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Reproductive endocrinology of the largest Dasyurids: Characterization of ovarian cycles by plasma and fecal steroid monitoring. Part II. The spotted-tailed quoll (*Dasyurus maculatus*)

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Abstract

Dasyurids exhibit a range of breeding patterns from semelparity through to an aseasonally polyestrous strategy, but detailed information on the reproductive endocrinology of many species is unavailable. This study aimed to extend our comparative understanding by characterizing the ovarian cycle of the spotted-tailed quoll (*Dasyurus maculatus*) through measurement of plasma progesterone, and also to investigate fecal sex steroid monitoring as an alternative, non-invasive technique. Longitudinal profiles revealed a biphasic pattern of plasma progesterone, with a significant pro-estrous pulse (0.97 ± 0.3 ng/ml) up to several weeks prior to onset of the luteal phase (LP). This pro-estrous period was associated with a predominantly cornified vaginal smear, onset of estrus behaviors and copulation. Mean luteal values for plasma progesterone were several fold higher (2.18 ± 1.10 ng/ml) than during the follicular phase (FP) (0.75 ± 0.02 ng/ml), and were sustained for approximately one month. Fecal progestagens and plasma progesterone were significantly positively associated during the estrous cycle. During the breeding period average concentrations of fecal total estrogens and pregnanediol (PgD) were significantly elevated. Ovarian activity during the FP was marked by increases in fecal estrogens, and rises in PgD which were sustained during the LP. In non-mated females the mean duration of the FP was significantly extended, being approximately twice as long (19.4 ± 4.0 d) as for mated females (8.3 ± 1.9 d) indicating coitus has some role in timing of ovulation in this species. This study has provided important new information on the reproductive biology of the female spotted-tailed quoll, and further demonstrated the usefulness of non-invasive endocrine techniques for monitoring ovarian cycles in marsupials.

Keywords: Marsupial; Dasyurid; Reproduction; Progesterone; Progestagens; Estrogens

1. Introduction

The Dasyuridae are a taxonomically diverse family, represented by more than fifty extant species, most of which live in Australia (Krajewski and Westerman, 2003). Dasyurids have evolved a wide range of life history strategies characterized at one extreme by a strictly regulated monoestrous cycle in females and the abrupt post-mating death finale of males (*e.g. Antechinus*) through to aseasonally polyestrous species which produce several litters each year (Lee et al., 1982; Krajewski et al., 2000; McAllan, 2003). Despite considerable focus on the reproductive biology of the semelparous Antechinus (Woolley, 1966; Selwood, 1980, 1985; Taggart and Temple-Smith, 1991), and detailed studies of several other dasyurids (Hill and O'Donoghue, 1913; Fletcher, 1985; Hinds, 1989; Selwood and Woolley, 1991) data on many species is still unavailable (Krajewski et al., 2000). There has been little additional research on the reproductive endocrinology of dasyurids in nearly 20 years.

Large marsupial carnivores have undergone major anthropogenic declines, and are predisposed to extinction events by a number of factors including naturally low population densities, ecological specialization and low lifetime

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reproductive effort, the latter being related to their relatively short lifespan (1–6 years) (Jones et al., 2003). All quoll species found in Australia are now listed by the IUCN (Low Risk—Vulnerable), and the two New Guinea species are of unknown conservation status (Jones et al., 2003).

The spotted-tailed quoll (*Dasyurus maculatus*) is the largest species of quoll, the second largest marsupial carnivore, and is endemic to Australia. Spotted-tailed quolls are found in Tasmania and on mainland Australia, where they persist in fragmented zones within the south-eastern states and far north Queensland. Populations on mainland Australia have experienced a major decline since European settlement, primarily through habitat fragmentation and loss (Jones et al., 2001, 2003), and they are currently listed as Threatened nationally in all states where they occur (Rare: Tasmania; Vulnerable: national, Victoria; Endangered: Queensland).

Detailed information on reproductive biology is necessary for understanding the behavioral life history strategies and ecology of spotted-tailed quolls, and also a priority for conservation (Temple-Smith, 2003). Some basic information is available on their breeding biology (Fleay, 1935; Fleay, 1940; Settle, 1978; Conway, 1988) and reproductive anatomy (Flynn, 1910,1911; Pearson and De Bavay, 1953), but because there have been no endocrine studies, important information on the frequency, duration and mechanisms of the species' estrous cycle is largely inferred (Croft, 1982; Conway, 1988; Collins et al., 1993).

Most marsupials are solitary (Russell, 1984), but the spotted-tailed quoll is considerably less abundant (Jones and Barmuta, 1998) and less social (Croft, 1982; Collins et al., 1993; Belcher and Darrant, 2004) than other dasyurids. The species also occupies relatively large home ranges and females are territorial (Belcher and Darrant, 2004; Claridge et al., 2005). We hypothesized that female quolls may be induced ovulators to allow increased opportunity for fertilisation upon encountering a male, and that this would be reflected in differences between mated and non-mated estrous cycles.

Most previous research into reproductive endocrinology of marsupials has relied on measurement of plasma progesterone and/or estradiol (reviewed in Tyndale-Biscoe and Renfree, 1987). Species studied include dasyurids such as the kowari and eastern quoll (*Dasyuroides byrnei* Fletcher, 1985; *Dasyurus viverrinus* Hinds, 1989), brown antechinus (*A. stuartii* Hinds, 1990) and brush-tailed phascogale (*Phascogale tapoatafa* Millis et al., 1999). More recently, fecal steroid monitoring has been applied as an alternative, non-invasive technique to monitor reproductive cycles in marsupials including the chuditch (*Dasyurus geoffroii*) (Stead-Richardson et al., 2001) and the squirrel glider (*Petaurus norfolcensis*) (Woodd et al., 2006).

The central aim of this study was to characterize the estrous cycle in the spotted-tailed quoll through measurement of plasma progesterone. A second aim was to apply fecal sex steroid analysis as an alternative non-invasive technique for monitoring ovarian cycles in this species as a basis for future applications of such techniques to *in situ* conservation and captive breeding. This study will contribute to our overall understanding of the diversity of reproductive patterns among the dasyurids, and complements research into the reproductive endocrinology of the closely-related Tasmanian devil (*Sarcophilus harrisii*), described in a companion paper (Hesterman et al., 2008).

2. Materials and methods

2.1. Study animals and husbandry

Ten female spotted-tailed quolls (1–3 years old) were housed at Featherdale Wildlife Park (FWP Doonside, NSW; $n = 3$) and Trowunna Wildlife Park (TWP Mole Creek, TAS; $n = 7$) during 2000–2001. Because there were few spotted-tailed quolls in captivity in Tasmania at the onset of the study in May 2000, in July of that year two wild quolls with pouch young were trapped from the Mole Creek district and relocated to TWP under permit. They were captured in wire-cage carnivore traps, aged and inspected for health, sex and status and individually marked by ear tattoo prior to transfer into captivity.

Quolls were maintained under similar conditions at both wildlife parks. They were fed a variety of meats including kangaroo or wallaby, possum, rabbit and chicken, and a variety of beef, mutton, poultry and fish at FWP. Additional items were provided for nutrition and enrichment at TWP. These included a prepared mix of grated carrot, apple, pumpkin seeds, egg and insectivore mix (Wombaroo Food Company, Mt Barker, SA) and, occasionally, commercially available cat biscuits. Water was available *ad libitum* at both sites.

Study animals were housed either in outdoor enclosures or pens with outside access, so exposed to natural variations in photoperiod, except for two quolls at TWP which were kept indoors under a natural lighting regime for a limited period of approximately three months. Animals kept outside were housed on natural substrate and those indoors were maintained on a wooden floor spread with eucalyptus mulch. All had access to climbing structures, native plants and other natural materials. Dens or nest boxes were available for shelter, and the number of retreats provided met or exceeded the number of animals per enclosure.

2.2. Experimental design

Adult quolls were housed individually, due to the species' solitary nature in the wild (Belcher and Darrant, 2004), with the exception of two females with unweaned young. Juveniles were separated from their dams at the beginning of March (prior to the onset of the breeding season) and maintained in mixed sex groups or separately until June. These young were included in the study as spotted-tailed quolls are known to become sexually mature in their first year (Conway, 1988).

Immediately prior to the onset of the breeding season females were placed into a specifically designed mating arena for 2–3 d per week. The mating arena consisted of three adjacent wire enclosures (each $\sim$3 m $\times$ 5 m $\times$ 4 m), with adjoining doors. The female was housed in the central pen and a male was kept in each of the two enclosures on either side of her.

To compare mated and non-mated estrous cycles females at TWP were assigned to different treatments groups during the breeding season (Table 1).

Treatment A: Females permitted full access/housed with males during estrus ($n = 4$ individuals, 5 estrous cycles).

Treatment B: Females with no physical access to males during estrus ($n = 7$ individuals, 10 estrous cycles).

To compare estrous cycles *within* individuals, individuals were assigned to different treatments during successive estrous cycles (STQ #02, #06, #07) or in different years (STQ #01). Four female spotted-tailed quolls from the non-mated group were paired with males during a different estrus.

Table 1
Experimental treatment of captive female spotted-tailed quolls during the breeding season

ID #	Treatment group	
	Estrous cycle	Estrous cycle 2
01 (Yr I)	A	A
(Yr II)	B^{+}	B
02	B	A^{+}
03	B	—
04	B	B^{+*}
05	B	A^{+}
06	B	B^{+}
07	B^{*}	A^{+}

Treatment A, paired with male/s during estrus; Treatment B, no physical access to males at estrus. +, vaginal smear and asterix (*) full isolation indoors under natural photoperiod. Note quoll #01 was monitored during two consecutive breeding seasons.

All females were monitored for behavioral and physical signs of estrus (vocalizations, thickened neck and pouch development (Settle, 1978; Conway, 1988; Collins et al., 1993). Vaginal smears were collected from three females paired with males during estrus, and from three other females during one of their non-mated cycles (Table 1). The first group of females were housed adjacent to males for 2–3 days, and permitted access to males when behavioral observations (vocalizations, crouching) indicated they were receptive (Settle, 1978). The adjoining door between the female and the 'preferred' male (based on the female's soliciting behaviors) was opened, and access to the adjacent male prevented with a large, solid screen. Copulation was confirmed by behavioral observation (observer presence or video recording: cameras positioned to cover both outdoor and nestbox activity), or detection of sperm in the vaginal smear. Pairs were separated after copulation and/or when heightened aggression between them was apparent. Female spotted-tailed quolls are reported to accept several males at estrus (Conway, 1988), so females were immediately returned to the central enclosure, and a new male exchanged for the one with which they had been paired. When a female ceased to show interest in males, she was returned to her individual enclosure. To ensure both groups had similar exposure to males, females from the second group were also placed into the central arena for an equivalent duration, but were not permitted physical access to males.

2.3. Sample and data collection and analyses

2.3.1. Plasma collection

At TWP, during the 2001 breeding season, blood samples were collected when animals were handled to obtain data on reproductive status (see below). Quolls were captured by hand or use of a large net. They were restrained unanaesthetised in a sack during sample collection and examination. A peripheral ear vein was pricked with a disposable Stat-Let© lancet and 75–150 μl blood was collected via a heparinised capillary tube. Samples were taken between 0730 and 0930 or 1500–1700 h except when individuals were being captured for husbandry, when blood was collected opportunistically. Samples were kept at 4 °C until centrifuged; the plasma was frozen (−20°C) until radioimmunoassay. Study animals were bled at ~10 day intervals from May to September. To reduce the potential impact of stress on successful rearing of young, handling and blood sampling of females was minimized during the post-mating period.

2.3.2. Fecal collection

Fecal samples were collected between May 2000–December 2001 (TWP); and October 2000–July 2001 (FWP). To ensure the individual identity of samples when pairs were housed together, small colored plastic beads (1 mm diameter) were mixed into a mincemeat ball and fed to the study animals on the day before sample collection. Frequency of fecal collection varied depending on the time of year and breeding status of individual animals. Immediately prior to and during the mating season (May–September), samples were obtained up to three times per week, whereas during the rest of the year samples were collected weekly. Fecal collection, storage and processing for analysis followed the methods outlined in Hesterman et al. (2008).

2.3.3. Additional data and sampling

Vaginal (urogenital) smears were collected from all females paired with males, and also from two non-mated individuals. Smears were obtained from the posterior vaginal sinus by introduction of a small cotton swab through a glass speculum (70 mm length × 3 mm ø). Smears were air-dried, fixed and then stained with acid fuchsin and toluidine blue (Dix and Billings, 1969). Stained smears were examined for percentage of intermediate (IE) and superficial/cornified epithelials (SE), leucocytes and presence of spermatozoa. Pouches were monitored for condition/presence of young, when not constrained by management.

2.3.4. Plasma progesterone analyses

Plasma progesterone was measured by radioimmunoassay (RIA), as detailed in Hesterman et al. (2008). Serial dilutions of quoll plasma ran parallel to the progesterone standard curve. Recovery of cold progesterone had a mean recovery within 10% of expected values. All samples were included in a single assay ($n = 74$). The intra-assay coefficient of variation was 9.5%.

2.3.5. Fecal sample processing and enzyme-immunoassay of steroids

Lyophilized fecal samples (0.1 g) were mixed with distilled water (0.9 ml) and methanol (4.0 ml). After vortexing and centrifugation, 1.0 ml of the methanol extract was transferred into a new vial, mixed with a $NaHCO_3$ solution and re-extracted with diethylether, following previously described methods (Schwarzenberger et al., 2000). Assay buffer was added to the extract residue prior to enzyme-immunoassay (EIA). Immuno-reactive progesterone and estrogen metabolites were assayed using previously established group-specific assays (Schwarzenberger et al., 1997). Samples were analyzed for 20α-OH-pregnanes (antibody: 5β-pregnane-3α-20α-diol 3HS:BSA; trivial name pregnanediol), 20-oxo-pregnanes (antibody: 5α-pregnane-3β-ol-20-one 3HS:BSA), and total estrogens (antibody: oestradiol-17β-OH 17-HS:BSA). Preliminary testing showed that, for fecal pregnanes the use of 20α-OH-pregnanes (pregnanediol PgD) was most appropriate for the spotted-tailed quoll, with concentrations being excreted in consistently higher levels than 20-oxo-pregnanes. EIAs were validated by demonstrating parallelism between standard curves and serial dilutions of the fecal extracts, and by showing that fecal values followed the same trend as the values obtained with the plasma progesterone assay. The intra- and inter-assay coefficients of variation for the assays were <10% and <15%, respectively.

2.3.6. Terminology

Non-conceptive cycles in marsupials have been described variously as non-pregnant, pseudopregnant or failed pregnant. Following Hesterman et al. (2008), the terms "non-mated" and "mated" are used to avoid ambiguity, because some females may have produced young, but lost them prior to pouch checking.

2.4. Interpretation of hormone data

Stages of the estrous cycle were defined as the follicular phase (FP), luteal phase (LP), anestrous and inter-estrus (period between beginning of FP to onset of next FP). Baseline values were generated by averaging values of plasma progesterone concentrations obtained from three mature study animals during the non-breeding season ($n = 21$ samples). For plasma progesterone monitoring, onset of estrous was readily determined because concentrations rose characteristically at pro-estrous, as reported for several other dasyurids (Hinds, 1981; Fletcher, 1985). Increases above the group mean baseline + one SD (*i.e.* 0.07 ng/ml) that were maintained

for at least 5 days were considered indicative of onset of the follicular phase (FP). The LP was confirmed when plasma progesterone concentrations increased two SD above baseline levels (*i.e.* 0.09 ng/ml).

Where samples were taken $\geqslant$7d apart, the durations of successive stages of the cycle were calculated by counting the days elapsed between the two samples, halving the result and adding it to the duration of the phase either side. The onset and length of the inter-estrus period were similarly defined, taking the days elapsed between the final two consecutive low plasma progesterone values (LP) and adding half the result to the days before subsequent significant increase (FP) in progesterone.

For fecal steroids, group baseline values were calculated by averaging steroid concentrations in six adults during the non-breeding season ($n = 33$ samples), as described for plasma progesterone. The FP was defined as the period during which fecal estrogens were elevated above the mean baseline + one SD for the group (*i.e.* 7.6 ng/g), and remained elevated for at least two consecutive samples. As for plasma progesterone, fecal pregnanes often showed a characteristic pro-estrous pulse that approached mean values. The LP was defined through a sustained increase in progestagen concentrations above the group mean for PgD (*i.e.* >4456.5 ng/g). The end of the LP was identified by a decline in pregnane concentrations to below the group mean values for a minimum of 2 weeks.

2.5. Comparison between plasma and feces

To allow comparison between the profiles of plasma progesterone and fecal progestagen concentrations, temporal alignment of samples was necessary. Because the lag period corresponds with the approximate passage of food (Schwarzenberger et al., 1996), fecal samples were displaced from the plasma results by 24 h—based on the appearance of the colored plastic beads fed to spotted-tailed quolls to individually identify scats.

2.6. Statistical analyses

All data are presented as means ± SE, except where indicated otherwise. Student's unpaired *t* tests were used to compare estrous characteristics and the duration of estrous cycles. Analysis of variance (ANOVA) with Tukey's post-hoc comparison was used to detect temporal changes in grouped data. Plasma progesterone profiles were compared with those of its fecal metabolites using linear regression on log-transformed data. Statistical analyses were performed using SPSS (SPSS Inc. 1998, Chicago IL), Version 13 package.

3. Results

Estrous cycles were recorded in all seven female quolls sampled during the breeding season. Longitudinal profiles showed that animals typically underwent estrus twice in each season, and reproductive activity lasted approximately four months, from mid-May until late September.

3.1. Estrous cycle characteristics

Mean duration of the estrous cycle varied between treatment groups (Table 2). There was a significant difference in the mean duration of the non-mated and mated estrous cycle ($t_{(11)} = -3.221$, $P = 0.008$). This was due to the extended length of the FP in non-mated females ($t_{(14)} = -2.516$, $P = 0.025$). Estrous cycles did not follow each other closely. After the LP, a variable period of ovarian quiescence was observed prior to onset of the next estrous cycle (30.3 ± 6.9 d, range = 7 –59; $n = 6$). This resulted in an inter-estrus interval of around 2.5 months between successive follicular phases. Intervals were highly variable, both between individuals and between cycles of the same individual.

3.2. Plasma progesterone

Mean plasma progesterone concentrations for female spotted-tailed quolls sampled during the breeding period (1.2 ± 0.41 ng/ml) were significantly higher than during the non-breeding period (0.1 ± 0.05 ng/ml) ($t_{(59)} = -2.285$, $P = 0.028$). Individual profiles (*e.g.* Fig. 1) demonstrated a biphasic pattern: there was a brief rise (0.97 ± 0.3 ng/ml) in concentrations about one week prior to onset of the LP, accompanied by behavioral estrus, a predominantly cornified vaginal smear and copulations for paired females. Copulations were observed immediately prior to, during and following this pro-estrous peak in plasma progesterone concentrations. Mating usually occurred over only 1–2 days but was extended up to a week when females accepted two different males at estrus. After the pro-estrous pulse, plasma progesterone concentrations dropped and remained low for several days, but then rose again at luteal onset. During the LP, mean

Table 2
Characteristics of the estrous cycle in the spotted-tailed quoll as assessed by changes in fecal (estrogens and progestagens) and plasma (progesterone) sex steroid concentrations

	FECES Mean length ± SE (Days)	Number of cycles(# individuals)	PLASMA Mean length ± SE (Days)	Number of cycles (# individuals)
Follicular phase (FP)*				
Non-mated	19.4 ± 4.0 (range 9–39)	12 (6)	15.2 ± 1.4 (range 11–18)	6 (4)
Mated	8.3 ± 1.9 (range 5–12)	6 (4)	8.5	1 (1)
Luteal phase (LP)				
Non-mated	23.5 ± 1.2 (range 18–27)	9 (6)	20.7 ± 1.4 (range 19–24)	4 (4)
Mated	23.8 ± 1.7 (range 20–28)	4 (4)	—	
Estrus cycle (FP + LP)*				
Non-mated	41.1 ± 2.3 (range 31–52)	8 (5)	35.3 ± 2.5 (range 30–40)	4 (3)
Mated	29.2 ± 2.8 (range 23–39)	4 (4)	—	
Inter-estrus (FP–FP)	71.1 ± 9.1 (range 49–125)	7 (5)	39.5 ± 3.3 (range 30–44)	4 (4)

Asterix indicates significant difference between non-mated and mated groups ($P < 0.05$).

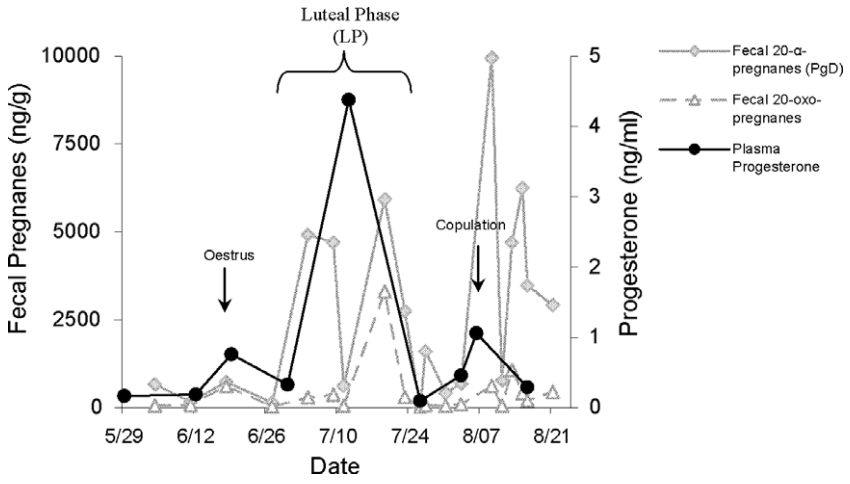

Fig. 1. Plasma progesterone (ng/ml) and fecal progestagen (20-α- and 20-oxo-pregnanes) profiles in a female spotted-tailed quoll sampled during the breeding season. Arrows indicate timing of behavioral estrus in the first cycle (housed alone) and copulation in the second cycle (paired with male).

plasma progesterone concentrations were several fold higher (2.18 ± 1.1 ng/ml) than FP levels (0.75 ± 0.02 ng/ml), with peak values in individual females of up to 8.61 ng/ml.

3.3. Comparison between plasma progesterone and fecal metabolites

Fecal progestagens and plasma progesterone during the estrous cycle were significantly positively associated ($P < 0.05$) (regression equation: $y = 0.69 \times -3.10$; $R^2 = 0.72$), and tracked each other (Fig. 1). PgD (20-α-OH-pregnanes) were excreted in consistently higher concentrations than 20-oxo-pregnanes throughout the cycle. Fecal PgD and 20-oxo-pregnanes were significantly associated ($P < 0.01$) (regression equation: $y = 1.08x + 1.17$, $R^2 = 0.73$). Mean PgD concentrations were 3860.2 ± 241 ng/g; mean 20-oxo-pregnanes were 336.67 ± 38.6 ng/g; total estrogens were 13.02 ± 2.4 ng/g.

3.4. Fecal steroid monitoring

During the breeding period average fecal sex steroid concentrations in female quolls were significantly elevated compared to during the non-breeding period (PgD *non-breeding* $= 133.5 \pm 17.58$ ng/g, *breeding* $= 5525.2 \pm 1244.77$ ng/g ($t_{(158)} = -2.203$, $P = 0.029$); estrogens *non-breeding* $= 4.0 \pm 0.74$ ng/g, *breeding* $= 17.6 \pm 2.70$ ng/g ($t_{(156)} = -2.569$, $P = 0.011$)). Group profiles for faecal pregnanediol (PgD) and total estrogens demonstrate the general pattern of sex steroids during the estrous cycle in the spotted-tailed quoll (Fig. 2). Estrogens were in highest concentrations during the FP, approximately one week prior to luteal onset and declined steadily thereafter ($F_{(3,\ 85)} = 1.147$, $P = 0.235$). There was a significant temporal difference in faecal PgD between days −10 to 30 from luteal onset ($F_{(3,\ 85)} = 2.994$, $P = 0.035$). Faecal PgD had an inverse pattern to fecal estrogens until day 20 from luteal onset,

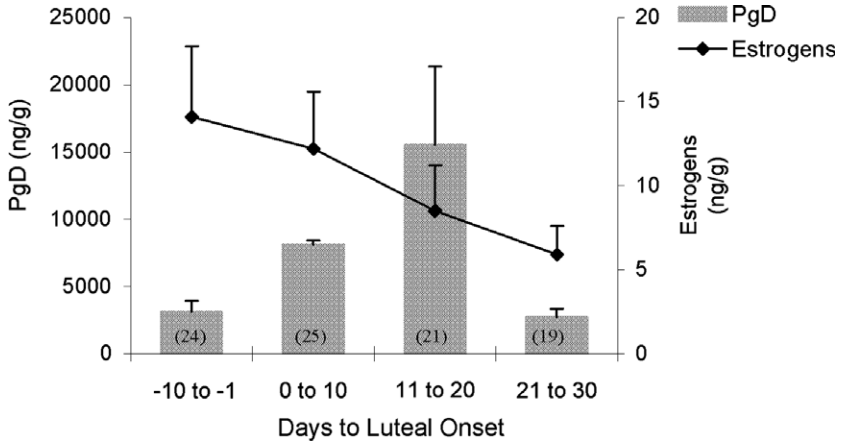

Fig. 2. Grouped mean fecal pregnanediol (PgD) and total estrogen concentrations (ng/g) between days −10 and 30 days from luteal onset in seven female spotted-tailed quolls. Sample sizes indicated within bar.

with highest concentrations in the latter part of the luteal phase (days 11–20). Faecal PgD concentrations were markedly lower during days 21–30 after luteal onset ($P = 0.059$).

3.4.1. Non-mated estrous cycle

Individual profiles show that up to three weeks prior to onset of the LP, small pulses of fecal estrogens were apparent, but fecal PgD concentrations remained relatively low (Fig. 3). Fecal progestagen concentrations climbed very rapidly within days after luteal onset and remained elevated during the LP, then declined sharply and approached baseline values between days 25 and 30. During the LP, fecal estrogen levels fluctuated at low concentrations.

All females underwent a second estrus, indicated by elevations in estrogen excretion coincident with an estrus smear (>95% cornified cells). Time elapsed between estrus periods varied, occurring between days 35–40 for most individuals ($n = 4$), but lasting 65 and 91 days for two study animals. In two females (#04 and #06), there was no subsequent elevation in PgD concentrations following the second FP (Fig. 3).

3.4.2. Mated estrous cycle

Mean concentrations of fecal estrogen were significantly higher in females that were paired with a male during estrus (*mated*: 26.2 ± 6.37 ng/g, *non-mated*: 12.4 ± 1.69 ng/g; $t_{(119)} = 2.466$, $P = 0.015$); however, there were no differences in the mean concentrations of PgD between the treatment groups (*mated*: 6392.7 ± 2255 ng/g, *non-mated*: 4585.3 ± 1518 ng/g; $t_{(121)} = 0.690$, $P = 0.491$).

The four female spotted-tailed quolls mated with males exhibited a similar endocrine pattern to that observed during the non-mated estrous cycle, with a pre-luteal pulse in fecal estrogens during the FP and a sustained increase in PgD during the LP (Figs. 4 and 5). Copulations usually occurred over two–three days, but the interval between mating and luteal onset varied widely between individuals (8.5 ± 3.8 d) in females that ovulated during that cycle. Progestagens peaked around 21 days after first mating at concentrations up to 100-fold higher than those recorded at estrus. Fecal PgD concentrations then dropped precipitously, and were low in three of the four females by day 25 from mating. We could not confirm births for any of the quolls.

Individual longitudinal fecal hormone profiles show that major peaks in estrogen excretion were associated with an estrus smear and coincided with times of copulation (Figs. 4 and 5). Anovulatory cycles (no sustained rise in PgD after mating) occurred in three of the four mated individuals. One female exhibited recurrent waves of fecal estrogen excretion 10–20 days apart over an approximate two month period prior to eventual ovulation, despite repeated bouts of mating with two different males (Fig. 5).

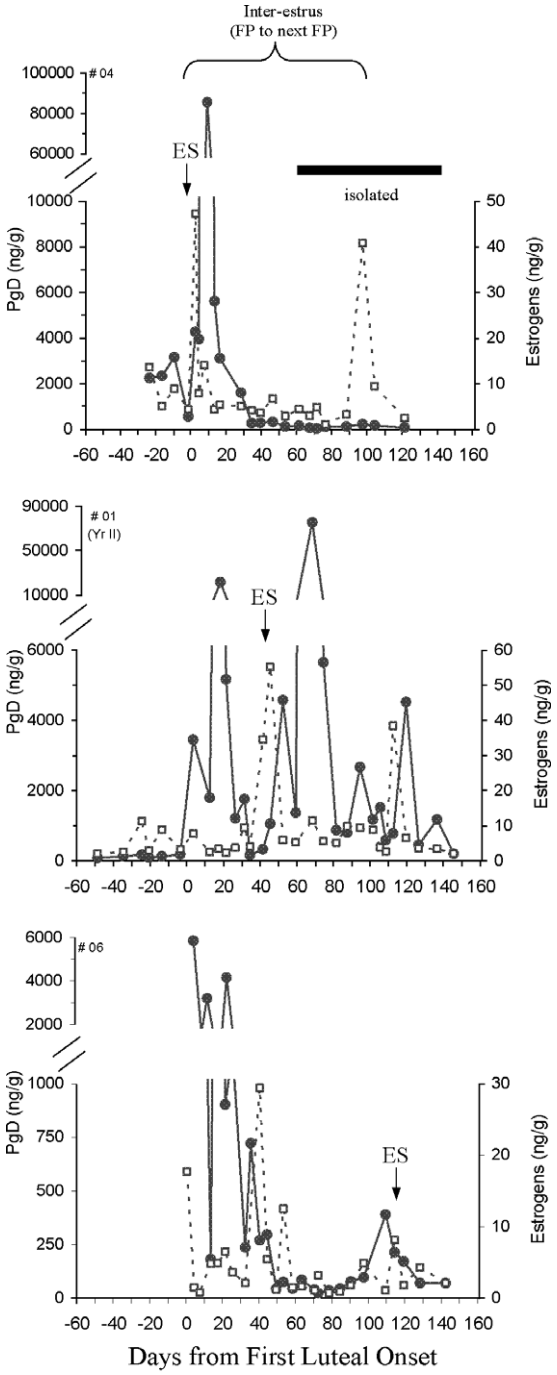

Fig. 3. Longitudinal profiles of fecal pregnanediol (PgD) (●) and total estrogen (□) concentrations (ng/g) during the breeding season in three non-mated female spotted-tailed quolls, aligned from onset of the first luteal phase. Arrows indicate an estrus smear (ES). Solid bar shows period when female #04 (top) was isolated indoors under natural photoperiod. Female #06 (bottom) was not sampled prior to first luteal onset. Note variable inter-estrus periods between individuals.

4. Discussion

4.1. Characteristics of the estrous cycle

Patterns of plasma and fecal progestagens were significantly associated, indicating that fecal progestagens can

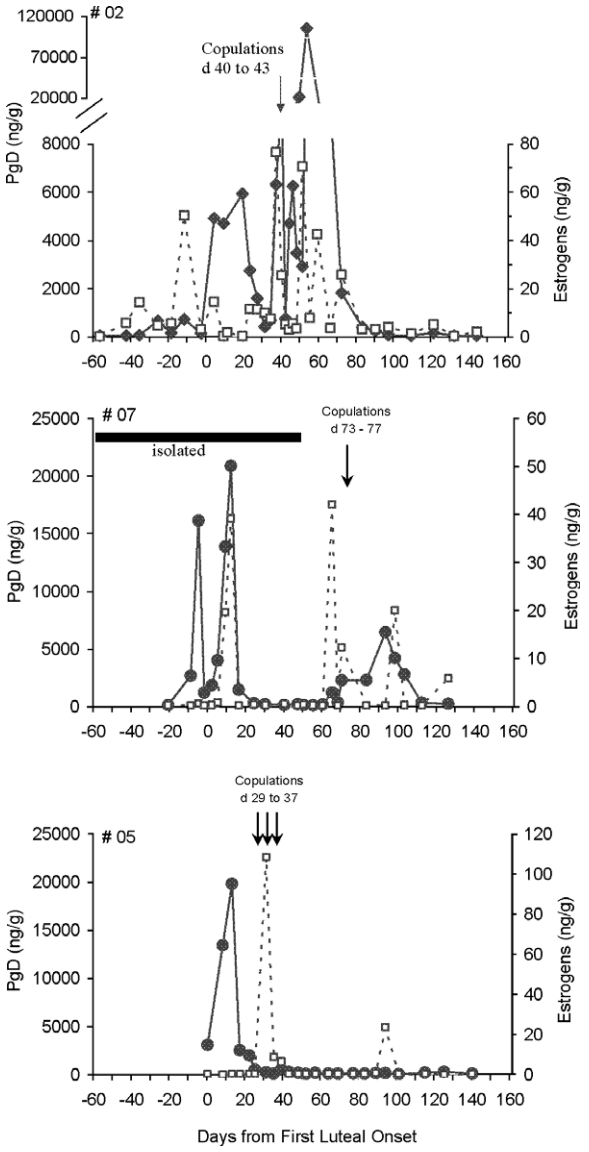

Fig. 4. Longitudinal profiles of fecal pregnanediol (PgD) (●) and total estrogen (□) concentrations (ng/g) in three female spotted-tailed quolls that were mated during their second estrus in the breeding season. Data are aligned from first luteal onset; female (#05) was not sampled prior to that time. Arrows denote periods of copulation. No births were confirmed. Note axis on different scales to account for individual variations in hormone concentrations. Solid bar shows period when female #07 was housed indoors under natural photoperiod.

be used as a relative measure of concurrent changes in plasma progesterone concentrations. Plasma and fecal steroid monitoring of spotted-tailed quolls showed that although there was some individual variation, on average the estrous cycle lasted approximately 38 days (range 35–52 d). Settle (1978) reported a 21 day cycle length for the species based only on vaginal cytology. Our endocrine study showed that the average duration of the estrous cycle in spotted-tailed quolls was similar to that of the eastern quoll (Hinds, 1989) and the Tasmanian devil (Hesterman et al., 2008).

Longitudinal profiling confirmed that spotted-tailed quolls are seasonally polyestrous, as is typical for other iteroparous dasyurids including other quolls (Tyndale-Biscoe and Renfree, 1987; Hinds, 1989) and the devil (Hesterman et al., 2008). Spotted-tailed quoll routinely exhibited two estrous cycles during the reproductive season, although one individual had three estrus periods—an occurrence previously reported by Collins et al. (1993). The kowari and the devil also routinely undergo two cycles (Fletcher, 1985; Hesterman et al., 2008). In spotted-tailed quolls the inter-estrus interval (onset of FP to next FP) was ~70 days (range ~49 to 125 d), similar to variable at our observations for the devil (Hesterman et al., 2008). Collins et al. (1993) reported a shorter, less variable interval of ~50 days (range 36–58 d) for the spotted-tailed quoll based on time elapsed between bouts of female receptivity. In kowari, Fletcher (1985) also found the inter-estrus period to be variable. By comparison, in the eastern quoll estrous cycles are of consistent duration and follow one another closely (Hinds, 1989).

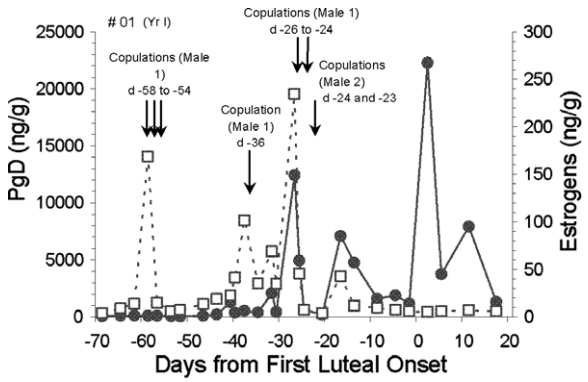

Fig. 5. Longitudinal profile of fecal preganediol (PgD) (●) and total estrogen (□) concentrations (ng/g) in a mated female spotted-tailed quoll. Arrows denote episodes of copulation by two different males. Data are aligned from luteal onset; no birth was confirmed.

Most marsupials are spontaneous ovulators (Tyndale-Biscoe and Renfree, 1987; Hinds et al., 1996) with very few exceptions: the brush-tailed bettong (*Bettongia penicillata*) (Hinds and Smith, 1992), the American opossum (*Monodelphis domestica*) and the koala (*Phascolarctos cinereus*) (Johnston et al., 2000). In the bettong and opossum, estrus can be induced by the presence of a male, but the koala is a true 'reflex' ovulator, requiring the physical act of mating to induce a luteal phase. In our study of spotted-tailed quoll, luteal phases occurred in both mated and non-mated cycles, indicating that this species is not an induced ovulator. Interestingly, ovulation occurred in a female housed indoors under complete isolation, but failed to occur in three other unpaired individuals and two mated females, despite episodes of confirmed copulation. However, mated and non-mated cycles did differ significantly in the duration of the follicular phase—being approximately twice as long in non-mated females. This finding indicates that vaginal/cervical stimulation during coitus does play some role in the timing of ovulation in this species.

4.2. Pattern of the estrous cycle

4.2.1. The follicular phase

In the spotted-tailed quoll, the follicular phase lasted approximately two weeks, similar to the range reported for other dasyurids (Tyndale-Biscoe and Renfree, 1987; Hinds, 1989; Hesterman et al., 2008). Both plasma progesterone and fecal progestagen concentrations in the spotted-tailed quoll exhibited a relatively low, brief pro-estrous pulse. A similar biphasic pattern has been observed in several other dasyurids including the eastern quoll (Hinds, 1989), kowari (Fletcher, 1985) and Tasmanian devil (Hesterman et al., 2008).

Fecal total estrogens were excreted in highest concentrations during pro-estrous and peaked during the pro-estrous pulse of plasma progesterone/fecal progestagens, as described for the devil (Hesterman et al., 2008). In spotted-tailed quolls and devils, multiple peaks in fecal total estrogens occurred during this period, similar to the wave-like pattern in fecal oestradiol-17β excretion observed at estrus in the chuditch (Stead-Richardson et al., 2001). During this period of heightened estrogens, we observed cornified vaginal smears and the onset of estrus behaviors, including copulation. Multiple copulations usually occurred over a period of several days, as previously reported for the spotted-tailed quoll (Edgar and Belcher, 1995) and most other dasyurids (Fletcher, 1985; Tyndale-Biscoe and Renfree, 1987; Hinds, 1989; Stead-Richardson et al., 2001; Hesterman et al., 2008).

Following the pro-estrous peak in estrogens and progesterone/progestagens, sex steroid concentrations returned to minimal levels for around a week, prior to a rise associated with onset of the luteal phase. This extended 'ovulatory interval' appears to be characteristic of dasyurids, and has been reported for the eastern quoll (Hinds, 1989), kowari (Fletcher, 1985) and brown antechinus (Selwood, 1980). Mean duration of this 'ovulatory interval' in spotted-tailed quolls (7.1 ± 1.3; range 2 – 12 d) was considerably longer than that reported for those species, but very similar to that of the devil (3–9 d) (Hesterman et al., 2008). This extended timeframe from copulation to ovulation may have a role in permitting matings with several different males, and resultant multiple paternity (Taggart et al., 2003).

4.2.2. The luteal phase

The pattern of progesterone/progestagens during the luteal phase was similar to that observed in other dasyurids (Tyndale-Biscoe and Renfree, 1987; Hinds, 1989; Hinds and Selwood, 1990; Millis et al., 1999; Hesterman et al., 2008). As observed in the Tasmanian devil (Hesterman et al., 2008), fecal estrogen concentrations fluctuated at lower levels during the luteal phase, a pattern that may be related to incomplete suppression of ovarian activity. In most marsupials, plasma progesterone concentrations drop within days after completion of the estrous cycle (Tyndale-Biscoe and Renfree, 1987), and similarly, in spotted-tailed quolls progesterone/progestagen concentrations returned to low levels soon after the end of the luteal phase.

4.3. Comparison of the mated and non-mated estrous cycle

As in most other marsupials, there were no differences in the mean duration or amplitude of steroid profiles between mated and non-mated females (Fletcher, 1985; Tyndale-Biscoe and Renfree, 1987; Hinds, 1989, 1990; Hinds and Selwood, 1990; Hesterman et al., 2008). It is unclear why none of the mated female spotted-tailed quoll in this study did not produce young, but profiles did show that copulation was not always followed by ovulation. For uncertain reasons, captive breeding success is low in several dasyurids, including this species (Conway, 1988; Fletcher, 1989; Roberts et al., 1993; Stead-Richardson et al., 2001). Selwood (1983) found that fertilization fails in around 25% of ovulations in the brown antechinus, and breeding records for spotted-tailed quoll indicate a conception rate of only around 50% in captivity (Collins et al., 1993), although losses of pouch young—especially at early stages of pouch life—are difficult to quantify. Two of the four mated females were only one year old, and a study of wild spotted-tailed quoll suggests that successful breeding may not occur until they reach two years of age (Belcher 2003; Belcher & Durrant 2004).

This study has described the reproductive endocrinology of the female spotted-tailed quoll, *D. maculatus*, documenting the characteristics of the estrous cycle in this Threatened species. It complements our recent research into the closely-related Tasmanian devil (Hesterman et al., 2008) and extends comparisons with other marsupials, as well as demonstrating the usefulness of fecal steroid measurements for monitoring ovarian cycles in carnivorous marsupials. This technique provides a useful, non-invasive method for regular monitoring of reproductive status in spotted-tailed quolls, and may assist in elucidating the reasons for their limited breeding success in captivity.

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