THE ROYAL SOCIETY

doi 10.1098/rspb.2000.1369

Semelparity in a large marsupial

Meri Oakwood[1]†, **Adrian J. Bradley**[2] **and Andrew Cockburn**[1]*

[1]*Evolutionary Ecology Group, Division of Botany and Zoology, Australian National University, Canberra ACT 0200, Australia*
[2]*Department of Anatomical Sciences, University of Queensland, Brisbane, Queensland 4072, Australia*

Complete mortality of males after mating is known in several small dasyurid and didelphid species (up to 300 g) and has previously been suggested to be a consequence of their small size and their inability to sequester sufficient fat reserves for an intense rut in the winter. Males of these species use increased corticosteroid levels to allow protein catabolism, enabling them to support their mating effort with other body reserves. However, increased corticosteroid levels have negative consequences such as anaemia, gastrointestinal ulceration, immune suppression and disease. The Australian dasyurid *Dasyurus hallucatus* shows complete male die off after mating in tropical savannah, yet males of this species may weigh as much as 1120 g and continue to eat during the rut. Die off in *D. hallucatus* shows many similarities to that in the smaller species including weight loss, fur loss, parasite infestation, increased testosterone levels and anaemia. However, in contrast to smaller species, there is no evidence of elevated corticosteroid levels or gastrointestinal ulceration. Consequently, the phenomenon of male die off after mating lacks a universal explanation.

Keywords: life-history evolution; marsupial; eutherian; semelparity; *Dasyurus hallucatus*; Dasyuridae

1. INTRODUCTION

The distinction between semelparity and iteroparity is one of the fundamental life-history dichotomies. Although semelparity has evolved frequently in plants, invertebrates and fishes, true semelparity in terrestrial vertebrates is thought to be restricted to marsupials in the distantly related groups Didelphidae and Dasyuridae (reviewed in Lee & Cockburn 1985; Cockburn 1997). Most of what we know of these species comes from studies of the genera *Antechinus* and *Phascogale*. In all well-studied species in these genera, females have a single, highly synchronized oestrus each year, during which males commit themselves totally to obtaining mates. Males die immediately after this short rut, so that in some populations all males are dead before the females give birth. The most common pathology is gastrointestinal haemorrhage, and endocrinological and pathological studies implicate failure of the immune and inflammatory systems as the ultimate cause of mortality. This in turn appears to be triggered by sustained high levels of corticosteroids, which arise because of increases in secretion, substantial reductions in corticosteroid-binding globulins and suppression of the usual adaptive negative feedback on adrenocorticotropin secretion (Bradley *et al*. 1975, 1980; McDonald *et al*. 1986; Bradley 1987, 1990).

This life history presents a dramatic contrast to that observed in other mammals. For example, in eutherian mammals, slow growth tends to be associated with longer life spans (Charnov 1993). Marsupials generally grow more slowly than their eutherian counterparts (Russell 1982; Zullinger *et al*. 1984; Lee & Cockburn 1985; Cockburn & Johnson 1988) so short reproductive spans are not predicted (Cockburn 1997). Evolutionary explanations of die off have stressed the adaptive advantages of elevated corticosteroid levels, which promote protein catabolism via gluconeogenesis. Use of protein as food storage may allow small mammals with a limited ability to store energy as fat to sustain mating effort when forced to mate in winter, when food is not abundant (Lee & Cockburn 1985), and in aggregations some distance from the home range with which the male is familiar (Lazenby-Cohen & Cockburn 1988). This interpretation is consistent with other examples of vertebrate semelparity, such as the hormonal changes that allow anadromous fishes, including salmon and eels, to migrate into new habitats in order to mate.

However, this interpretation has been challenged by the observation that post-reproductive senescence may occur in all didelphids and dasyurids (Cockburn 1997). Most notably, complete male die off has been reported in a savannah population of the northern quoll (*Dasyurus hallucatus*), a dasyurid that weighs up to 1120 g (Dickman & Braithwaite 1992) and possesses specialized areas for fat storage in the tail. Here, we report a detailed long-term study of this population and conclude that many characteristics of die off in male antechinuses and other dasyurids are absent in quolls. This suggests that the original explanation for male die off in antechinuses cannot be generalized to other marsupials, and probably represents the consequences rather than the causes of semelparity. We propose that marsupials suffer acute developmental constraints on life-history evolution that predispose them to the evolution of semelparity.

2. METHODS

(a) *Study area*

The research was conducted in lowland savannah of Kakadu National Park, Northern Territory, Australia. Live trapping and radiotracking occurred at Kapalga Research Station (12°43′ S, 132°25′ E) at a site located in eucalypt open forest and woodland, with rocky hills up to 77 m high and small seasonal creeks.

(b) *Collection and examination of road kills*

A 10 km stretch of the Arnhem Highway between South Alligator and Kapalga Research Station was monitored from

---

*Author for correspondence (andrew.cockburn@anu.edu.au).
†Present address: Department of Ecosystem Management, University of New England, Armidale, New South Wales 2351, Australia.

Received 4 August 2000 *Accepted* 24 October 2000

February 1993 to May 1995 by M.O. and two volunteers, and the remaining 78 km were checked from April 1994 to May 1995 by four other volunteers. Within each monitoring period, roads were inspected on at least ten days per fortnight. Inspections were made at dawn (and also in the early evening) as carcasses are quickly destroyed by diurnal scavengers, particularly raptors. Additional bodies were collected from nearby areas opportunistically.

The digestive tracts of 16 road-killed males were examined both macroscopically and microscopically for evidence of gastrointestinal ulceration.

Testes were obtained from three road-killed males after the mating season in 1994 and preserved in formalin. The tunics were removed and the lengths and widths were measured. Sections were then taken and stained with haematoxylin and eosin prior to examination to determine whether spermatogenesis had failed, as has been recorded for some other dasyurids (e.g. Kerr & Hedger 1983).

(c) *Trapping and radiotracking*

Live trapping was carried out on two consecutive nights per fortnight from November 1992 to December 1993 on a 22.5 ha grid and on two consecutive nights per month from January 1994 to February 1995 at the same site with the grid expanded to 35 ha. Twenty male quolls were fitted with radio-collars (Sirtrack, NZ) for periods of between one and 56 days and tracked to their dens during the day (Oakwood 1997). This allowed additional focal trapping of specific individuals to monitor the timing of various reproductive events. Radiotracking also allowed determination of the date and cause of death for several individuals. During trapping, quolls were individually marked with ear tags and weighed. Age was estimated by a combination of month of capture, weight, body measurements and incisor wear (Oakwood 2000). Tail circumference was measured at the tail base as an index of individual condition (since northern quolls store fat in their tails), and scrotal width was used as an indicator of testicular development and sperm production. The abundance of lice was assessed. Small blood samples were obtained from the ears of trapped quolls by venepuncture with a new 25 gauge needle and bleeding directly into a heparinized capillary tube. After examination, quolls were released immediately at the site of capture.

(d) *Physiological investigation*

Haematocrit was determined using a Clements microhaematocrit centrifuge and reader. Cortisol and testosterone concentrations were measured in separate 20 µl plasma samples using a direct ^{125}I cortisol radioimmunoassay (Pantex Catalogue number 031, Santa Monica, California, supplied by Immunodiagnostics, Camperdown, Australia) and a direct ^{125}I testosterone radioimmunoassay (Pantex Catalogue number 135), respectively. Curve fitting was performed using the AssayZap program (Universal Assay Calculator, Biosoft, Cambridge, UK). A typical curve for cortisol, using calibrators in duplicate, ranges from 0.01 to 128 ng ml^{-1} and for testosterone from 0.01 to 25.6 ng ml^{-1}. In order to establish the reliability of these assays in *D. hallucatus* plasma, serial dilutions of *D. hallucatus* plasma were used in the assays. Plasma pools that gave high results for cortisol and testosterone in the assays were chosen and serial dilutions were carried out using charcoal-stripped *D. hallucatus* plasma. Charcoal (25 mg ml^{-1} Norit A activated charcoal, Sigma Chemical Company, St Louis, MO, USA) was added and the plasma was incubated at 37 °C for 30 min before centrifugation to remove all traces of charcoal. The assay curves for both standard calibrators and the diluted *D. hallucatus* plasma calibrators were compared and the parallelism was considered acceptable with regression analysis giving a probability of 0.0001 for the relationships. The cross-reactivity with corticosterone was 58% and with 5 α-dihydrotestosterone 6.9%. Both inter- and intra-assay variation were always less than 10%. The lowest detectable mass of cortisol was 200 pg, which is equivalent to a cortisol concentration of 10 ng ml^{-1}. The recovery of added cortisol gave a mean in excess of 99%. The lowest detectable mass of testosterone was 2 pg, which is equivalent to a testosterone concentration of 0.1 ng ml^{-1}. The recovery of added testosterone gave a mean in excess of 99%.

3. RESULTS

In total, 79 quolls (41 males) were live trapped at Kapalga over 4267 trap nights. The oldest male trapped was 14 months old. Out of the 20 males that had radiocollars fitted, radiotracking allowed the determination of the date and proximate cause of death for eight. The mean age at death for these eight males was 11 months, with the oldest dying at 12 months of age. In addition to these eight males, 63 dead quolls (mostly road kills) were collected. Out of these, 38 were males, 16 were females and nine could not be sexed due to decay or physical damage by vehicles and/or predators. The oldest male road kill was 17 months old.

(a) *Timing of male die off*

Out of the 26 adult males ($\geqslant$7 months old) that were caught on the trapping grid, 85% subsequently disappeared between April and June. Mating occurs in late May and early June, so most males disappeared before and during mating. Although some of this reduction in male numbers could be due to these males leaving the area, it would be expected that some males would remain. However, no adult-sized males were trapped between September and December, despite 1414 trap nights during this period. Moreover, no individual male that was trapped in a given year was re-trapped the following year, and all males trapped in November and December were juveniles. This occurrence of discrete male generations (figure 1) confirms earlier suggestions of complete post-mating mortality in this species.

To verify the timing of male deaths, in 1994 eight males were radiotracked during the rut until their deaths. All died between 31 May and 4 July. These eight males were killed by motor vehicles (three), predators (three: two probably by dingoes, *Canis lupus dingo*, and one by an olive python, *Liasis olivaceus*) and unspecified post-mating mortality (two). The latter two animals were found lying dead in logs, in very poor condition but without any obvious sign of predation. Both were too badly decayed for useful post-mortem examination.

Most road-killed males were collected between April and June, around the mating season, though collection effort was constant throughout the year (Oakwood 2000). Although a surge in male road kills could be caused by increased dry-season tourist traffic at this time of year, there was no corresponding surge in the number of female road kills. Road kills are not directly equivalent to die off. However, they provide an easily detectable

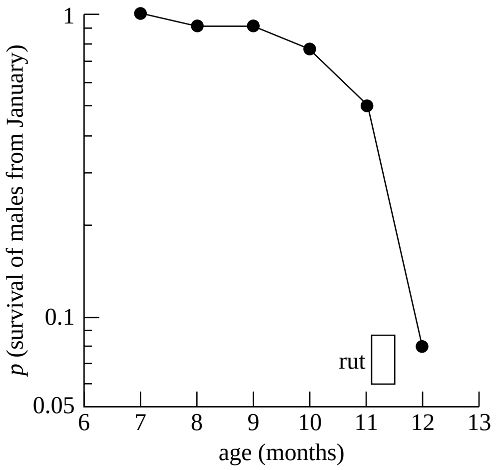

Figure 1. Percentage of males that were present on the trapping grid at seven months of age (January; $n = 26$, 1993 and 1994 cohorts combined) that continued to be trapped, in relation to age. Death was confirmed for eight of these males by radiotracking (all 11–13 months old at death).

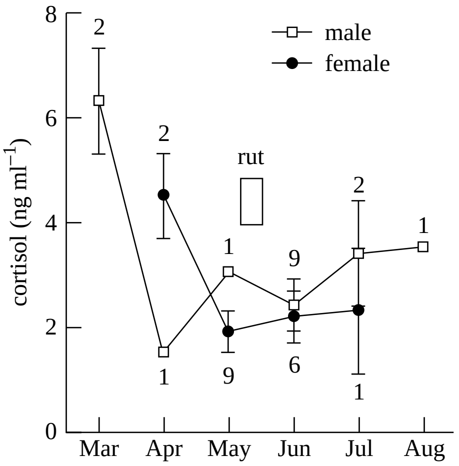

Figure 2. Change in mean (±s.e.m.) plasma cortisol levels for male (open squares) and female (filled circles) *D. hallucatus* between March and August. Values above and below the points indicate number of individuals.

indication of the timing of maximum male mobility, during female oestrus, and the subsequent die off. Thus, few *D. hallucatus* males survived longer than two weeks after mating.

(b) *Physiological and physical changes associated with die off*

Although the proximate causes of death for most males were motor vehicles or predators, physiological debilitation is probably the ultimate cause. As for other dasyurids, the occasional discovery of uninjured moribund males suggests that seasonal die off has a physiological basis.

Further physiological investigation was motivated by observations of the well-studied and closely related *Antechinus* and *Phascogale* (Lee & Cockburn 1985; McDonald *et al.* 1986; Bradley 1987, 1997). Symptoms observed repeatedly in these genera include a marked decline in body condition, anaemia, blooms of previously benign parasites, diseases that are symptomatic of immunosuppression, a suite of endocrine changes that cause elevated cortisol titres, ulceration and haemorrhage of the gastrointestinal wall, and complete spermatogenic failure before the onset of the breeding season.

We found evidence of both a decline in condition and immunosuppression. Male *D. hallucatus* reached their maximal body weight, tail circumference and testes' width at nine to ten months old (March/April). During and after the mating season, they lost body weight, their tail circumference narrowed (Oakwood 1997) and their scrotal width declined (Oakwood 2000). Males also exhibited fur loss, infestation with lice (*Boopia uncinata*) and formation of cutaneous lesions on the tail, rump and soles of the feet during the mating season. In June, a significantly greater number of males were observed to have lice infestations (85%) than in May (23%, $\chi^2 = 11.9$, d.f. $= 1$, $p < 0.001$). In contrast, only 8% of females carried lice in June ($n = 13$). A single male caught at another site at Kapalga in August 1992 was also infested. Lice were not observed on males or females at any other time of year (Oakwood & Spratt 2000).

However, we found no evidence of marked endocrinological changes or spermatogenic failure. The tubules of the testes of males after mating were still patent and contained spermatogenetic cells. Primary and secondary spermatocytes, spermatids and spermatozoa were recognizable in all three specimens. Even more surprisingly, male *D. hallucatus* cortisol levels were lowest at the beginning of the mating season and similar to those of females (figure 2), although the plasma-testosterone concentration in male *D. hallucatus* was significantly higher during the mating period in late May (1.7 ± 0.2 s.e. ng ml^{-1}) than during the pre-mating (0.6 ng ml^{-1}) and post-mating (0.7 ± 0.2 s.e. ng ml^{-1}) periods ($F_{2,6} = 5.5$, $p < 0.04$). As the mating season progressed, males became dramatically more anaemic (figure 3) but none of the 16 males examined in June–August showed any evidence of ulceration, suggesting that the anaemia was not caused by gastrointestinal haemorrhage.

4. DISCUSSION

Consistent with reports from smaller dasyurid species where male die off has been well documented, male *D. hallucatus* exhibit elevated androgens, weight loss, loss of fur and proliferation of parasites during the mating period.

However, there are some distinct differences. First, in *Antechinus agilis*, spermatogenesis fails before the annual rut (Kerr & Hedger 1983). In *D. hallucatus*, although there was regression of testicular size after the mating season, spermatogenic cells were still observed in June and August, suggesting that the testes are still patent and that males would be capable of reproducing in a second year if they survived that long (see also Taggart & Temple-Smith 1994).

Second, in the smaller species, free cortisol in males increases dramatically to a peak just before death while females show a decrease at that time, an effect that results in part from an increase in the concentration of corticosteroid-binding globulin in the plasma of pregnant females

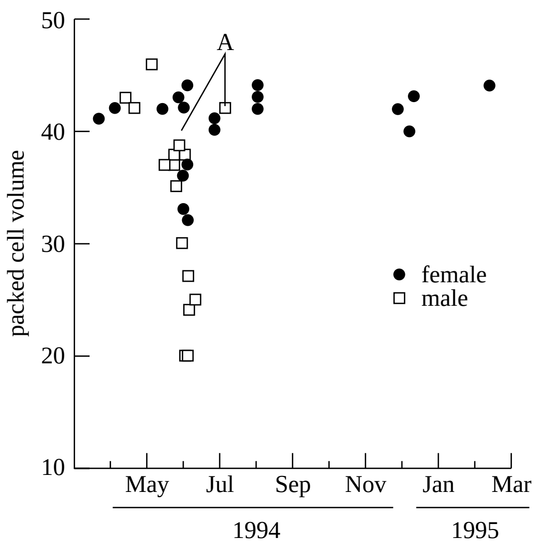

Figure 3. Intra-annual changes in the packed cell volume for male (open squares) and female (filled circles) *D. hallucatus*. One male (A), unusual in that he was re-trapped a month after the mating period (early July), increased his packed cell volume back to a normal value of 42% (from a relatively high 39% at the end of May); he had regrown some hair on his back and increased his body weight. He was not caught again.

(Bradley 1987). In contrast, male *D. hallucatus* showed no increase in cortisol levels as breeding commenced; nor did male levels differ from female levels. Schmitt *et al.* (1989) report similar results, finding no evidence for a change in free corticosteroid levels, total plasma cortisol or corticosteroid-binding globulins associated with reproduction. In both studies, there is a tendency for female cortisol levels to be higher than those of males during the rut.

The absence of spermatogenic failure is not entirely surprising as it is likely to be a consequence of the evolution of die off rather than part of the causative chain. Once die off has evolved to be obligate, as in *Antechinus* and *Phascogale*, there will be no selection for the maintenance of spermatogenesis beyond the rut.

However, elevated cortisol levels are thought to be a crucial causative component of die off. Smaller species such as *A. agilis* are forced to attract or pursue mates in winter, when food is least available, at considerable distances from their usual feeding range (Lee & Cockburn 1985; Lazenby-Cohen & Cockburn 1988, 1991). Males have inadequate fat supplies to sustain them throughout the rut so they store energy as protein and catabolize the tissue via gluconeogenesis, which is promoted by elevated cortisol levels. Although such a stress response is universal among mammals (and other vertebrates), elevated cortisol levels are normally corrected by negative feedback on adrenocorticotropin secretion. In *Antechinus* and *Phascogale* this normal negative feedback malfunctions in males, an effect that probably involves selective hippocampal damage (Bradley 2001). These species not only pay the penalty of a dramatic decline in body condition but in addition prolonged elevation of cortisol also leads to suppression of the immune and inflammatory responses, so the proximal cause of death is usually an outbreak of an otherwise benign disease (e.g. listeriosis) or gastrointestinal haemorrhage.

The generality of this interpretation is undermined by the absence of elevated cortisol levels during breeding in *D. hallucatus*, so a universal explanation for the mechanism of die off remains obscure. Certainly larger mammals such as male *D. hallucatus* should be able to store greater fat reserves than smaller dasyurids, both because of their larger body size and because they have specialized caudal fat storage. Furthermore, because of surface-to-volume considerations, thermal homeostasis in the larger species should be less challenged by adverse weather conditions. The precipitate decline in condition in this species therefore remains paradoxical. Cockburn (1997) has recently argued that post-reproductive senescence may be a universal feature in the two large clades of carnivorous marsupials, the Didelphidae and the Dasyuridae. In these families, male die off has evolved at least six times (Cockburn 1997; Armstrong *et al.* 1998). However, other manifestations of senescence, such as declines in female fertility and survival, appear to be ubiquitous, including in the well-studied *Didelphis*, which is also large. These results suggest that die off may be the result of an unexplained phylogenetic predisposition in these species towards post-reproductive senescence. According to this view, the use of elevated cortisol levels to facilitate the use of protein stores may be a secondary modification resulting from this tendency, rather than the adaptive cause of mammalian semelparity.

We thank the Commonwealth Scientific and Industrial Research Organization (CSIRO) for allowing the use of Kapalga Research Station as a base for fieldwork and Parks Australia North for granting permission to collect road kills. We also thank R. Booth for advice about blood sampling, R. Eager for field assistance, D. Pritchard for examining gut tissue for evidence of ulceration, P. Woolley for examining the testes and S. Barker for identifying the lice. This research was supported by a Commonwealth Postgraduate Research Award (M.O.), an Australian Nature Conservation Agency travel grant, CSIRO/Australian National University Collaborative grants, the M. A. Ingram Trust, an Ethel Mary Read Award and the Australian Geographic Society.

REFERENCES

Armstrong, L. A., Krajewski, C. & Westerman, M. 1998 Phylogeny of the dasyurid marsupial genus *Antechinus* based on cytochrome-*b*, 12S-rRNA, and protamine-P1 genes. *J. Mamm.* **79**, 1379–1389.

Bradley, A. J. 1987 Stress and mortality in the red-tailed phascogale, *Phascogale calura* (Marsupialia: Dasyuridae). *Gen. Comp. Endocrinol.* **67**, 85–100.

Bradley, A. J. 1990 Failure of glucocorticoid feedback during breeding in the male red-tailed phascogale *Phascogale calura* (Marsupialia: Dasyuridae). *J. Steroid Biochem.* **37**, 155–163.

Bradley, A. J. 1997 Reproduction and life history in the red-tailed phascogale, *Phascogale calura* (Marsupialia: Dasyuridae): the adaptive-stress senescence hypothesis. *J. Zool.* (*Lond.*) **241**, 739–755.

Bradley, A. J. 2001 Stress, hormones and mortality in small dasyurid marsupials. In *Predators with pouches: the biology of carnivorous marsupials* (ed. M. Jones, C. Dickman & M. Archer). Sydney, Australia: CSIRO Publishing. (In the press.)

Bradley, A. J., McDonald, I. R. & Lee, A. K. 1975 Corticosteroid binding globulin and mortality in a dasyurid marsupial. *J. Endocrinol.* **70**, 323–324.

Bradley, A. J., McDonald, I. R. & Lee, A. K. 1980 Stress and mortality in a small marsupial (*Antechinus stuartii* Macleay). *Gen. Comp. Endocrinol.* **40**, 188–200.

Charnov, E. L. 1993 *Life history invariants: some explorations of symmetry in evolutionary ecology.* Oxford University Press.

Cockburn, A. 1997 Living slow and dying young: senescence in marsupials. In *Marsupial biology: recent research, new perspectives* (ed. N. Saunders & L. Hinds), pp. 163–171. Sydney: University of New South Wales Press.

Cockburn, A. & Johnson, C. N. 1988 Patterns of growth. In *The developing marsupial: models for biomedical research* (ed. C. H. Tyndale-Biscoe & P. A. Janssens), pp. 28–40. Berlin: Springer-Verlag.

Dickman, C. R. & Braithwaite, R. W. 1992 Postmating mortality of males in the dasyurid marsupials, *Dasyurus* and *Parantechinus*. *J. Mamm.* **73**, 143–147.

Kerr, J. B. & Hedger, M. P. 1983 Spontaneous spermatogenic failure in the marsupial mouse *Antechinus stuartii* Macleay (Dasyuridae: Marsupialia). *Aust. J. Zool.* **31**, 445–466.

Lazenby-Cohen, K. A. & Cockburn, A. 1988 Lek promiscuity in a semelparous marsupial, *Antechinus stuartii* (Marsupialia: Dasyuridae)? *Behav. Ecol. Sociobiol.* **22**, 195–202.

Lazenby-Cohen, K. A. & Cockburn, A. 1991 Social and foraging components of the home range in *Antechinus stuartii* (Dasyuridae: Marsupialia). *Aust. J. Ecol.* **16**, 301–307.

Lee, A. K. & Cockburn, A. 1985 *Evolutionary ecology of marsupials.* Cambridge University Press.

McDonald, I. R., Lee, A. K., Than, K. A. & Martin, R. W. 1986 Failure of glucocorticoid feedback in males of a population of small marsupials (*Antechinus swainsonii*) during the period of mating. *J. Endocrinol.* **108**, 63–68.

Oakwood, M. 1997 The ecology of the northern quoll, *Dasyurus hallucatus.* PhD thesis, Australian National University, Canberra, Australia.

Oakwood, M. 2000 Reproduction and demography of the northern quoll, *Dasyurus hallucatus*, in the lowland savanna of northern Australia. *Aust. J. Zool.* **48**, 519–539.

Oakwood, M. & Spratt, D. M. 2000 Parasites of the northern quoll *Dasyurus hallucatus* (Marsupialia: Dasyuridae) in tropical savanna, Northern Territory. *Aust. J. Zool.* **48**, 79–90.

Russell, E. M. 1982 Patterns of parental care and parental investment in marsupials. *Biol. Rev.* **57**, 423–486.

Schmitt, L. H., Bradley, A. J., Kemper, C. M., Kitchener, D. J., Humphreys, D. F. & How, R. A. 1989 Ecology and physiology of the northern quoll, *Dasyurus hallucatus* (Marsupialia, Dasyuridae), at Mitchell Plateau, Kimberley, Western Australia. *J. Zool.* (*Lond.*) **217**, 539–558.

Taggart, D. A. & Temple-Smith, P. D. 1994 Comparative studies of epididymal morphology and sperm distribution in dasyurid marsupials during the breeding season. *J. Zool.* (*Lond.*) **232**, 365–381.

Zullinger, E. M., Ricklefs, R. E., Redford, K. H. & Mace, G. 1984 Fitting sigmoidal equations to mammalian growth curves. *J. Mamm.* **65**, 607–636.

Proc. R. Soc. Lond. B **268**, 407–411 (22 February 2001)

Semelparity in a large marsupial

Meri Oakwood, Adrian J. Bradley and Andrew Cockburn

In figure 2 (p. 409), the symbols on the lines of the graph were transposed. The shorter line with fewer data points represents males and the longer line with more data points represents females. The symbols in the key remain correct. The corrected figure is reproduced below together with its caption.

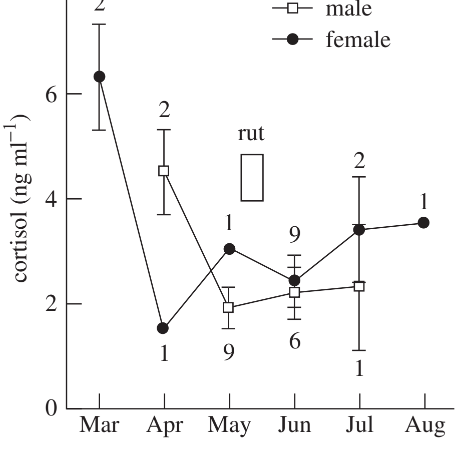

Figure 2. Change in mean (± s.e.m.) plasma cortisol levels for male (open squares) and female (filled circles) *D. hallucatus* between March and August. Values above and below the points indicate number of individuals.