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Research article

Using non-invasive faecal hormone metabolite monitoring to detect reproductive patterns, seasonality and pregnancy in red river hogs (*Potamochoerus porcus*)

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Abstract

Few studies have been conducted on red river hog (*Potamochoerus porcus*) reproductive biology in zoos. Furthermore, in spite of regular breeding efforts in zoos, reproductive success has been relatively poor for this species, particularly in the North American population. In this study, we used faecal hormone metabolite monitoring to analyse near daily samples from two males and three females over several years to gain insight into their patterns of reproductive hormone secretion. Both a progesterone and a testosterone enzyme immunoassay (EIA) were validated and subsequently used to monitor reproductive patterns, seasonality, ovulatory activity and a successful pregnancy. The findings indicate that female red river hogs are seasonally polyoestrous. Regular cycles were observed from approximately December through August and an annual period of anoestrous was observed from approximately September until December. Average cycle length for all females was 23 days ± 1.19, range 13–30 days. Androgen excretion patterns of the two males did not show clear seasonal patterns. Only one male experienced an increase in androgen levels (141.53 ± 45.55 ng/g) corresponding with the female seasonal oestrous period. There was, however, some evidence of possible androgen suppression between the two males, and a potential 'boar effect' on a young female upon first introduction to a male. Ultimately, this information may increase our understanding of this species' reproductive biology and serve as a baseline for more in-depth follow-up studies to identify specific patterns associated with reproductive success.

Introduction

The red river hog (*Potamochoerus porcus*) is a member of the Suidae family and is native to equatorial West Africa. Its home range extends from Senegal to southeastern Zaire. Its habitat preference is rainforest and gallery forest, but it is adaptable to most habitats that provide adequate cover, food, and water availability. Like most porcines, red river hogs are social animals and typically live in groups of 15–20 individuals of various ages (Grubb 1993). The species is deemed locally abundant and at this point is not endangered or threatened. However, populations in the wild are decreasing in non-protected areas due to deforestation and hunting (Olivier 1995). Additionally, red river hogs do occupy a section of the IUCN Status Survey and Conservation Action Plan for Pigs, Peccaries, and Hippos (Vercammen 1993) where future research and management recommendations are addressed.

Overall, surprisingly few publications are available on this charismatic species. A field study conducted in Nigeria by Oduro (1989) determined habitat preference and home range and reported on several other ecological and behavioral variables. Only one other study, conducted at several European zoos, has recently investigated some aspects of red river hog reproductive physiology in captivity (Berger et al. 2006).

In spite of long-term breeding efforts at various zoos around the world, overall breeding success at the beginning of this study was reported to be relatively poor, particularly in the North American zoo population. An Association of Zoos and Aquariums (AZA) Population Management Program (PMP) was founded to assist with the management of red river hogs held in North American zoos. According to the most recent PMP report (Holland and Putnam 2013), the target population of 190 animals is within reach, yet there is still a high average mean kinship. At present the genetic diversity is calculated to be at 83.16% of the founder population, indicating some level of inbreeding. There are currently eight founder animals in the population. Any value lower than 90% is generally associated with reduced reproductive success (Holland and Putnam 2013).

In addition, several social and behavioural factors have been reported, mostly anecdotally, as potential causes of historically poor breeding success. For example, in North American zoos, red river hogs have typically been housed in adult pairs rather than attempting group housing. According to the 2003 Wild Pig & Peccary Taxon Advisory Group (Holland 2003), seven out of 10 pairings were unsuccessful. It appears that under these circumstances it can be difficult to find individuals that will be compatible with each other. Also, it has been reported that when paired individuals are housed together continuously for prolonged periods, they may lose interest in each other and not breed, or in other cases even show excessive aggression and fighting that will prohibit pair or small group formation altogether.

Higher latitude climates and zoo management regimens are remarkably different from the species' natural environment. It has been reported that red river hogs in the wild show some seasonality in reproductive patterns with most births occurring during the end of the dry seasons (February and May–August) in their home range (Beaune et al. 2012). In zoos such patterns appear to be less obvious based on breeding records, although peaks of births seem to occur March through May according to the studbook (Holland 2012). Thus, several questions regarding red river hog reproductive physiology arise: What are the general patterns of cyclicity in North American zoo-housed red river hogs? Do North American zoo-housed red river hogs experience physiological seasonality, and if so, what factors may be associated with seasonality? Do males and females show similar patterns of reproductive hormone production?

Due to the limited number of founder animals, high average mean kinship, and potential compromised reproduction, zoos are currently attempting to increase genetic diversity by introducing additional wild-caught founder individuals into the North American zoo population. However, though imports may be restricted due to concerns about disease transmission (Vercammen 1993), if breeding success is achieved with potential wild-caught founders it would be beneficial. In order to accomplish this goal we urgently need more information on this species' reproductive physiology and basic patterns of reproduction. In this study, we validated a non-invasive hormone monitoring technique for red river hogs and analysed near daily samples from two males and three females over several years to gain insight into their patterns of reproductive hormone secretion across time and seasons. Ultimately, this information may help to increase our understanding of this species' reproductive biology and serve as a baseline for more in-depth follow-up studies to identify specific patterns associated with reproductive success.

Methods

Study animals

Five adult red river hogs (2.3) were studied over a period of si years at Chicago's Brookfield Zoo (Table 1). Animals were house individually or in male/female pairs until the birth of Rhea's hoglet at the end of the study; however, all individuals had olfactor contact and occasionally visual contact with each other. Currentl two of the study individuals (Jivi and Rhea) and their hoglets ar housed together in the Habitat Africa Forest exhibit and barn (i. off exhibit holding area). Jivi and Rhea occupy stalls 4, 5 and (Figure 1). However, co-housing arrangements and individual changed a couple of times during the course of the study a outlined below. Indoor enclosures follow the natural light/dar cycle of the outside area in Chicago, Illinois, USA. Artificial lightin is used, but windows are located throughout the building allowin natural light inside. The indoor temperature is maintained a approximately 22° C year round. The animals are always indoor during the winter months, while in the summertime, they are give access to outside yards during the day, weather permitting, with outside temperature of 15° C or above. Their daily diet consists of sweet potato, apple, romaine lettuce, spinach, grain, and grass/alfalfa mix. Water is available ad libitum.

Table 1. Age of study animals at start of study and time period of data collection.

Name (sex)	ISIS#	Age at start of study	Birthplace	No. of samples collected	Data collection
Nutmeg (f.)	875	8 yrs	San Diego Zoo (captive)	458	1 December 2001–2 June 2003
Meka (f.)	676	6 ½ yrs	San Diego Zoo (captive)	973	29 May 2001–13 April 2004
Rhea (f.)	1545	8 yrs	Pretoria So. Afr. (captive)	1427	6 December 2003–30 December 2007
Ekundu (m.)	672	7 yrs	San Diego Zoo (captive)	532	29 May 2001–12 January 2003
Jivi (m.)	855	6 yrs	Guinea (wild)	1272	29 November 2001–5 December 2007

Male and female introductions occurred throughout the time of study. No tranquillising medications were used during male/female introductions. In July 2001–October 2001 Meka and Ekundu were introduced and housed together, usually in stalls 4–6. Increasing aggression was observed in October 2001, and the keepers were forced to separate the pair permanently. Ekundu was then introduced to Nutmeg in February 2002. A 'howdy fence' was installed in-between stalls 4 and 5 on 22 February 2002 to give one-to-one visual and tactile access. They were given access to several stalls together the first day, but each following day they were only given visual access to each other. No matings were documented, but Nutmeg did show breeding interest and courtship behaviours**.** In April 2002, introductions between Nutmeg and Ekundu ceased and breeding efforts focused on Meka

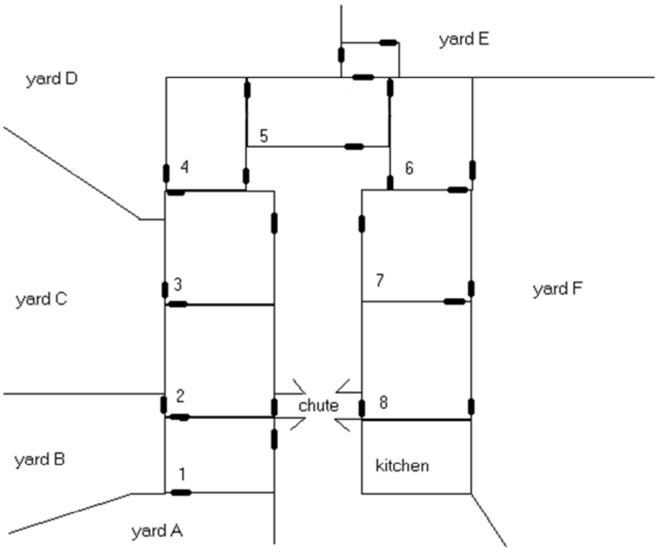

Figure 1. Schematic drawing of red river hog enclosure at Brookfield Zoo.

and Jivi. Nutmeg was transferred to Peace Reserve on 28 October 2003 and Ekundu was transferred to Kansas City, MO on 5 April 2003 (Figure 1).

The next pairing occurred between Meka and Jivi, who were placed together for September 2002–September 2004. Jivi is a wild-caught male, which makes him a high priority breeding partner from the PMP perspective. This pair was usually housed in stalls 4–6 and they were together during most of the above mentioned time period aside from a few occasional separations intended to increase breeding interest. Normally these separations lasted a couple of months. Several mating attempts were observed, but no successful copulations. The pair was subsequently separated; Jivi was housed in stall 6 and Meka in stall 3.

Rhea is the most recent arrival at Brookfield Zoo. She arrived in October 2003 and was initially housed alone in stall 3 and moved to stall 4 in September 2004 (Figure 1). There was an attempted introduction between her and the other female, Meka. The introduction had to be abandoned due to the high level of aggression between the two females.

The final pairing occurred between Jivi and Rhea April 2005–present. From April to June 2005 they were introduced regularly during the day with little aggression, and following June 2005, they were left alone together overnight in stalls 4–6 and rarely separated. Jivi and Rhea mated successfully on 18–21 December 2006 and conception occurred on18 December 2006 according to the hormone data, resulting in Brookfield Zoo's first successful red river hog pregnancy, and the birth of three (2.1) healthy hoglets on 15 April 2007. Jivi, Rhea and the hoglets were housed together in stalls 4–6 and only separated during examinations.

Faecal hormone metabolite extraction and assay analyses

Faecal samples were collected by zookeepers on a close to daily basis, and stored in a regular freezer at –20° C until the time of analysis. Green food colouring (Gordon Food Service, Wyoming, MI) was added to various food items for one of the pair-housed individuals to distinguish individual faeces.

Faecal hormone metabolites (progestagens and androgens respectively) were extracted using 80% ethanol in dH2O. First, 0.5 g (±0.05 g) of each faecal sample was weighed out (Mettler balance, model #AB104-5) into 16 x 125 mm polypropylene tubes. Then, 5 ml of 80% ethanol solution was added to each extraction tube. Each tube was vortexed and placed on a rotator (Labline Maxi Rotator, model #4631/Fisher) overnight (14–18 hrs). Tubes were then centrifuged for 15 minutes at 1500 rpm (Marathon 3000R centrifuge, model #120). For each sample, 1 ml of supernatant was pipetted into 1 ml of assay buffer (0.1M phosphate buffered saline containing 1% BSA, pH 7.0) into 12 x 75mm polypropylene tubes to produce a 1:10 dilution. Extracts were stored frozen at –20° C until assay analyses.

All female red river hog samples were assayed using a previously established in-house progesterone EIA (Atsalis et al. 2004) and male samples were assayed using an established in-house testosterone EIA (Marneweck et al. 2013). Antibodies and conjugates were prepared and supplied by Coralie Munro, MS (Dept of Clinical Endocrinology, University of California–Davis, Davis, CA).

Parallelism and recovery tests were used for the validation of the testosterone and progesterone enzyme immunoassays for the study species. To establish parallelism, serial two-fold dilutions of a sample pool were tested for comparison displacement curves. Recovery of exogenous hormone was measured by spiking a baseline diluted sample with the five highest standards, each containing a known amount of hormone. The percent recovery was calculated by dividing the measured concentration of hormone by the expected concentration of hormone multiplied by 100.

For the progesterone EIA, the antibody (CL425) was diluted 1:6,000. Working HRP dilution was 1:16,600, standard range was 0.05 to 12.5 ng/ml, and sample volume was 50 ul per well. Assay sensitivity was 0.05 ng/ml and intra-assay and inter-assay coefficients of variation were 6.5% and 16.9% at 32.6% binding and 8.5% and 21.6% at 73.3% binding, respectively. Recovery of exogenous progesterone (0.39–6.25 ng/ml) was 80.71% ± 21.17 % ($y=0.24+0.57x$, $r^2=0.997$). The cross reactivity for the progesterone antibody are 100% progesterone, 55% 5a-pregnen-3,20-dione and <0.1% pregnanediol, androstenedione and corticosterone.

For the testosterone EIA, the antibody (R156) was diluted 1:20,000. Standard range was 0.39 to 10 ng/ml. Working HRP dilution was 1:20,000 and sample volume was 100 ul per well. Sample incubation time was 2 h. Assay sensitivity was 0.039 ng/ml and intra-assay and inter-assay coefficients of variation were 7.9% and 19.7% at 40.5% binding and 9.5% and 17.4% at 70.8% binding, respectively. Recovery of exogenous testosterone (0.312–5 ng/ml) was 121.84%±11.32% ($y=0.25+0.1.08x$, $r^2=0.98$). The cross reactivity for the testosterone antibody are 100% testosterone, 20% 5α-dihydrotestosterone, 0.78% androstenedione, 0.19% androsterone, and <0.1% DHEA, progesterone, oestradiol-17B, pregnenolone and cortisol.

All faecal hormone metabolite values are reported as mean (± SD) ng/g wet weight.

Data analysis

Ovulatory activity was inferred from the measured faecal progestagen metabolite concentrations. Oestrous cycles were determined by first calculating baseline faecal progestagen metabolite levels during the cycling season and second, using the last day of measured progestagen metabolites above baseline of the first cycle through the next value measured above baseline in the second cycle (Figure 2). This method was repeated throughout the cycling period. To determine individual baseline faecal hormone metabolite levels for female progestagens and male androgens, the data set was averaged and any values falling outside of ± 1.5 standard deviation were removed. This was repeated until no values remained outside the standard deviation (Brown et al. 1994). The cycling season was established for each female by using the first date above baseline through the last date above baseline on the final cycle of the season (Moriera et al. 2001). The non-cycling season was marked by at least two months of baseline progestagen metabolite concentrations and observed and inferred anoestrus. Pregnancy was diagnosed by sustained elevated levels of faecal progestagens exceeding 30 days.

Potential associations between male androgen levels and females' cycling and non-cycling progestagen metabolite levels were assessed by using Spearman's rank-order correlation tests (Cody and Smith 1997). Since hormonal data sets were non-normally distributed, non-parametric statistical testing was used for all tests. Tukey's HSD tests were applied to determine potential differences in the means of each male's androgen level across the four main seasons (winter: December, January, February; spring: March, April, May; summer: June, July, August; autumn: September, October, November) (Cody and Smith, 1997). Additionally, Student's t-tests were run to determine any significant differences between female cycling data. All statistical analyses were performed using JMP, version 8 (2008, SAS Institute Inc.). We applied a $P \leq 0.05$ significance level for all statistical tests.

Results

Female reproductive physiology

During the study period, 14 cycles were observed in Nutmeg. Her average cycle length was 22 ± 2.46 days with a range of 19–26 days and her baseline faecal progestagen metabolite concentration was 66.99 ± 1.94 ng/g. Meka was observed cycling 20 times with a mean cycle length of 23 ± 4.01 days and a range of 13–30 days. Her

Table 2. Red river hog oestrous cycle frequency, mean cycle length, range, baseline progestagen concentrations, cycling (oestrous periods) and non-cycling (anoestrous periods) fecal progestagen data for each study female.

Red river hog	No. of cycles	Baseline progestagen (ng/g)	Avg. cycle length	Std dev	Range	Avg. progestagen (cycling) (ng/g)	Avg. progestagen (non-cycling) (ng/g)	Highest progestagen peak (ng/g)
Nutmeg	14	66.99	22 days	+/- 2.46	19–26 days	170.25	37.26	1037.66
Meka	20	73.07	23 days	+/- 4.01	13–30 days	236.82	65.91	2389.54
Rhea	27	73.76	23 days	+/- 2.93	16–28 days	237.33	68.26	1115.77

baseline faecal progestagen metabolite concentration was 73.07 ± 0.78 ng/g. The newest female in the group, Rhea, who arrived at Brookfield Zoo in 2003, cycled 27 times during the study time. Her mean cycle length was 23 ± 2.93 days with a range of 16–28 days and her faecal baseline progestagen metabolite concentration was 73.76 ± 0.23 ng/g (Table 2).

When all cycles are averaged across females the mean cycle length is 23 days and the average overall baseline progestagen metabolite concentration is 71.27 ± 3.73 ng/g. Though each female had a 22–23 day average cycle length, there was no significant relationship between any of the individual's cycling data sets (Rhea–Meka: t = -0.419, P = 0.680; Rhea–Nutmeg: t = -1.775, P = 0.101; and Meka–Nutmeg: t = -1.778, P = 0.101). Based on these data the three females monitored in this study show a seasonally polyoestrous pattern, cycling multiple times during the year from approximately December through August followed by a period of anoestrous from around September until December (Figure 3). Overall, the females follow similar patterns of cyclicity and synchrony. In 2002, Nutmeg cycled 3 more times after Meka's cycling ceased, but then both began cycling the next season within a month of each other. Interestingly, Rhea began cycling a couple of months after her arrival at the zoo after being housed in close proximity to Meka, who had already begun her cycling season.

One pregnancy was diagnosed and monitored (Figure 4). Mating between Jivi and Rhea was observed at the onset of Rhea's first oestrous cycle following a 4-month anoestrous period. Faecal progestagen metabolite levels started increasing on day 15, remaining around 1000 ng until day 75. This was proceeded by a steady elevation, peaking at approximately 6400 ng three days prior to parturition. Values plummeted to baseline immediately following parturition on 15 April 2007. Gestation length was 119 days and produced three healthy hoglets, two males and one female.

Males: androgen levels, female oestrous and seasonality

The baseline faecal androgen concentration for Ekundu was 252.07 ± 0.61 ng/g, yet Jivi's baseline concentration was only 62.97 ± 0.51 ng/g, a near four-fold difference. Spearman's rank-order tests used for data analysis to detect differences in male faecal androgens during female's cycling and non-cycling season revealed that Jivi's faecal androgen concentrations were significantly higher during Nutmeg's oestrous periods, but interestingly, this relationship was not observed with the other two females. During the female's anoestrous periods, Jivi showed a strong inverse correlation with Meka and a significant positive correlation with Rhea. Surprisingly, Ekundu's faecal androgens showed no significant correlation with

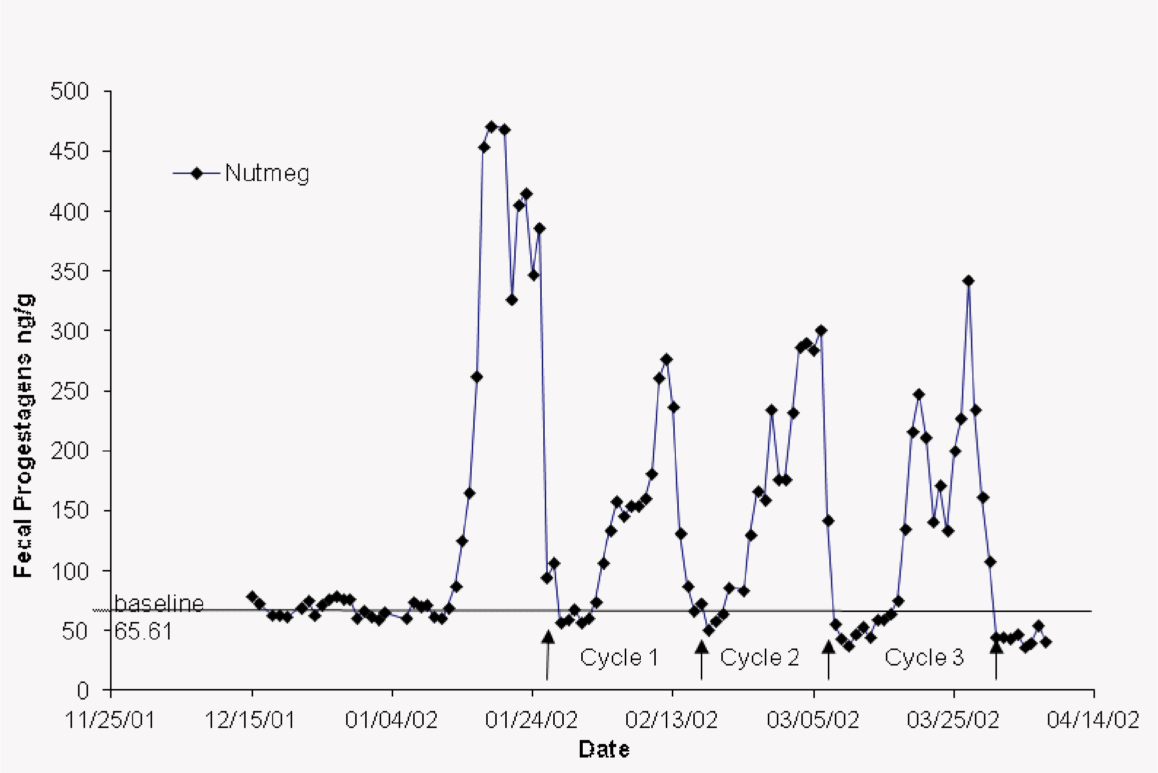

Figure 2. Calculation of the red river hog oestrous cycle. The arrows point to the first value that falls below baseline and thus the first day of each new oestrous cycle.

Table 3. Spearman's rank-order correlation coefficients presented by study animal for all tests conducted between male androgen and female progestagen concentrations during oestrous and anoestrous periods. Italics show significant correlations.

	Oestrous periods		Anoestrous periods	
Animal ID	Ekundu	Jivi	Ekundu	Jivi
Nutmeg	r_s = 0.0908 P = 0.5464	*r_s = 0.2865* *P = 0.0163*	r_s = 0.3047 P = 0.9062	r_s = 0.0013 P = 0.8421
Meka	r_s = 0.2804 P = 0.2345	r_s = 0.0633 P = 0.4624	r_s = 0.0921 P = 0.2459	*r_s = -0.9117* *P = 0.0043*
Rhea	N/A	r_s = 0.1917 P = 0.3746	N/A	*r_s = 0.2465* *P = 0.0068*

any of the females during their oestrous or anoestrous periods (Table 3).

For seasonality, Jivi showed a significant difference between summer and all other seasons of the year, with summer androgen concentrations being much higher than any other time of year. In addition, Jivi's faecal androgen concentrations were significantly lower in the autumn than in the spring. Ekundu's summer faecal androgens concentrations were also significantly different from concentrations measured during all other seasons, but unlike Jivi, Ekundu's summer concentrations were lower than concentrations measured during any other times of the year (Figure 5).

Social effects

Ekundu was the only male in the group until November 2001 when the wild-caught juvenile male, Jivi, arrived. Jivi's average androgen concentrations during his first five months were 114.76 ± 62.85 ng/g while Ekundu's were 324.87 ± 137.33 ng/g, nearly three times higher. In August 2002, Jivi's androgen concentrations averaged 220.05 ± 78.53 ng/g, while Ekundu's concentrations showed their lowest values, averaging 96.54 ± 28.26 ng/g. After Ekundu's departure on 5 April 2003, Jivi's androgen concentrations appeared to stabilise at a fairly low concentration, averaging 90.25 ± 48.74 ng/g for the remainder of the collection period.

A possible boar effect may have been observed when Ekundu was introduced to Meka in July 2001. She showed her first clear oestrous cycle in August 2001 and began cycling regularly in December 2001 (Figure 6).

Nutmeg and Ekundu were introduced to each other repeatedly between February and April 2002. Ekundu's faecal androgen concentrations averaged 346.34 ± 120.84 ng/g during their introduction period, but following their separation, his androgen concentrations decreased over several weeks, averaging 259.85 ng/g in May 2002 and 213.05 ng/g in June 2002. Partial matings were observed, but no complete copulations occurred. Introductions ceased due to lack of successful mating and to focus efforts on a new breeding pair.

Two females, Meka and Rhea, were introduced to each other for potential group housing in June 2004 with poor results. Interestingly, in spite of substantial aggression between the two females, that eventually required their separation, both were

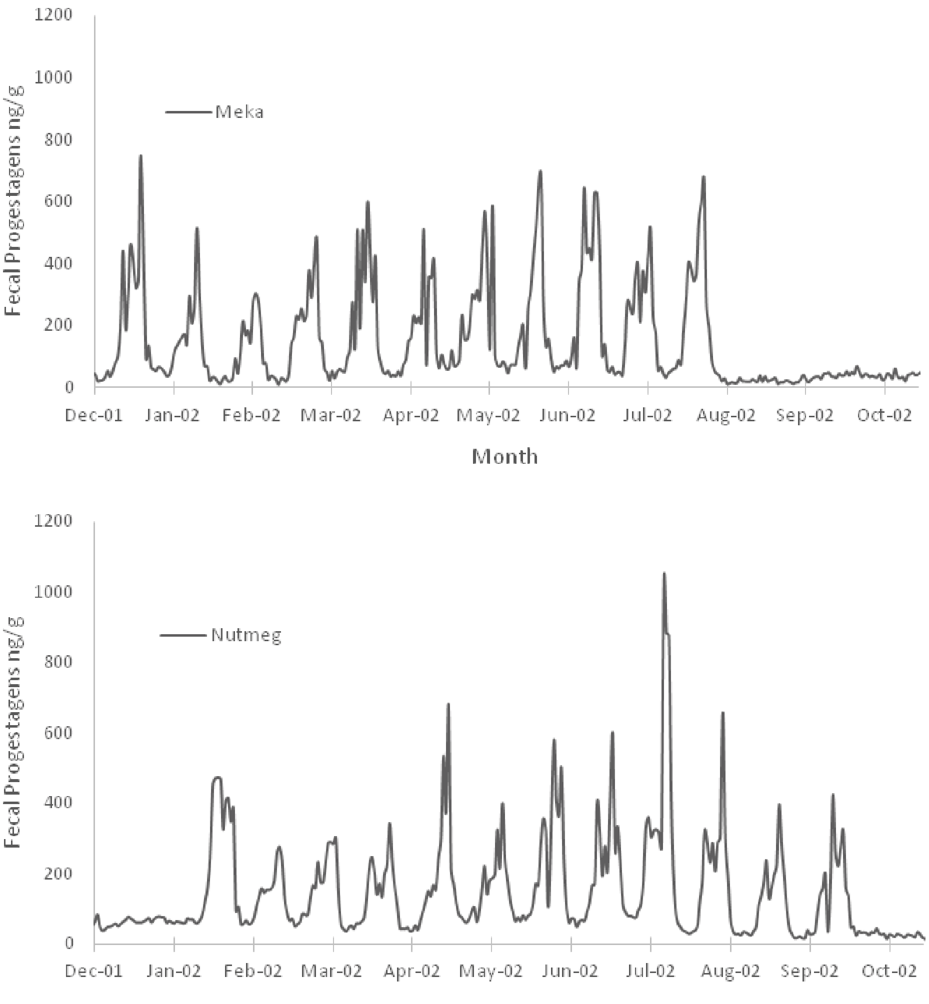

Figure 3. Faecal progestagen data for Meka and Nutmeg from December 2001 to October 2002.

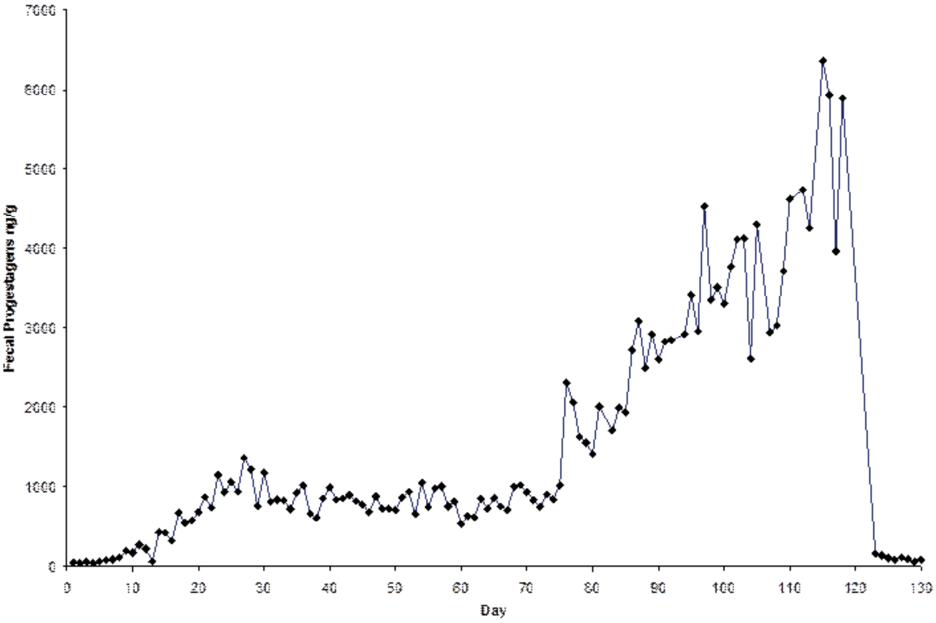

Figure 4. Pregnancy profile for a red river hog female. Mating and conception occurred on 18 December 2006 and parturition on 15 April 2007. Gestation length was 119 days.

observed to cycle regularly and no change was detected in their hormone levels due to these aggressive interactions.

Discussion

Monitoring hormones non-invasively has become an essential tool in studying reproductive patterns and aiding in the conservation of wildlife (Schwarzenberger and Brown 2013; Kersey and Dehnhard 2014). Information provided by longitudinal hormone monitoring can help with various aspects of species management, both in the wild and in zoo settings. Although red river hogs are not currently endangered or threatened, their habitat is continuously declining and their breeding success in captivity has been sporadic and inconsistent. Relatively little is known about their reproductive biology to date.

The average oestrous cycle for the females in this study was 23 days, which is more consistent with the oestrous cycle length of domestic pigs, 21 days (Hughes and Varley 1980). The seven red river hogs in the Berger et al. (2006) study had cycle lengths of 34–37 days, but it is important to note that sample collection frequency was 1–3 samples per week for 3–10 months and collection ceased between April and July. More research would be necessary to determine the reason for these differences, but it may be an effect of living under different climatic conditions.

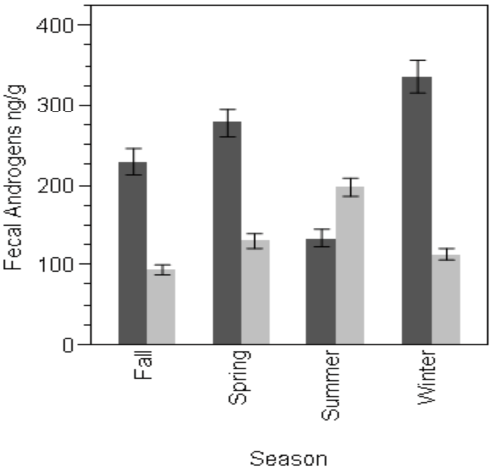

Figure 5. Average male red river hog faecal androgen concentrations by season. Dark grey bars represent Ekundu, light grey represents Jivi.

Synchrony of oestrous cycles is also a phenomenon common in Suidae that was seen occasionally in this study. Observations on wild boars in France showed synchrony in oestrous cycles within the group, with or without a male present (Delcroix et al. 1990). This phenomenon is believed to be initiated by female pheromones and driven by the benefit of farrowing synchrony, which elicits nursing synchrony and protection against predation (Pederson 2007). Suidae have finely tuned olfactory senses and it was determined by Love et al. (1993) that synchrony was probably due to pheromones. Meka and Nutmeg showed similar patterns of cyclicity, as well as Rhea and Meka. We were able to monitor a successful pregnancy from conception to birth with close to daily samples. We documented a gestation length of 119 days, which is very close to the 121–129 day gestation length reported by Berger et al. (2006).

The lack of reproductive success prior to the final successful breeding may in part have been due to various social issues observed over the years. It is known that disruptive social interactions can impair reproductive function (Connor 2001). At various times during attempted breeding introductions and same-sex introductions, a lot of aggressive incidents were observed and these introductions had to be abandoned. For example, many encounters between Ekundu (the male that did not breed successfully) and two of the females were marked by high levels of aggression. Also, attempted introductions for group housing (e.g. between two females, Meka and Rhea) did not succeed due to aggression.

Introducing unrelated females to each other may be fraught with difficulty and other means of pursuing group housing may be needed. It is known that this species is highly social in the wild and some zoos (e.g. Zoo Pretoria) house them in larger groups.

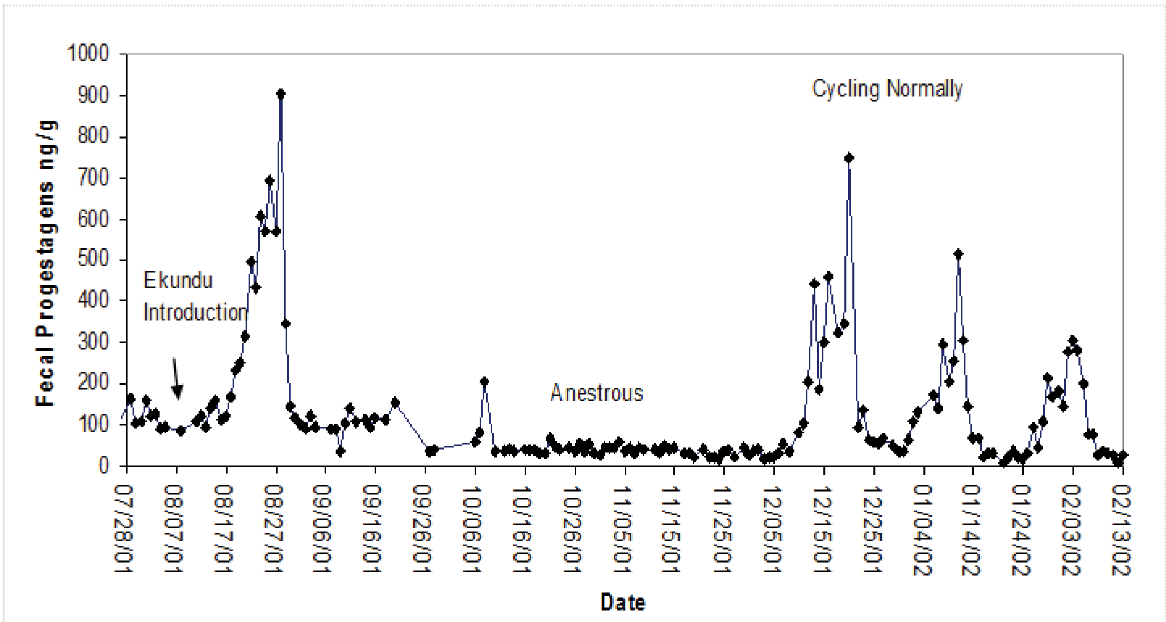

Figure 6. Red river hog female Meka's first cycles as a juvenile when she may have experienced a 'boar effect'. The introduction of male Ekundu may have been a stimulus for her first oestrous cycle in August 2001. She then entered an anoestrous period until the breeding season began again in December 2001.

Thus living in abnormal social conditions may be a factor in lack of successful breeding (Berger et al. 2006). In domestic pigs, it has been documented that many situations that lead to poor reproductive success appear to be caused by inadequacies in the social and physical environment. Environments that provide comfort and positive interactions will produce better breeding (Connor 2001).

Our study corroborates an earlier report (Berger et al. 2006) that red river hog females are indeed seasonally polyoestrous. Reproductive seasonality is a phenomenon observed in a wide variety of mammals and is typically a direct reflection of food availability and energy status (Lu et al. 2011; Bronson 1989). Suids that live under temperate climate conditions tend to breed seasonally and are mainly regulated by photoperiod (Berger et al. 2006). In domestic pigs, a decrease in fertile periods is seen in the summer and autumn (Peltoniemi et al. 2000), which coincides with the results of this study. Another study done on wild boars showed the timing of reproduction is greatly influenced by the availability of food (Love et al. 1993). Seydack (1992) observed that wild red river hogs give birth mainly at the end of the dry season and onset of the rainy season. In their natural equatorial African habitat, red river hogs are dependent on rainfall patterns and therefore nutrition (Berger et al. 2006). The seasonal rainfall pattern of their home range, which correlates to the availability of food, is mostly likely the main factor in seasonal reproduction of red river hogs in the wild.

There have been several published accounts about the significant effect of photoperiod on seasonal reproduction. For example, in domestic pigs, photoperiod instigates an endocrine response in the body by releasing melatonin from the pineal gland that then influences reproductive activity (Love et al. 1993). Interestingly, inserting melatonin implants causes continuous oestrous cycles with no seasonal anoestrous period, proving that photoperiod is one primary cause of reproductive seasonality in domestic pigs (Bassett 2001). According to Negus and Berger (1987) and Beehner et al. (2006), obligate seasonal breeders use a natural cue such as photoperiod to initiate every reproductive cycle.

In our study females stopped cycling in August and started again in December, apparently cued into the light cycle changes happening in northern zoo environments. Berger et al. (2006) found female red river hogs cycled from December until summer and hypothesised that they were probably cued to begin cycling by a change in the photoperiod. It could therefore be concluded that female red river hogs are obligatory seasonal breeders if photoperiod changes are present. Potentially other environmental cues (e.g. rainfall) could act as cues for seasonal changes in their natural habitat.

Males may, however, be affected to some extent by social and environmental cues. Social animals such as pigs often require necessary social cues and development in order to ensure normal sexual behaviour in adults. Hormone levels can be affected by a variety of social factors, such as new individuals joining a group, individuals leaving a group, changes in group composition and housing arrangements, or, as previously reported for Suidae, an effect of being exposed to new individuals of the opposite gender, e.g. the boar effect (Hughes et al. 1997). Conversely, hormone changes themselves can cue the onset of social behaviours, such as breeding attempts.

Androgen levels of the two red river hog males we monitored did not show as clear a cyclicity. However, the younger male, Jivi, who also bred and reproduced successfully during the study period, did show some signs of possible seasonality in androgen production, with somewhat higher levels of androgen production during a female's oestrous period. When Jivi first arrived at the zoo his androgen levels fluctuated frequently. This may have been associated with social issues, given that an older and established male was already present. It has been documented that male androgen levels can be correlated with the ranking of males within a group (Creel et al. 1997). Interestingly, once Ekundu was transferred out of the zoo, Jivi's androgen levels fluctuated less, remaining at a lower concentration overall and somewhat tracking the females' oestrous cycles.

For females, a so called 'boar stimulus' appears to be a significant component in cueing mating responses (Behan and Watson 2005).

Similarly, the Vandenbergh effect in mice occurs when immature female mice are exposed to a male and experience an accelerated first oestrus (Vandenbergh 1967). Also, it has been found that shortly after the introduction of a boar, the sow's oxytocin levels significantly increase (Kotwicka et al. 1995). Oxytocin may serve a role in sexual receptivity and assist in the transport of sperm in the uterus (Langendijk et al. 2005). Sexual activity in boars is increased by the presence of other boars (Tanida et al. 1990), therefore boars isolated from sows during development achieve fewer copulations. The daily exposure to boars of high sexual motivation to sows will produce a cycle in the female within 25 days 60% of the time (Hughes and Varley 1980). This effect may have been observed in one of our females, Meka. Although it is important to consider that the onset of puberty can occur as early as 6–7 months (Prunier and Meunier-Salaun 1989), when Meka was one and a half years old she was introduced to a male and shortly thereafter showed her first definite oestrous cycle.

Lastly, due to a lack of a genetically diverse pool of individuals in captivity, inbreeding has also been an issue. The inbreeding coefficient was substantial a few years ago due to the low number of founder animals, with eight out of nine potential founders not having had breeding success (Holland 2003). Currently there is still a high average mean kinship, probably due to lower genetic diversity. It is documented that inbreeding causes poor reproductive success and high neonatal mortality. An increase in successful breedings of valuable founders in the captive population is vital to the health and genetic diversity of the population (Holland 2003; Holland and Putnam 2013). Understanding species-specific reproductive patterns and the factors that influence them helps us to establish sustainable captive populations of rare exotic species.

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References

Atsalis S., Margulis S.W., Bellem A., Wielebnowski N. (2004) Sexual behavior and hormonal estrus cycles in lowland gorillas (*Gorilla gorilla*). *American Journal of Primatology* s62: 123–132.

Bassett J.M., Bray C.J., Sharpe C.E. (2001) Reproductive seasonality in domestic sows kept outdoors without boars. *Reproduction* 121: 613–629.

Beaune D., Bollache L., Fruth B., Bretagnolle F. (2012) Bush pig (*Potamochoerus porcus*) seed predation of bush mango (*Irvingia gabonensis*) and other plant species in Democratic Republic of Congo. *African Journal of Ecology* 50(: 509–512.

Beehner J.C., Onderdonk D.A., Alberts S.C., Altmann J. (2006) The ecology of conception and pregnancy failure in wild baboons*. Behavioral Ecology* 17: 741–750.

Behan J.R., Watson P.F. (2005) The effect of managed boar contact in the post-weaning period on the subsequent fertility and fecundity of sows. *Animal Reproduction Science* 88: 319–324.

Berger E.M., Leus K., Vercammen P., Schwarzenberger F. (2006) Faecal steroid metabolites for non-invasive assessment of reproduction in common warthogs (*Phacochoerus africanus*), red river hogs (*Potamochoerus porcus*) and babirusa (*Babyrousa babyrussa*). *Animal Reproduction Science* 91: 155–171.

Bronson F.H. (1989) *Mammalian Reproductive Biology*. Chicago: University of Chicago Press.

Brown J.L., Wasser S.K., Wildt D.E., Graham L.H. (1994) Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. *Biology of Reproduction* 51: 776–786.

Cody R.P., Smith J.K. (1997) *Applied Statistics and the SAS Programming Language*, 4th edn. Upper Saddle River, NJ: Prentice Hall.

Connor M.L., Orzechowski K. (2001) *Environmental Factors Affecting Breeding Herd Performance*. Manitoba: Manitoba Agriculture, Food, and Rural Initiatives.

Creel S., Marusha Creel N., Mills M.G.L., Monfort S.L. (1997) Rank and reproduction in cooperatively breeding African wild dogs: behavior and endocrine correlates. *Behavioral Ecology* 8: 298–306.

Delcroix I., Mauget R., Signoret J.P. (1990) Existence of synchronization of reproduction at the level of the social group of the European wild boar (*Sus scrofa*). *Journal of Reproduction and Fertility* 89: 613–617.

Grubb P. (1993) The Afrotropical suids: *Potamochoerus*, *Hylochoerus* and *Phacochoerus*. In: Oliver W.L.R. (ed.). *Pigs, Peccaries and Hippos Status Survey and Action Plan*. Gland: IUCN, 66–75.

Holland J. (2003) North American red river hog population. *Wild Pig & Peccary Taxon Advisory Group* 3(2).

Holland J. (2012) *Red River Hog North American Regional Studbook*. Los Angeles: AZA.

Holland J., Putnam A. (2013) *Red River Hog (*Potamochoerus porcus*), Population Analysis and Breeding and Transfer Plan.* Chicago: AZA Population Management Center.

Hughes P.E., Philip G., Siswadi R. (1997) The effect of contact frequency and transport on the efficacy of boar effect. *Animal Reproduction Science* 46: 159–165.

Hughes P., Varley M. (1980) *Reproduction in the Pig*. Boston: Butterworth & Co. Ltd.

Kersey D.C., Dehnhard M. (2014) The use of noninvasive and minimally invasive methods in endocrinology for threatened mammalian species conservation. *General and Comparative Endocrinology* 203: 296–306.

Kotwicka G., Sobczak J., Koziorowski M. (1995) Effects of opioid peptides, indomethacin and age on oxytocin and prolactin release during mating in sows. *Reproduction in Domestic Animals* 30: 257–263.

Langendijk P., Soede N.M., Kemp B. (2005) Uterine activity, sperm transport, and the role of boar stimuli around insemination in sows. *Theriogenology* 63: 500–513.

Love R.J., Evans G., Klupiec C. (1993) Seasonal effects on fertility in gilts and sows. *Journal of Reproduction and Fertility* 48: 191–206.

Lu A., Beehner J.C., Czekala N.M., Koenig A., Larney E., Borries C. (2011) Phytochemicals and reproductive function in wild female Phayre's leaf monkeys (*Trachypithecus phayrei crepusculus*). *Hormones and Behavior* 59: 28–36.

Marneweck D.G., Ganswindt A., Rhodes S., Bellem A., Bryant J., Wielebnowski N., Dalerum F. (2013) Reproductive endocrinology of zoo-housed aardwolves. *Acta Theriologica* 58: 223-232.

Moriera N., Montiero-Filho E.L.A., Moraes W. (2001) Reproductive steroid hormones and ovarian activity in felids of the *Leopardus* genus. *Zoo Biology* 20: 103–116.

Negus N.C., Berger P.J. (1987) *Mammalian Reproductive Physiology: Adaptive Responses to Changing Environments*. New York: Plenum Press.

Oduro W. (1989) *Ecology of the Red River Hog in Southern Nigeria*. PhD thesis. Ibadan, Nigeria: The University of Ibadan.

Oliver W. L. R. (1995) Taxonomy and conservation status of the suiformes – an overview. *IBEX Journal of Mountain Ecology* 3: 3–5.

Pederson L.J. (2007) Sexual behavior in female pigs. *Hormones and Behavior* 52: 64–69.

Peltoniemi O.A.T., Tast A., Love R. (2000) Factors effecting reproduction in the pig: seasonal effects and restricted feeding of the pregnant gilt and sow. *Animal Reproduction Science* 60/61: 173–184.

Prunier A., Meunier-Salaun M.C. (1989) The influence of boar stimulation on puberty attainment in tethered and group penned gilts. *Theriogenology* 32: 949–959.

Schwarzenberger F., Brown J.L. (2013) Hormone monitoring: an important tool for the breeding management of wildlife species. *Wiener Tierärztliche Monatsschrift* 100: 209–225.

Seydack A., Bigalke R.C. (1992) Nutritional ecology and life history tactics in the bushpig (*Potamochoerus porcus*): development of an interactive model. *Oecologia* 90: 102–112.

Tanida H., Miyazaki N., Tanaka T., Yoshimoto T. (1990) Sexual behaviours of boars under multi-sire mating in summer and fall. *Journal of Animal Science Technology (Jpn.)* 62: 271–276.

Vandenbergh J.G. (1967) Effect of the presence of a male on the sexual maturation of female mice. *Journal of Endocrinology* 81: 345.

Vercammen P., Seydack A.H.W., Oliver W. (1993) *The Bush Pigs*. In: Oliver W. (ed.). *Status Survey and Conservation Plan. Pigs Peccaries and Hippos.* Gland, Switzerland: IUCN/SSC Pigs and Peccaries Specialist Group.