present study focuses on repeated longitudinal measurement of testosterone concentrations in the faeces of male manatees. The overall goal of this research was to validate the radioimmunoassay (RIA) of faecal testosterone in the Florida manatee for a longitudinal study of hormone concentrations obtained from identified captive individuals. Longitudinal faecal collections would allow for a qualitative description of hormonal fluctuations within an individual, and these could indicate seasonal patterns. We hypothesized that testosterone levels were higher during warmer months of the year and higher in the plasma and faecal samples of adult male manatees compared to male calves, female juveniles, and female adults (nonpregnant and pregnant). Opportunistically collected samples from wild manatees were analyzed to allow comparisons among different reproductive groups because the captive animals utilized for these studies consisted of adults housed in single-sex groups. We characterized faecal testosterone concentrations of manatees to test for differences in mean hormone concentrations among locations where animals were housed. Values from wild animal samples were compared with seasonal values measured in captive animal samples.

Materials and Methods

Animals

Captive adults were maintained in single-sex groups at several sites in Florida: SeaWorld Florida (SWF) (9 males), Lowry Park Zoo in Tampa (2 males), and Walt Disney World Resorts, Inc., Epcot, The Living Seas (3 males) (see Table 1). Captive animals provided long-term repeated samples for monitoring fluctuations in hormone concentrations over time. Samples collected from wild manatees were from individuals in the Crystal River area and from necropsied animals throughout the state (males: 20 adult, 16 juvenile, and 9 calves). Externally, gender can be distinguished easily when viewing the animal's ventrum by measuring the distance of the genital aperture to the anus, which is greater in males than in females (Bonde et al., 1983; Husar, 1977). Collections from wild individuals were opportunistic, and repeated samples from the same individuals were not possible. The data include a comparison with female samples—12 captive and 58 wild adults.

Animals were divided into three age categories: adult, juvenile, or calf. These age estimate categories were based on life history reports, observations of physiological events (e.g., nursing calf or pregnant female), or total body length, as defined by the Sirenia Project, Florida Caribbean Science Center, U.S. Geological Survey (USGS). A calf is defined as 80-245 cm, with an age range of 0 to 2+ years; a juvenile is 246-265 cm, with an age range of 1.5 to 4 years; and an adult is 266->325 cm, with an age range of 3 to 6+ years. The use of body length as an age estimate for Sirenia

Table 1. Background information on captive male manatees included in analysis

Location	Name/ID	Sex	Age during study	Reason for captivity	Reproduced successfully	Other comments
Epcot	Chester LS-Tm-0191	Male	4-5	Captive born	No	DOB 13/9/91
Epcot	Hurricane LZP-1004321	Male	12-13	Captive born	No	DOB 23/11/83
Epcot	Gene LZP-100378	Male	20-21	Rescued as a large calf	Yes	208 cm at rescue on 16/2/77
Lowry	New Bob LZP-100518	Male	2-3	Orphaned calf	No	Rescued on 3/1/93
Lowry	Hugh LZP-100414	Male	11	Captive born	Unknown	DOB 28/6/84
SeaWorld	Group*	9 males	Range 2-7	6 orphaned; 3 captive born	No	

* Group includes Webster/SWF-Tm-9121B, Slip/SWF-Tm-9122CB, Doc/HS 9201, Spike/SWF-Tm-9114B, Mo/SWF-Tm-9417B, Little Joe/SWF-Tm-8911B, Dakota/SWF-Tm-9305B, Brian/SWF-Tm-9324B, and Hunter/SWF-Tm-9503B; DOB = date of birth.

Project's manatee photo-identification system was determined using data from Odell (1977), O'Shea & Reep (1990), and Marmontel (1993). Length of wild animals was estimated using an object of known length such as an underwater writing tablet.

Faecal Collections

Faecal samples of approximately 5 g or more were collected weekly from captive individuals for one year from Epcot (August 1995-November 1996) and SWF (November 1996-November 1997), and six months from Lowry Park Zoo (August 1995-February 1996) ($n = 472$ samples assayed). Samples were collected throughout the day using a handheld pool net to retrieve samples either directly from the animal as it was voided or from the water within minutes of excretion. Due to gut transit time, the faecal sample hormone values in many species is typically representative of circulating concentrations from one to two days prior to the faecal collection date (Palme et al., 1996; Rees, 1982; Warner, 1981; Wasser et al., 1996). In the case of Florida manatees, the hormone values may actually represent concentrations from five to eight days prior to the collection date (Best, 1981; Larkin, 2000; Lomolino & Ewel, 1984). When possible, colored corn was fed to captive manatees to increase the reliability of faecal identification from individuals and improve collection time by marking the faeces from different individuals with an identifiable color (Larkin, 2000). Samples could be identified from specific individuals at both Lowry and Epcot; however, the large number of individuals and other additional logistical problems prohibited this at SeaWorld. Thus, samples from SeaWorld had to be treated as one group. Faecal collections from wild manatees ($n = 102$ samples assayed) were opportunistic and did not involve repeated samples from known individuals. Samples from wild animals were treated as groups by age (adult, juvenile, or calf) and gender. Samples were stored in plastic bags on ice in the field and stored at -20° C until assayed.

Plasma and Necropsy Collections

Sixteen plasma samples (adult female=7, pregnant=1, juvenile=2, adult male=3, and calf=3), along with matching faecal samples (adult female=8, pregnant=5, juvenile=2, adult male=3, and calf=4) were collected on the same day by veterinarians during routine medical examinations or by individuals participating in the capture, tag, and release program of wild manatees. These samples, like the wild samples, did not allow for repeated sampling from the same individual and were not collected on a set schedule. Plasma samples from captive manatees were not necessarily from the same individuals from which repeated faecal collections were being made. In three cases, additional faecal samples were collected from an animal, but not additional plasma samples.

Faecal samples were collected from manatees that were brought into the Manatee Salvage Program, Florida Wildlife Research Institute (FWRI) Marine Mammal Pathobiology Laboratory in St. Petersburg, Florida, and necropsied within approximately 72 h of death. Faecal samples were collected and treated as mentioned above for captive and wild animals.

Steroid Radioimmunoassay

Manatee faecal samples were removed from the freezer and freeze dried in a lyophilizer

(Freezemobile 3, VirTis Co., Gardiner, NY). Several different solubilization solutions were tried, but the most efficient consisted of 5 ml citrate buffer at pH 3.7 and 5 ml 100% ethanol added to 0.25 g dried faecal sample, which were put on a rotating mixer at room temperature for 12-24 h to solubilize the steroids from the faecal mass. This was centrifuged, and an aliquot of this solution was extracted with 4 ml of ethyl ether and stirred for 1 min. The aqueous phase was frozen in a bath of methanol and dry ice. The organic phase was decanted and completely dried with air blown into each sample tube. This procedure was repeated for a double extraction.

The technique utilized to measure hormone concentrations from faecal and plasma samples in the Florida manatee was a Testosterone Double Antibody ^{125}I Radioimmunoassay Kit purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). The protocol provided with the kit was followed directly except for two aspects. First, a total counts tube was used with only the ^{125}I radiolabeled hormone at the same amount as given to all other tubes in the assay; and secondly, the amount of both faecal and plasma sample utilized in the assay differed from what was recommended. Instead, the volume of sample to be used in each plasma and faecal assay was determined by dilution curves of a representative pooled sample and optimized to displace the ^{125}I testosterone at 50% binding. In brief, the assays utilized a standard curve, which included the following tubes in duplicate: total counts (Tc), nonspecific binding (NSB), baseline with no steroid (B0), plus 7 tubes with increasing concentrations of standard steroid: 100, 200, 500, 1,000, 2,500, 5,000, and 10,000 ng/100µl. Once the standard or sample solutions were pipetted into tubes, the radiolabel and first antibody were added. This was stirred and incubated at 37° C as indicated in the protocol. Then, the second antibody or precipitate was added and centrifuged for 20 min. The solution was decanted, and the remaining pellet was counted for radiation levels. All samples were measured in duplicate. The data were used quantitatively to identify fluctuation patterns in hormone concentrations.

The faecal assay (25 µl at 1:40 before extraction) and the plasma assay (50 µl neat) were used to measure testosterone concentrations. The mean extracted recovery of a known amount of radiolabeled testosterone added to a subset of individual faecal samples was 66 ± 11% SD. The range of detectable concentrations of testosterone from the faecal solution was 120.8-36,240 ng/g and from plasma samples ranged 1-300 ng/ml. The identified crossreactivities for the testosterone antiserum were as follows: 3.4% for 5α–dihydrotestosterone, 2.2% for 5α–androstane-3β, 17β–diol, 2.0% for 11-oxotestosterone, < 1.0% for 6β–hydroxytestosterone, 5β–androstane–3β, 17β–diol, 5β–dihydrotestosterone, androstenedione, epiandrosterone, and < 0.01% for all other steroids examined. The faecal testosterone internal standard (0, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, and 10.0 ng/ml) had a calculated linear curve of y = 1673.6 + 1.5289x where x equals the log concentration and y equals the bound. The internal standard curve had a correlation coefficient of 0.99 with the standard curve. The dilution curve of the faecal solution (50, 100, 200, and 500 µl) was parallel to the standard curve, which was confirmed by a test of homogeneity of variance. The plasma internal standard utilized the same concentrations of testosterone as the faecal samples, and the calculated linear equation was y = 189.16 + 0.78694x. The curve was parallel to the standard curve with a correlation coefficient of 0.99. A plasma dilution curve (25, 50, 100, and 200 µl) was tested for homogeneity and was parallel to the internal standard. Inter-assay and intra-assay coefficients of variation (CV) were measured for faecal testosterone samples: 21.4% and 3.0%, respectively. Inter-assay CV was calculated utilizing the ED 50 value for each standard curve provided by *Beckman ImmunoFit EIA/RIA®* program, and intra-assay CV calculations were measured from samples run in duplicate. Plasma samples were run in a single assay with an intra-assay CV of 1.6%.

Analyses of Hormonal Parameters

Raw data from the gamma counter (LKB-Wallac 1282 CompuGamma, LKB Wallac, www.Wallac.fi/) were initially analyzed utilizing *Beckman ImmunoFit EIA/RIA® Version 3.1* (Copyright ® 1989-1991, Beckman Instruments Inc., Microsoft Corp.). This program provided statistical regressions for the standard curve produced in each assay. The data were then log transformed to provide the best fit for a normal distribution. ANOVA and Tukey pair-wise contrasts indicated that male and female concentrations for testosterone (female, $p = 0.18$, male, $p = 0.83$) were similar between the wild and necropsy values; thus, these two groups were pooled. Regression analysis was used to determine relationships between faecal and plasma hormone concentrations. A *t*-test was utilized to determine the differences between adult male and female testosterone concentrations. Due to the assay minimum and maximum detectable concentrations, low faecal values were truncated at 120.8 ng/g and high values at 36,240 ng/g. For monthly comparisons where the data did not meet normality or equal variance assumptions, a Kruskal-Wallis ANOVA on Ranks was run with Dunn's Method for all pairwise multiple comparisons. A General Linear Model test was run to identify the source of variation among wild age groups. *Sigma Stat Version 2.03* (SPSS, Inc.)

and *SAS Version 6.12* (SAS Institute, 1989), were used to conduct these analyses. All tests were conducted at the $\alpha = 0.05$ level of significance.

Results

Faecal and Plasma

Comparisons were made between faecal hormone concentrations and matching plasma concentrations to validate the faecal radioimmunoassay. Mean plasma testosterone concentrations across age and reproductive groups indicate higher concentrations in adult males compared to male calves and females (Figure 1A). Similarly, faecal hormone concentrations exhibited higher mean testosterone values for adult males compared with females and younger manatees (Figure 1B). A regression analysis of the data on 16 matching faecal and plasma testosterone samples of the same individual animals showed a significant correlation ($R^2 = 0.69$; $p < 0.05$; Figure 1C).

Gender

We sought to determine if faecal testosterone concentrations could distinguish male and female adult manatees. Values from all adult manatees, male and female, wild and captive, throughout the year were compared. The total range of male faecal testosterone concentrations were 120.8-36,240 ng/g dry weight; whereas, females ranged 120.8-5,919 ng/g. Captive animals with multiple samples per individual were represented by their mean; whereas, wild animals were indicated by their single sample value, thus, each individual was represented by a single value. With the single value for each individual, male faecal testosterone concentrations ranged from 120.8-36,240 ng/g with a mean of 10,238 ng/g ($n = 37$); whereas, mean female faecal testosterone concentrations ranged from 120.8-1,248 ng/g with a mean of 294 ng/g ($n = 70$). A *t*-test indicated male testosterone values were significantly higher ($p < 0.001$) than female values (Figure 2). Of individual adult mean values, 62% of males had higher testosterone concentrations than all adult females measured.

Location

Comparisons were made among all captive manatees (by location) and all wild manatees to identify variations in faecal hormone concentrations associated with the living environment. A two-way ANOVA indicated no significant difference between locations alone ($p = 0.11$), but the monthly pattern was significantly different ($p < 0.001$). The results suggest the range of values between locations is similar, but some environmental factors throughout the year may influence temporal fluctuations between captive facilities. These data indicate that values from different locations should not necessarily be pooled together for all types of analyses. A confounding factor is that all age classes were included in this comparison due to the limited number of samples.

Age

The majority of faecal samples from captive animals were collected from adults. Faecal samples from wild manatees provided a wider range of ages and were collected opportunistically throughout the year. However, larger numbers of wild collections were possible during the winter months due to access at warm water refuges. Means were calculated for the different age classes (adult, juvenile, or calf). The highest values were measured in adults, followed by juveniles, and then calves (Figure 3). Although a General Linear Model test did not show a significant difference between the ages, the increased variance was due to seasonal hormone changes ($p = 0.007$).

Seasonality

To identify seasonal fluctuations in hormone concentrations, monthly means were obtained for all samples collected from captive male manatees as well as opportunistic samples from wild manatees. Data from Epcot males indicated statistically higher testosterone concentrations in February and March compared to July, followed by a rise again in September (Figure 4). Males at SWF had higher testosterone concentrations in March that were statistically greater than values in November, December, January, February, April, May, and August. Although all months are not represented in the data from the two males at Lowry, a significant autumnal peak in September is followed by decreasing values through December. To allow for some comparison with the captive data, all wild male data were graphed by month; however, all months were not sampled (Figure 5). There is a trend of increasing concentrations from January through March, but these values are overshadowed by the concentration in July. The highest values are generally in adults, except for in one juvenile.

Discussion

This is the first time samples were taken over an extended period from captive and wild manatees to elucidate biologically meaningful testosterone data related to age, gender, and season. We used radioimmunoassays of faecal hormones, a noninvasive means of measuring steroid values, because there are logistical restrictions to blood collections with this endangered species. Repeated faecal collections from captive manatees were readily available, and limited sampling from wild animals could provide

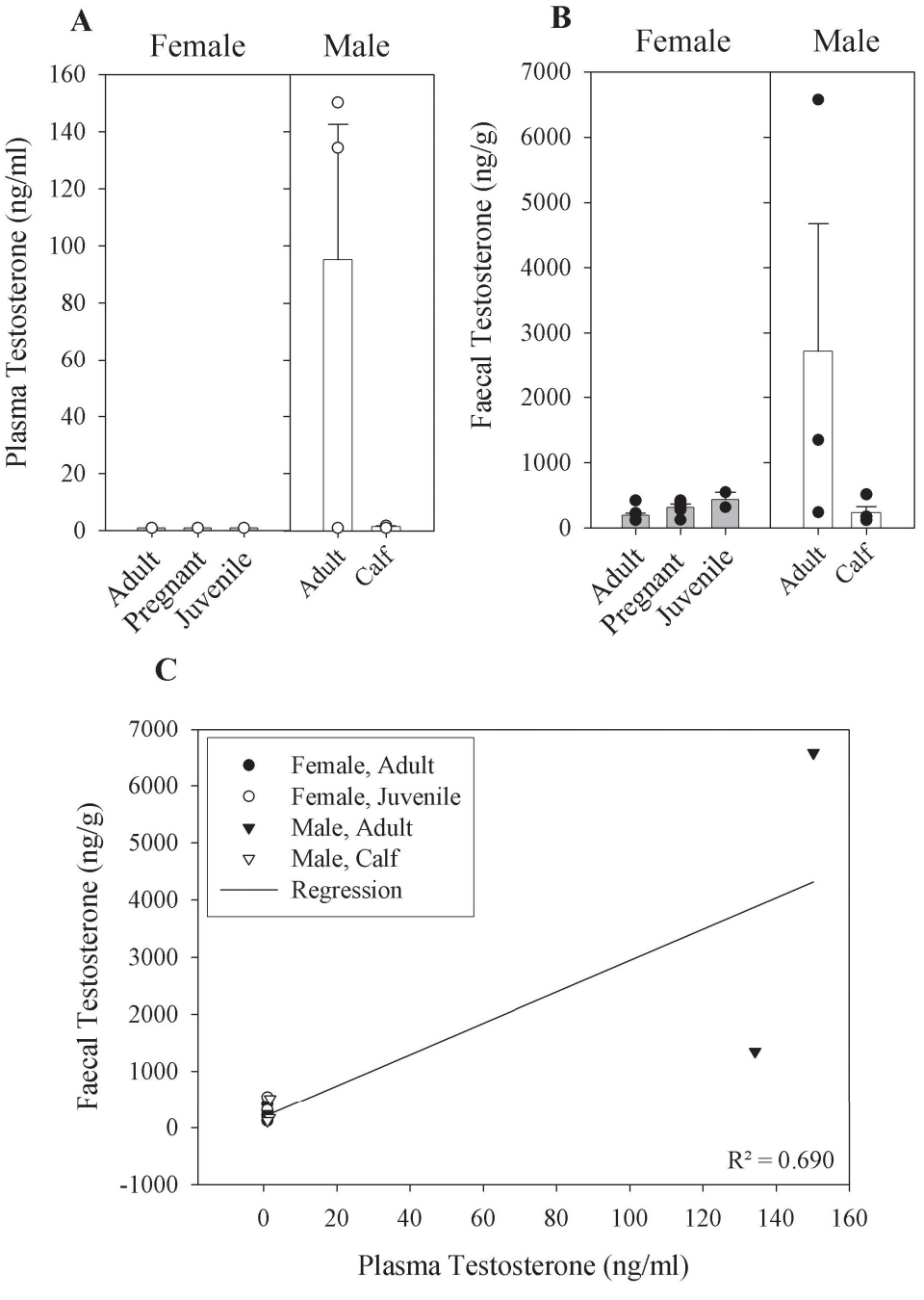

Figure 1. Plasma (A) and faecal (B) concentrations of testosterone collected simultaneously from the same individual, with samples grouped by reproductive state; the bars indicate mean values, whiskers indicate ± SE, and circles (white = plasma, black = faecal) indicate individual animal values. The number of individuals in each group are as follows: Plasma: adult female=7, pregnant=1, juvenile=2, adult male=3, and calf=3; faecal: adult female=8, pregnant=5, juvenile=2, adult male=3, and calf=4. Regression analysis of male and female faecal and plasma testosterone concentrations is presented (C). Each data point represents a single individual that had both faecal and plasma samples collected on the same day.

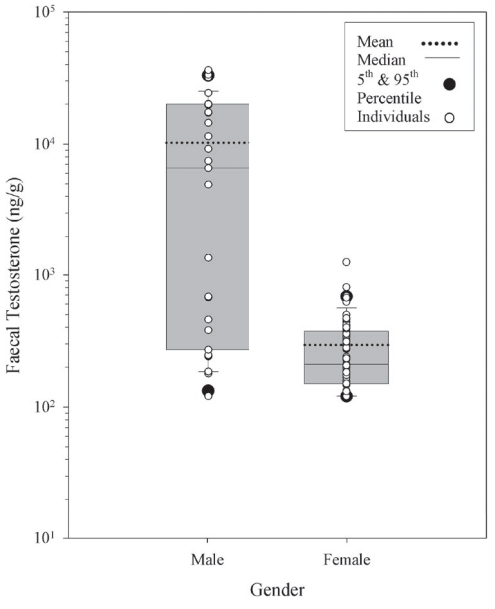

Figure 2. Box plots of faecal testosterone concentrations in male and female adult manatees; samples were collected throughout the year. Captive animals with multiple samples per individual are represented by their mean; whereas wild animals are indicated by their single sample value, thus each individual is represented by a single value. The top and bottom of each gray box indicate the 25th and 75th percentiles. The whiskers above and below the gray boxes indicate 10th and 90th percentiles. The black circles indicate the 5th and 95th percentiles. White circles indicate individual values.

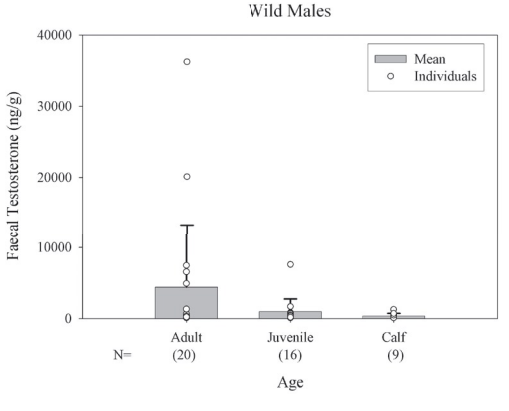

Figure 3. Wild male manatee faecal testosterone concentrations grouped by age; samples were collected throughout the year. The number of samples contributing to each mean value is indicated below the age. The bars indicate the mean ± SE.

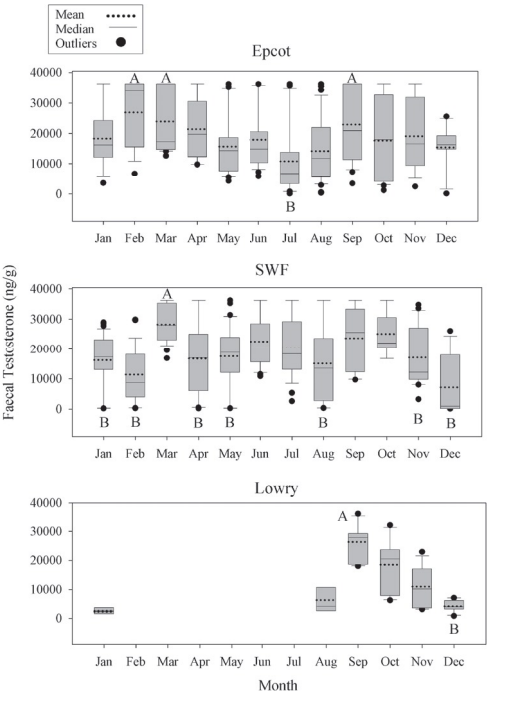

Figure 4. Box plots of Epcot, SWF, and Lowry male manatee monthly faecal testosterone concentrations; months without boxes are months when no samples were collected. The top and bottom of each gray box indicate the 25th and 75th percentiles. The whiskers above and below the gray boxes indicate 10th and 90th percentiles. Black dots indicate individual outliers. Statistically significant differences between months, as calculated by a Kruskal-Wallis ANOVA on Ranks, are indicated by different letters.

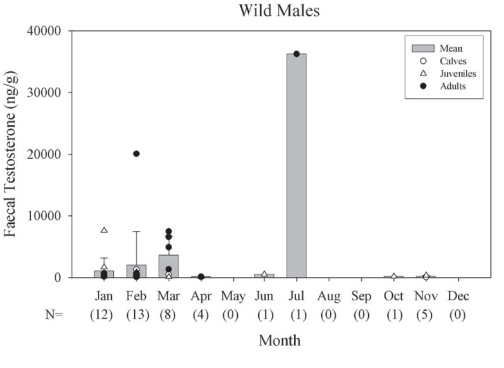

Figure 5. Wild male manatee mean monthly faecal testosterone concentrations ± SE; the number of faecal samples per month is indicated below the month. The age groups are distinguished by different symbols.

direct comparisons with captive values. This technique was successfully validated in the laboratory and was able to recognize biologically meaningful characteristics, such as higher testosterone concentrations in adult males compared to other age and gender groups.

Despite the low number of animals and the inability to collect repeated matching faecal and plasma samples from the same individuals, it is apparent that adult male manatees generally have higher concentrations of faecal and plasma testosterone than females and sexually immature animals. The regression analysis (Figure 1C) relied heavily on two data points, but suggested that a larger sample size may corroborate a correlation between faecal and plasma concentrations within individual animals. A time delay between the faecal and plasma concentrations is a factor that can affect the correlation. Larkin (2000) observed a mean gut transit time in the Florida manatee of approximately seven days, which is similar to transit times measured by others: six days by Lomolino & Ewel (1984) and five days by Best (1981). Unless males exhibit pronounced seasonal reproductive activity, individual testosterone concentrations are not expected to fluctuate cyclically or to the same degree as observed in female estradiol or progesterone concentrations. Identifying the time differential between circulating hormones and those that have passed through the digestive tract of the manatee, utilizing serial blood and faecal collections, is another key step for correlating plasma and faecal hormone concentrations.

The Florida manatee is not a strong seasonal breeder, and individuals can breed throughout most of the year (Hartman, 1979). It is most likely that manatees are similar to dugongs (*Dugong dugon*) in having a diffusely seasonal breeding period (Marsh et al., 1984a, 1984b, 1984c). Seasonal data presented here indicate that captive manatees have increased hormone concentrations in the spring and/or fall, depending upon location. Comparisons of faecal testosterone concentrations across the different environmental and housing locations of manatees in the study suggest that the overall range of concentrations measured did not differ significantly but that patterns over time at each location were significantly different. It may be that the shift in seasonal hormone peaks among the different captive groups is reflective of different environmental factors at each of the zoological facilities. For example, the Living Seas at Epcot is an indoor facility with salt water, but SeaWorld and Lowry Park have outdoor pools with fresh water. Variables, such as diet and environmental stressors (e.g., water temperature, social influences), can profoundly affect hormone concentrations (Adams et al., 1994; Arts et al., 1992; Moberg, 1985; Wasser et al., 1993), and husbandry protocols can vary among facilities. The data from captive animals support the hypothesis that manatees have increased hormone concentrations during the warmer months of the year, but determining the factors that influence peaks and lulls in hormone concentrations will require larger numbers of animals, both captive and wild.

The samples collected from wild manatees do not provide data that clearly demonstrate seasonality in testosterone concentrations. Wild male manatees represented (Figure 5) appear to have lower faecal testosterone concentrations compared with captive values (Figure 4); however, the majority of samples were collected during winter months when the animals are most easily accessed in the warm water refuges. Winter is when wild animals are energetically stressed due to cold water temperatures, decreased food availability, and increased risk of mortality due to cold stress; thus, reproductive activity is reduced (Ackerman et al., 1995; Boyd et al., 1999; Hernandez et al., 1995; Irvine, 1983; Marmontel, 1995; Odell et al., 1981; O'Shea & Hartley, 1995; O'Shea & Langtimm, 1995; O'Shea et al., 1985; Rathbun et al., 1995). A larger wild sample size throughout the year would be needed to confirm whether captive manatees actually have higher faecal testosterone concentrations during the nonwinter months of the year or not, as suggested by the single adult male sample collected in July (Figure 5). Additional differences between wild and captive environments include captive animals being housed in single sex groups, dependence on how individuals react to captivity, availability of nutritious food, types of food, veterinary care, and elimination of predatory and other natural threats, such as cold water temperatures, to the animal's well-being. Often, reproduction can occur at a much younger age and more frequently in captive animals than in the wild (Asa, 1996; Laws, 1973; Odell et al., 1995). These factors must be taken into consideration when analyzing various data sets.

Studies of Florida manatee endocrinology and reproductive physiology are still in their infancy, yet more is known of the Florida manatee than any other Sirenian. This is the first study with this species to use a noninvasive faecal hormone RIA technique as a tool to monitor long-term hormone concentrations for determining seasonal hormone fluctuations, and gender identification. Aspects of the data presented here are suggestive, with some questions still remaining. Data collected from wild manatees should be expanded to allow for more complete comparisons between captive and wild values. Husbandry methods, including training to obtain repeated blood samples from captive animals, should be supported. Collecting behavioural data will be an important additional tool for future studies. Faecal hormone RIAs can be invaluable for monitoring the Florida

manatee both in captive and wild settings. This is especially true when working with an endangered species where there are logistical obstacles not present when working with more common species. Of course, the status of being endangered suggests a sense of urgency, and, thus, all information available potentially can be significant in understanding the ecology and natural history of the Florida manatee.

Acknowledgments

We thank the many people and organizations that participated in this study, including Maggie Stoll for collecting samples; Jon Wiebe and Carla Wieser for their assistance with the endocrine techniques; Daniel Odell, Randy Runnells, and staff at SeaWorld Florida; Mark Barringer and staff at the Walt Disney World Resorts, Epcot, The Living Seas; David Murphy and Debbie Halin at Lowry Park Zoo, Tampa; Robert Bonde, Lynn Lefebvre, and staff at Sirenia Project, part of the U.S. Geological Survey (USGS), Biological Resources Division – Florida Caribbean Science Center; and the staff at the Florida Wildlife Research Institute Marine Mammal Pathobiology Laboratory in St. Petersburg, Florida. Funding for this project was received from the College of Veterinary Medicine at the University of Florida, Save the Manatee Club, and the U.S. Fish and Wildlife Service. We thank those who critically reviewed this manuscript before publication. All faecal and blood samples collected in this study were covered under Endangered Species Permit PRT-791721, issued to USGS's Sirenia Project. Tissue samples were covered under the Department of Environmental Protection (DEP) Endangered Species Permit PRT-773494. The University of Florida project IACUC approval number to utilize animal tissue was A164. This work also printed as College of Veterinary Medicine Journal Series No. 625.

Literature Cited

Ackerman, B. B., Wright, S. D., Bonde, R. K., Odell, D. K., & Banowetz, D. J. (1995). Trends and patterns in mortality of manatees in Florida, 1974-1992. In T. J. O'Shea, B. B. Ackerman, & H. F. Percival (Eds.), *Population biology of the Florida manatee* (pp. 223-258). Washington, DC: U.S. Dept. of the Interior, National Biological Service.

Adams, N. R., Abordi, J. A., Briegel, J. R., & Sanders, M. R. (1994). Effect of diet on the clearance of estradiol-17β in the ewe. *Biology of Reproduction*, *51*, 668-674.

Alcock, J. (1983). Male mating systems. In R. Thormhill & J. Alcock (Eds.), *The evolution of insect mating systems* (pp. 230-268). Cambridge, MA: Harvard University Press.

Arts, C. J. M., Govers, C. A. R. L., VanDenBerg, H., Blankenstein, M. A., & Thijssen, J. H. H. (1992). Effect of wheat bran on excretion of radioactively labeled estradiol-17β and estrone-glucuronide injected intravenously in male rats. *Journal of Steroid Biochemistry and Molecular Biology*, *42*, 103-111.

Asa, C. S. (1996). Reproductive physiology. In H. Harris, D. G. Kleiman, M. E. Allen, K. V. Thompson, & S. Lumpkin (Eds.), *Wild mammals in captivity: Principles and techniques* (pp. 390-417). Chicago: The University of Chicago Press.

Bengtson, J. L. (1981). *Ecology of manatees* (Trichechus manatus) *in the St. Johns River, Florida*. Unpublished Ph.D. dissertation, University of Minnesota, Minneapolis. 126 pp.

Best, R. C. (1981). Foods and feeding habits of wild and captive Sirenia. *Mammal Review*, *11*, 3-29.

Bonde, R. K., O'Shea, T. J., & Beck, C. A. (1983). *Manual of procedures for the salvage and necropsy of carcasses of the West Indian manatee* (Trichechus manatus). Jacksonville, FL: U.S. Fish and Wildlife Service, Sirenia Project. 175 pp.

Boyd, I. L., Lockyer, C., & Marsh, H. D. (1999). Reproduction in marine mammals. In J. E. Reynolds III & S. A. Rommel (Eds.), *Biology of marine mammals* (pp. 218-286). Washington, DC: Smithsonian Institution Press.

Gomendio, M., Harcourt, A. H., & Roldan, E. R. S. (1998). Sperm competition in mammals. In T. R. Birkhead & A. P. Moller (Eds.), *Sperm competition and sexual selection (*pp. 667-755). San Diego: Academic Press.

Hartman, D. S. (1979). Ecology and behavior of the manatee (*Trichechus manatus*) in Florida. *The American Society of Mammalogists* (Special Publication), 1-153.

Hernandez, P., Reynolds, J. E., III, Marsh, H., & Marmontel, M. (1995). Age and seasonality in spermatogenesis of Florida manatees. In T. J. O'Shea, B. B. Ackerman, & H. F. Percival (Eds.), *Population biology of the Florida manatee* (pp. 84-97). Washington, DC: U.S. Department of the Interior, National Biological Service.

Husar, S. L. (1977). The West Indian manatee (*Trichechus manatus*). In *Wildlife research report*. Washington DC: U.S. Department of the Interior, Fish & Wildlife Services.

Irvine, A. B. (1983). Manatee metabolism and its influence on distribution in Florida. *Biological Conservation*, *25*, 315-334.

Larkin, I. V. L. (2000). *Reproductive endocrinology of the Florida manatee* (Trichechus manatus latirostris)*: Estrous cycles, seasonal patterns and behavior.* Unpublished Ph.D. dissertation in Physiological Sciences, University of Florida, Gainesville. 354 pp.

Laws, R. M. (1973). Effects of human activities on reproduction in the wild. *Journal of Reproduction and Fertility*, *19*, Supplement, 523-532.

Lomolino, M. V., & Ewel, K. C. (1984). Digestive efficiencies of the West Indian manatee (*Trichechus manatus*). *Biological Sciences*, *3*, 176-179.

Marmontel, M. (1993). *Age determination and population biology of the Florida manatee,* Trichechus manatus latirostris. Unpublished Ph.D. dissertation, University of Florida, Gainesville. 408 pp.

Marmontel, M. (1995). Age and reproduction in female Florida manatees. In T. J. O'Shea, B. B. Ackerman, & H. F. Percival (Eds.), *Population biology of the Florida manatee* (pp. 98-119). Washington, DC: U.S. Department of the Interior, National Biological Service.

Marsh, H. (1995). The life history, pattern of breeding, and population dynamics of the dugong. In T. J. O'Shea, B. B. Ackerman, & H. F. Percival (Eds.), *Population biology of the Florida manatee* (pp. 75-83). Washington, DC: U.S. Department of the Interior, National Biological Service.

Marsh, H., Heinsohn, G. E., & Channells, P. W. (1984a). Changes in the ovaries and uterus of the dugong, *Dugong dugon* (Sirenia: Dugongidae), with age and reproductive activity. *Australian Journal of Zoology*, *32*, 743-766.

Marsh, H., Heinsohn, G. E., & Glover, T. D. (1984b). Changes in the male reproductive organs of the dugong, *Dugong dugon* (Sirenia: Dugongidae), with age and reproductive activity. *Australian Journal of Zoology*, *32*, 721-742.

Marsh, H., Heinsohn, G. E., & Marsh, L. M. (1984c). Breeding cycle, life history and population dynamics of the dugong, *Dugong dugon* (Sirenia: Dugongidae). *Australian Journal of Zoology*, *32*, 767-788.

Moberg, G. P. (1985). Influence of stress on reproduction: Measure of well-being In G. P. Moberg (Ed.), *Animal stress* (pp. 245-267). Bethesda, MD: American Physiological Society.

Odell, D. K. (1977). *Age determination and biology of the manatee*. Washington, DC: U.S. Fish & Wildlife Service.

Odell, D. K., Forrester, D. J., & Asper, E. D. (1981). A preliminary analysis of organ weights and sexual maturity in the West Indian manatee (*Trichechus manatus*). In R. L. Brownell & K. Ralls (Eds.), *Workshop proceedings* (Special Publication) (pp. 52-65). Orlando: Florida Department of Natural Resources.

Odell, D. K., Bossart, G. D., Lowe, M. T., & Hopkins, T. D. (1995). Reproduction of the West Indian manatee in captivity. In T. J. O'Shea, B. B. Ackerman, & H. F. Percival (Eds.), *Population biology of the Florida manatee* (pp. 192-193). Washington, DC: U.S. Department of the Interior, National Biological Service.

O'Shea, T. J., & Hartley, W. C. (1995). Reproduction and early-age survival of manatees at Blue Spring, upper St. Johns River, Florida. In T. J. O'Shea, B. B. Ackerman, & H. F. Percival (Eds.), *Population biology of the Florida manatee* (pp. 157-170). Washington, DC: U.S. Department of the Interior, National Biological Service.

O'Shea, T. J., & Langtimm, C. A. (1995). Estimation of survival of adult Florida manatees in the Crystal River, at Blue Spring, and on the Atlantic coast. In T. J. O'Shea, B. B. Ackerman, & H. F. Percival (Eds.), *Population biology of the Florida manatee* (pp. 194-222). Washington, DC: U.S. Department of the Interior, National Biological Service.

O'Shea, T. J., & Reep, R. L. (1990). Encephalization quotients and life-history traits in the sirenia. *Journal of Mammalogy*, *71*, 534-543.

O'Shea, T. J., Beck, C. A., Bonde, R. K., Kochman, H. I., & Odell, D. K. (1985). An analysis of manatee mortality patterns in Florida, 1976-81. *Journal of Wildlife Management*, *49*, 1-11.

Palme, R., Fischer, P., Schildorfer, H., & Ismail, M. N. (1996). Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock. *Animal Reproduction Science*, *43*, 43-63.

Rathbun, G. B., Reid, J. P., Bonde, R. K., & Powell, J. A., Jr. (1995). Reproduction in free-ranging Florida manatees. In T. J. O'Shea, B. B. Ackerman, & H. F. Percival (Eds.), *Population biology of the Florida manatee* (pp. 135-156). Washington, DC: U.S. Department of the Interior, National Biological Service.

Rees, R. A. (1982). Gross assimilation efficiency and food passage time in the African elephant. *African Journal of Ecology*, *20*, 193-198.

Reid, J. P., Bonde, R. K., & O'Shea, T. J. (1995). Reproduction and mortality of radio-tagged and recognizable manatees on the Atlantic coast of Florida. In T. J. O'Shea, B. B. Ackerman, & H. F. Percival (Eds.), *Population biology of the Florida manatee* (pp. 171-191). Washington, DC: U.S. Department of the Interior, National Biological Service.

Reynolds, J. E., III (1981). Manatees of Blue Lagoon Lake, Miami, Florida: Biology and effects of man's activities. In R. L. Brownell & K. Ralls (Eds.), *Workshop proceedings* (Special Publication) (pp. 25-32). Orlando: Florida Department of Natural Resources.

Reynolds, J. E., Rommel, S. A., & Pitchford, M. E. (2004). The likelihood of sperm competition in manatees–explaining an apparent paradox. *Marine Mammal Science*, *20*, 464-476.

SAS Institute. (1989). *SAS/STAT user's guide, Version 6.* Cary, NC: SAS Institute Inc. 943 pp.

Tyack, R., & Whitehead, H. (1982). Male competition in large groups of wintering humpback whales. *Behaviour*, *83*, 137-154.

Warner, A. C. I. (1981). Rate of passage of digesta through the gut of mammals and birds. *Nutritional Abstracts and Reviews Series 'B,' 51*, 789-820.

Wasser, S. K., Papageorge, S., Foley, C., & Brown, J. L. (1996). Excretory fate of estradiol and progesterone in the African elephant and patterns of fecal steroid concentrations throughout the estrous cycle. *General Comparative Endocrinology*, *102*, 255-262.

Wasser, S. K., Thomas, R., Nair, P. P., Guidry, C., Southers, J., Lucas, J., Wildt, D. E., & Monfort, S. L. (1993). Effects of dietary fibre on faecal steroid measurements in baboons (*Papio cynocephalus cynocephalus*). *Journal of Reproduction and Fertility*, *97*, 569-574.

Wilson, E. O. (1975). *Sociobiology: The new synthesis*. Cambridge, MA: The Belknap Press, Harvard University. 697 pp.

Wittenberger, J. F. (1978). The evolution of mating systems in grouse. *Condor*, *80*, 126-137.