SEASONAL BREEDING

IN THE EASTERN QUOLL *DASYURUS VIVERRINUS*

(MARSUPIALIA : DASYURIDAE)

by

SALLY LEE BRYANT B. Sc. (Hons).

A thesis presented for the Degree of Doctor of Philosophy,

Department of Zoology, University of Tasmania,

Hobart, Tasmania, Australia.

September, 1988.

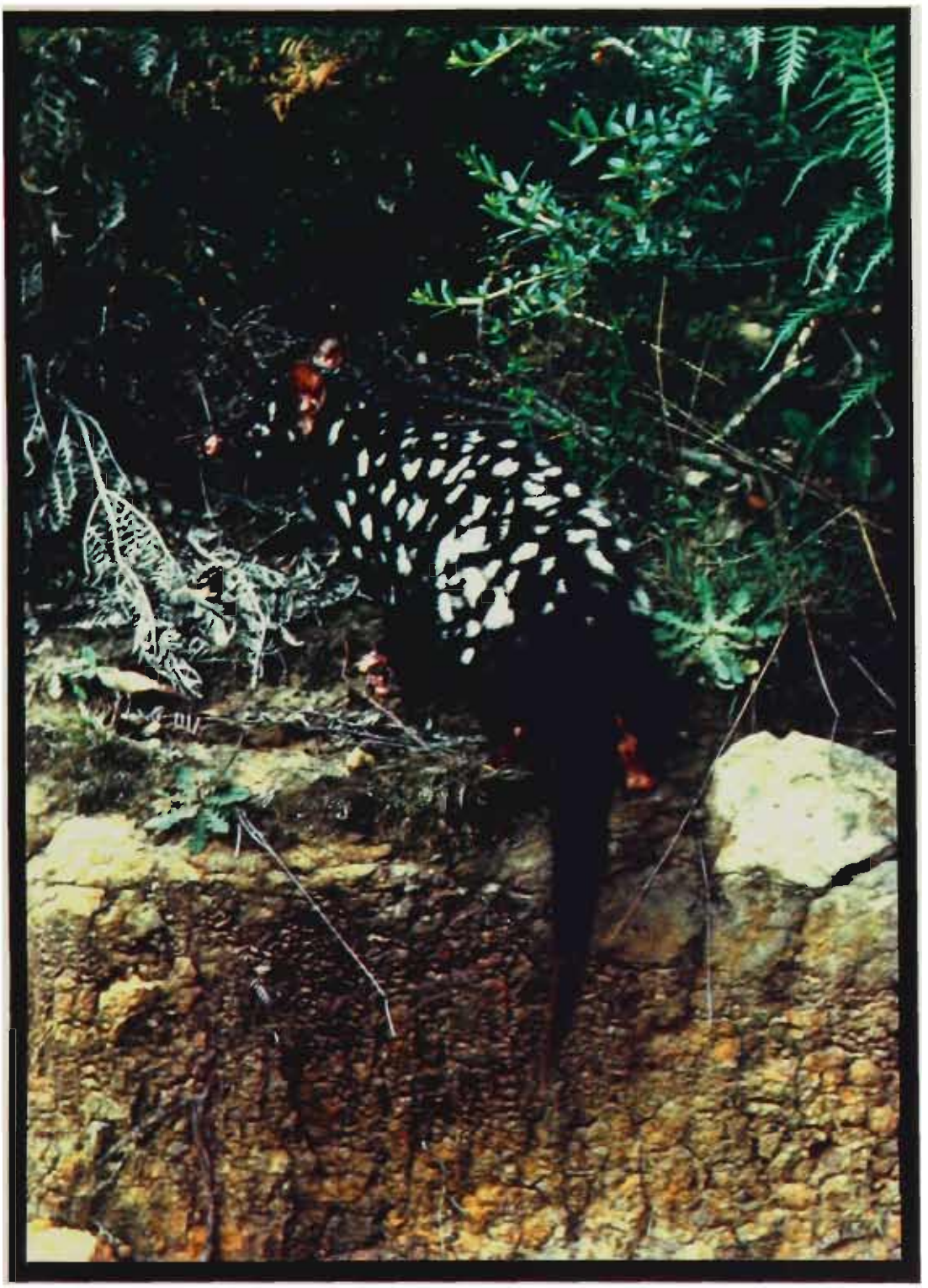

Plate 1a : A black Eastern quoll, *Dasyurus viverrinus.*

5. Access to, and copying of, thesis

The thesis copy lodged in the University Library shall be made available by the University for consultation but, for a period of two years after the thesis is lodged, it shall not be made available for loan or photocopying without the written consent of the author and in accordance with the laws of copyright.

After a thesis has been examined, the following authority will apply. Please complete your request, and sign below.

(i) I agree/~~do not agree~~ that the thesis may be made available for loan.

(ii) I agree/~~do not agree~~ that the thesis may be made available for photocopying.

(iii) I note that my consent is required only to cover tha two-year period following approval of my thesis for the award of my degree. After this, access to the Library copy will be subject only to any general restrictions laid down in Library regulations.

Signed: ..... Date: 30-09-88

Lodged in Morris Miller Central Library: 13 / 11 / 1989 from which date the two years embargo will apply.

SMED 12/86

DECLARATION

This thesis represents my own work and to the best of my knowledge contains no material published or written by another person except where specifically indicated.

Sally L Bryant

ABSTRACT

The Eastern quoll, *Dasyurus viverrinus,* is one of the larger members of the family Dasyuridae and is found only in Tasmania. The quoll has a short breeding period with mating occupying two to three weeks of every year. Males display seasonal cycles of body weight and testes size. Both parameters are maximal prior to breeding then decline during and after mating.

LH and testosterone concentrations fluctuate at basal levels for much of the year. A gradual rise in LH and testosterone occurs in April, two to three months before breeding. This probably functions to initiate spermatogenesis and to prepare the gonads and accessory glands for reproduction. Male quoll produce a peak in LH (mean 13.9 ng per ml) and testosterone (mean 5.0 ng per ml) during the mating period. This peak coincides with maximum number of animals in the area and is associated with an increase in activity and mobility of males at this time. The highest testosterone level occurs just prior to mating and declines during copulation. By the time the young are born, approximately 19 days after copulation, most males have basal levels of hormones.

Juvenile quoll increase in weight with age and have comparatively high levels of LH and testosterone when entering the population after weaning. These levels may be associated with the processes of maturation and also with agonistic behaviour encountered during dispersal.

LH, progesterone and prolactin secretion in the female quoll conform to the patterns shown by other marsupials. LH and progesterone levels are highest near the time of oestrus and ovulation while prolactin concentration increases throughout the lactation period. The endocrine cycles and pouch development of pregnant and non-pregnant females appear to be similar.

Experimental evidence suggests that photoperiod is the likely proximate cue regulating the breeding cycle of the male quoll. Males exposed to a long daylength increase in weight and have lower LH concentrations compared to control animals. Testosterone secretion and testes size appear unaffected by a change in photoperiod and may require either a longer exposure time or additional factors to influence these cycles.

Males in captivity generally have lower LH and testosterone levels compared to males in the wild. The hormonal profiles of captive male quoll are related to the degree of physical contact with the female. When males are housed with females, LH and testosterone levels are significantly higher than when males are housed near, or isolated from females. A cue from the female may be the stimulus initiating a peak in androgens in the male and therefore females may be responsible for the synchrony of the breeding cycle.

Cortisol levels are significantly lower in captive animals when physical contact is prevented. There is no evidence of an androgen dependent decrease in plasma CBG during breeding nor is there any increase in free cortisol associated with a decrease in MCBC. The breeding season did not appear to be a period of high stress nor was it characterised by major changes in plasma protein, albumin or triglyceride levels. This is consistent with the Eastern quoll being one of the long lived members of the Dasyuridae.

This project proposes that the seasonal breeding activity in the male quoll is broadly regulated by photoperiod. However, the androgen rise prior to mating is triggered by additional cues, mainly from the female. The synchrony of this rise is directly related to interspecific male aggression probably involving physical interaction. High androgen levels occur just before mating, enabling males to establish their dominance and position in the social hierarchy before pairing with females. This reproductive pattern enables the Eastern quoll to intensify its reproductive effort and the relatively short, sharp rise in androgens and free cortisol does not impose immediate constraints on the life expectancy of the animal. The role of the male throughout the breeding cycle therefore contributes much to the life history classification of this species.

ACKNOWLEDGMENTS

I am grateful to a number of people for their support and encouragement throughout this project.

My supervisor Dr. Randy Rose for his direction, criticism and friendship over many years in the zoology department. Thanks Randy.

Property owners Ken Rowe of Cradoc and Wally and Kathleen Alexander, 'Gum Flat', Ringarooma, for allowing me to trap quolls in truly beautiful places.

Department of Lands, Parks & Wildlife for their cooperation with permits and special transfer of animals.

To Lyn Hinds and Hugh Tyndale-Biscoe for making the facilities available at the CSIRO, Rangelands and for showing me every kindness and encouragement during my stay in Canberra.

To Terry Fletcher for his gift of iodinated LH and his friendship. And the INGRAM Trust for donating funds enabling me to complete the assay work.

Adrian Bradley for performing the corticosteroid assays and for his valuable discussions since joining this zoology department.

Eric Colquhoun and Roy Swain for their time and guidence with assay techniques and Ian McDonald, Bob Gemmell and Dan Irby for their helpful telephone advice with procedures.

Jim Kiss and Wayne Melrose for access to the facilities at the Public Health Department and Haematology Department, Royal Hobart Hospital.

Pitts Poultry for their endless supply of chickens and Rob Watkins of Chemistry, for his endless distillation of solvents.

To all the academic and technical staff particularly Barry Rumbold and Kit Williams who between them seem to know, have and supply everything.

My fellow students especially Dom, Rosie, Gwen and in particular David Pemberton, for their friendship and inspiration.

To my parents and extended Bryant and Alexander families who have always given me encouragement and support.

To Ian, Debbie and Madeleine Woodward with whom I shared a special part of this project.

Finally, thankyou to my husband Graeme and to Tim, who have waited patiently and wondered if life changes after a PhD. Yes and No !

CONTENTS

CHAPTER 1

INTRODUCTION

The family Dasyuridae is a diverse group of marsupials that occupies a wide range of environments and habitats. Although they vary in size from the pygmy planigales to the largest extant marsupial carnivore, the Tasmanian devil, fundamental biological features such as their predatory nocturnal behaviour, serology, dentition and morphology are similar. A collection of works titled 'Carnivorous Marsupials' (edited by M. Archer 1982) was the first comprehensive account of many life history aspects of the Dasyuridae. Two more recent reviews have discussed their breeding biology in comparison to other marsupial families (Lee & Cockburn 1985; Tyndale-Biscoe & Renfree 1987). While these works represent a significant contribution to our understanding of the Dasyuridae, the authors have highlighted the need for further investigations, especially in the area of reproduction and particularly with regard to the role of the male in the breeding cycle.

This study examines the pattern of reproduction in the marsupial Eastern quoll, *Dasyurus viverrinus* (Shaw 1800), with attention focused on the male of the species. The aim of the project is to describe the endocrinological and physiological changes associated with the breeding cycle and the investigate some of the mechanisms which influence reproductive activity.

1.1 REPRODUCTIVE PHYSIOLOGY OF THE MALE MARSUPIAL

The anatomy and physiology of the male reproductive glands are essentially the same for all marsupials (Biggers 1966; Waring *et al.* 1966; Setchell 1977; Temple-Smith 1984; reviewed by Tyndale-Biscoe & Renfree 1987). Most American and Australian marsupials have paired testes and epididymides carried externally within a prepenile scrotal sac. The testes join the abdomen via a stalk (or peduncle) which contains a cremaster muscle ensheathing two spermatic cords (Rodger & Hughes 1973). The blood vessels within the cord are arranged in a unique rete mirabile (Barnett & Brazenor 1958; Heddle & Guiler 1970; Temple-Smith 1984; Bryant & Rose 1985) which,

like its eutherian equivalent (the pampiniform plexus) functions primarily to traffic blood and secretions to and from the testes.

The anatomy and histology of the marsupial epididymides and testes and the process of spermatogenesis are similar to those of eutherian mammals (Greenwood 1923; Setchell & Carrick 1973; Setchell 1977; Tyndale-Biscoe & Renfree 1987). Differences occur among the marsupials in relation to spermatozoan ultrastructure, epididymal development, testicular vasculature and seminiferous tubule physiology. Some of these features have been used as phylogenetic and taxonomic characteristics to identify closely related species (Hughes 1965, 1982; Woolley 1975; Harding *et al.* 1977, Harding *et al.* 1982). Dasyurids are unique when compared to other marsupials because their testes have relatively few but large seminiferous tubules that produce very large sperm (Woolley 1975; Setchell 1977; Harding *et al.* 1982).

The marsupial accessory organs include a prostate and paired Cowper's glands but no seminal vesicles or coagulating (ampullary) glands (Rodger & Hughes 1973). The prostate is a complex organ divisible into three discrete regions (anterior, central, posterior), each of which performs a function similar to the separate eutherian glands (Tyndale-Biscoe & Renfree 1987). The accessory organs of the male Eastern quoll comprise a single elongated prostate and three pairs of Cowper's glands (Fletcher 1977).

A detailed account of the microscopic structure of the penis, testes, accessory glands and sperm of male Eastern quoll has found them to be basically similar to other dasyurid and marsupial species (Woolley 1975; Fletcher 1977, 1985; Hughes 1982). Ullmann (1984) described the differentiation of the testes of the quoll from day 3 to 2.5 months post-partum and Hill and Hill (1955) have shown that the testes descend into the scrotum at between 15 and 19 days of age.

1.1.1 Endocrine System

Steroidogenesis and the endocrine secretion of hormones in male mammals are coordinated and regulated by the hypothalamus (Turner & Bagnara 1976). The hypothalamus can respond to stimuli from the central nervous system or hormonal feedback, by releasing gonadotrophin-releasing hormone (Gn-RH). The releasing hormone acts on the anterior pituitary gland via the portal system causing it to synthesise and secrete FSH (follicle stimulating hormone or interstitial cell stimulating hormone) and LH

(luteinizing hormone). These hormones in turn regulate spermatogenesis by acting on the interstitial tissue (or Leydig cells) and the Sertoli cells within the seminiferous tubules of the testes. In most mammals FSH stimulates the formation and biosynthesis of an androgen binding protein (ABP) and a peptide, inhibin, from the Sertoli cells (Turner & Bagnara 1976). FSH, in conjunction with LH, induces spermatogenesis during puberty (de Kretser 1984). LH maintains spermatogenesis by acting on the plasma membrane of the Leydig cells to stimulate testosterone secretion (Short 1975; de Kretser 1984). Testosterone is secreted into the interstitial fluid and is either transported to the efferent duct of the epididymis, channelled into venous capillary blood or taken up into the seminiferous tubules by the binding activity of the Sertoli cells. In most mammals, testosterone and inhibin provide the negative feedback to the anterior pituitary which ultimately modulates the release of LH and FSH (Ganong 1965; Catling & Sutherland 1980; Evans *et al.* 1980; deKretser 1984).

Information concerning the identification and function of male marsupial (and monotreme) steroids including pituitary, pancreatic, thyroid and pineal hormones, is fragmentary but has been reviewed by a number of authors (Carrick & Cox 1977; McDonald & Waring 1979; Tyndale-Biscoe & Renfree 1987). Plasma testosterone and 5α-dihydrotestosterone are produced in the testes of many Australian marsupials and secreted at a rate comparable with some eutherian species (Carrick & Cox 1973, 1977; Setchell 1974; Sernia 1978; McDonald *et al.* 1981; Curlewis & Stone 1985a). Androgen levels have been measured in a few dasyurids, namely *Antechinus stuartii* (Bradley *et al.* 1980; Kerr & Hedger 1983; Scott 1986), *A. swainsonii* (McDonald *et al.* 1981), *A. minimus* (Wilson & Bourne 1984), *Dasyuroides byrnei* (Fletcher 1983), *Sminthopsis crassicaudata* (McDonald *et al.* 1981), *Phascogale calura* (Bradley 1987) and *Dasyurus hallucatus* (Schmitt *et al.* 1988). Comparative results are available for other marsupial species such as *Isoodon macrourus* (Gemmell *et al.* 1985), *Trichosurus vulpecula* (Carrick & Cox 1977; Cook *et al.* 1978; Allen & Bradshaw 1980; Curlewis & Stone 1985a), *Didelphis virginiana* (Harder & Fleming 1986) and several *Macropodinae* species (Lincoln 1978; Catling & Sutherland 1980; Inns 1982).

LH and testosterone are the two prime hormones reflecting reproductive activity in the male mammal and were monitored throughout

this study as the major indicators of reproductive activity of the Eastern quoll. The radioimmunoassay techniques (RIA) developed to measure these hormones are provided in Chapter 3 and their precision highlights the sensitivity required to detect the low concentrations which at times circulate in marsupials.

Marsupials, like most mammals, have steroids bound to macromolecules, usually in the form of albumin, corticosteroid-binding globulin (CBG) or sex hormone binding globulin (SHBG) (Sernia 1978; Gower 1979; Bradley 1982). The Dasyuridae is unusual in that it is one of the few mammalian families that lack SHBG. Instead steroids such as testosterone are bound preferentially to albumin with a high capacity and low affinity (Sernia *et al.* 1979; Bradley 1982). This means that at times, such as during the breeding period, the body tissues may be exposed to high concentrations of free steroid and this manifests itself as aggressive behaviour during mating (Lee *et al.* 1977; Lee & Cockburn 1985).

In addition to seasonal and behavioural changes in secretion, some hormones also display an episodic or diurnal cycle of release. These intrinsic fluctuations have been well documented in many species (Lincoln & Kay 1979; Balthazart *et al.* 1981; McLeod & Craigon 1985; Perret 1985) especially in the larger animals such as the Soay ram and other breeds of sheep (Lincoln & Short 1980; Ortavant *et al.* 1982; Pelletier *et al.* 1982; Almeida & Lincoln 1984; Pelletier 1986). The diurnal episode of testosterone, LH, melatonin, cortisol and prolactin have been investigated in a few marsupial species for example the Brushtail possum, *Trichosurus vulpecula*, (Than & McDonald 1973; Allen & Bradshaw 1980; Curlewis & Stone 1985a), Tammar wallaby, *Macropus eugenii*, (Harder *et al.* 1985, Hinds & Tyndale-Biscoe 1985; McConnell & Tyndale-Biscoe 1985) and Koala, *Phascolarctos cinereus* (McDonald 1986). During the breeding season, pulses of plasma testosterone in the male koala can vary between from 0.5 and 8.0 ng per ml over a period of hours (McDonald 1986). This represents a considerable fluctuation and is important when considering the extent to which other factors may influence hormonal secretion.

This project determined whether there were fluctuations in LH and testosterone secretion over a 24 hour period in the male quoll and also whether LH secretion was controlled by a negative feedback mechanism involving

testosterone. Such mechanisms could then be accounted for when interpreting the influence of external factors on the breeding cycle.

1.2 SEASONAL BREEDING

Seasonal breeding appears to be the general rule for the majority of mammals and birds (Sadlier 1969; Farner & Follett 1979; Lincoln & Short 1980; Temple-Smith 1984; Tyndale-Biscoe 1984) and marsupials living in temperate climates are no exception. The strategy of breeding seasonally is regarded as a concentration of reproductive effort enabling the young to be born at the most favourable period of the year. In marsupials this pattern is complicated somewhat because the young spend a portion of their life developing in the female's pouch, with some often spending additional time in a nest or den. The benefit gained by marsupials breeding seasonally is achieved only if the young are weaned at the most favourable time of the year.

Many female macropodid marsupials such as the Tammar, *Macropus eugenii,* and Bennett's wallaby, *M. rufogriseus*, combine embryonic diapause with seasonal breeding (Tyndale-Biscoe *et al.* 1986). Most female wallabies are polyoestrous and monovular, with a gestation period occupying almost the entire oestrous cycle (Tyndale-Biscoe & Renfree 1987). The breeding pattern in these species is controlled by two periods of quiescence, one caused through lactation by the suckling young and the second by seasonal factors possibly involving photoperiod.

In the few dasyurids investigated, most have either short or extended periods of breeding. The majority of species mature and breed in their first year with only a few males surviving to reproduce again in a second season. The females can be monoestrus, such as many *Antechinus* and *Phascogale* species, or they can be polyoestrus or a combination of both. The Eastern quoll is an example of this latter type. Oestrous cycles range from 24 days in *Sminthopsis murina* to 60 days in *Dasyuroides byrnei* with gestation periods ranging from 13 to 31 days respectively (Tyndale-Biscoe & Renfree 1987). A few species such as *Antechinus apicalia* and the Ningbing antechinus have longer gestation periods, estimated to be between 44 to 53 days, that possibly include a slow or arrested development of the embryo

(Woolley 1988).

Lee *et al.* (1982) recognised six distinct life history strategies among the Dasyuridae based on these reproductive parameters. The majority of species were placed in the first two categories. These included most *Antechinus* and also two larger members, the Northern and Western quoll, *Dasyurus hallucatus* and *D. geoffroii*. These species have a restricted breeding season; the females are monoestrus and very few males live past their first breeding season.

The Eastern quoll was placed in the third category together with the Tiger quoll, *Dasyurus maculatus,* Crested-tailed marsupial mouse, *Dasycercus cristicauda,* and probably the Tasmanian devil, *Sarcophilus harrisii.* These generally are the larger sexually dimorphic dasyurids that breed seasonally and give birth in winter. The males breed for more than one season and the females are polyoestrus and can undergo a second oestrous cycle if the young are lost early in lactation. The remaining three categories defined by Lee *et al.* (1982) contain the smaller sized dasyurids that either have extended periods of breeding or breed only when conditions are favourable. All the females are polyoestrus and some males and females survive to breed in a second year.

Even within one marsupial family such as the Dasyuridae, there is a diverse range of breeding strategies. It is not suprising then that the reproductive system of the male shows an equally diverse range of responses to the breeding season. The most commonly used indicators of male reproductive activity include changes in body weight and testes size, testicular histology, spermatogenesis, size of the accessory glands and levels of androgens. The Red deer, *Cervus elaphus,* for example, is a seasonally breeding animal that experiences complete testicular regression and spermatogenic arrest during the spring and early summer months. The male resumes spermatogenesis and maximal androgen secretion in time for the autumn rut. The female deer potentially is fertile for about six months of the year but the behavioural phenomenon of the 'rut' confines breeding to a few weeks in duration (Lincoln 1985).

Similar variability exists among the marsupials. In the Tammar wallaby and the Brushtail possum, spermatogenesis continues throughout the year with the male showing little or no seasonal change in testes and epididymis weight or seminiferous tubule diameter (Gilmore 1969; Hearn 1975;

Inns 1982; Gemmel *et al.* 1986). During the breeding season the male tammar undergoes a significant increase in prostate and Cowper's glands weight and an increase in peripheral testosterone and LH (Catling & Sutherland 1980; Inns 1982). The male possum also experiences a rise in plasma androgens and body weight (Curlewis & Stone 1985a; Gemmell *et al.* 1986) and a four to five fold increase in prostate weight during the breeding season (Gilmore 1969; McFarlane *et al.* 1986a). The increase in prostate weight in both species has been associated with a change in receptor concentration in the prostate coupled with elevated androgen levels (Cook *et al.* 1978; Inns 1982; Curlewis & Stone 1985b).

Most male Dasyuridae show dramatic changes in scrotal dimensions over the year. Testes attain their maximum size shortly before mating and then regress and in some species are aspermic by the completion of breeding (Woolley 1966, 1988; Inns 1976; Begg 1981a,b; Calaby & Taylor 1981; Cuttle 1982; Woolley & Ahern 1983; Read 1984; Wilson & Bourne 1984; Kitchener *et al.* 1986). *A. minimus* and *A. stuartii* show dramatic changes in testes, prostate gland, epididymis and Cowper's gland weight throughout the year. They also produce significant rises in plasma androgens during the three month period prior to mating (Woolley 1975; Kerr & Hedger 1983; Wilson & Bourne 1984). Similarly, the increase in body weight, scrotal size, energy consumption and metabolic activity in the Yellow-footed antechinus, *A. flavipes* has been positively correlated with the breeding cycle (Inns 1976).

In this study a population of Eastern quoll was monitored over consecutive breeding seasons in the wild to correlate hormonal activity with the changes in body weight and scrotal size. This work enables events such as mating and the emergence of weaned young to be followed and it also provides information on the natural population structure of the species. The data collected on social structure and biology from wild animals provides the basis for interpreting the information obtained from subsequent investigations using animals maintained in captivity. The results of this study are provided in Chapter 4.

1.3 FACTORS AFFECTING SEASONAL BREEDING

1.3.1 Photoperiod

Photoperiod or daylength is one proximate cue by which many seasonally breeding animals living in temperate regions regulate their reproductive cycle. Light acts as a signal either to synchronise an endogenous biological rhythm or to activate the neuroendocrine-gonadal axis in preparation for spermatogenesis, ovulation and the stimulation of secondary sex organs (Turek & Campbell 1979; Bronson 1985). Photoperiod is more than just an alternation of light and dark periods from day to night. There is a array of gradual increases and decreases in light over the year including sharper alterations in light during the equinoxes and solstices.

Clarke (1981) has summarised the way in which photoperiod exerts its effect on mammals as follows : environmental stimuli cause an alteration in the release of gonadotrophins and prolactin associated with the non-breeding season through (1) a change in the output of releasing and inhibiting factors by the hypothalamic neurones; (2) a change in the signal arising from the gonads such as a decrease in gonadal hormones; (3) a change in the response of the hypothalamo-hypophysial system to the gonadal signal, enhancing the effects in (1) above. The relationship between body function and the rate of change in daylength in most animals appears to be mediated by the secretion of melatonin from the pineal gland (Turek & Campbell 1979; Clarke 1981).

A great deal of experimental work on the effects of modifying daylength has been conducted on birds and on the larger mammals such as the Soay and Ile-de-France rams and various breeds of deer (Sadlier 1969; Lincoln & Short 1980; Lincoln 1985). Many animals such as hamsters, voles, ferrets and most temperate zone birds require long days to induce reproductive activity. Others, such as sheep, deer and mink are sensitive to some component of short days (Farner & Follet 1979; Turek & Campbell 1979). The testes size and the hormone levels of Soay rams are maximal in autumn during decreased daylength and regress in spring when daylength is increasing (Lincoln & Short 1980). Rams can be made to breed and rut more or less frequently per year by exposing them to altered day light cycles. Lincoln and Short (1980) have shown that the prerequisite for breeding is an abrupt change from long to short days which induces gonadotrophic secretion and

full sexual redevelopment. Rams exposed to a prolonged or unchanged photoperiod become photorefractive and discontinue breeding.

As the majority of marsupials are seasonal breeders (Tyndale-Biscoe 1984), photoperiod is a likely proximate factor controlling breeding activity. It is therefore interesting to speculate on the mechanisms through which photoperiod exerts its effect.

Most marsupials retain their young within a pouch after birth and have direct control over the growth and emergence of the young via lactation. As mentioned, the most critical period of reproduction for marsupials is not birth but the time of emergence of young from the pouch or den. Species with a long pouch life such as kangaroos and the Koala, or species such as the Eastern quoll and Tasmanian devil that have a relatively short pouch life but a lengthy den period, must respond to a feature that is present before conditions become suitable for rearing young. Photoperiod may be the most likely proximate factor controlling the cycle of breeding activity as it is a ratio fixed by latitude that occurs at the same time in every year, independent of other conditions.

In species with a short gestation period and pouch life, such as the bandicoots, a combination of factors such as temperature, food resources and photoperiod may combine to influence breeding activity. In some bandicoots the rate of change of minimum temperature and daylength is thought to be associated with the number of births (Barnes & Gemmell 1984). Similarly, sexual activity in the Australian bush rat,*Rattus fuscipes,* is influenced by a combination of photoperiod, temperature and food quality (Irby *et al.* 1984).

The role of photoperiod has been investigated in only a few species of marsupial. The female Tammar wallaby, *Macropus eugenii,* can respond within six days to an alteration in photoperiod by producing endocrine changes that cause a reactivation of the diapausing blastocyst (Sadlier & Tyndale-Biscoe 1977; Hinds & den Ottolander 1983; McConnell & Tyndale-Biscoe 1985). Tyndale-Biscoe (1980) suggested that it is the lunar phases, the summer and winter solstice and the autumn and vernal equinoxes that regulate the timing of breeding in this species. In the family Dasyuridae the effect of photoperiod has been investigated in the Fat-tailed dunnart, *Sminthopsis crassicaudata*, the Kultarr, *Antechinomys laniger* and several species of *Antechinus* (Godfrey 1969; Smith *et al.* 1978; Valente 1984; Dickman 1985, Dickman *et al.* 1987; McAllan & Dickman 1986; Scott 1986). The dunnart responds to a change

in daylength with a change in birth rate (Godfrey 1969; Smith *et al.* 1978) although the general timing of breeding in this species is also related to food supply (Morton 1978). Male and female *A. laniger* and *A. stuartii* have also been shown to respond to photoperiod cues although the responses are sometimes atypical and related to social conditions and olfactory communication (Dickman 1982; Valente 1984; Scott 1986).

Calaby and Taylor (1981) suggested that the reproductive cycle of two tropical dwelling dasyurids, *Antechinus bilarni* and *A. bellus,* were probably regulated by factors other than photoperiod. In their study the seasonal change in daylength was found to vary by as little as one hour over the year with light shifts amounting to 20 seconds or less in any given day. The annual monsoonal rainfalls or maximum and minimum temperatures were suggested as the more likely environmental variables affecting the breeding cycle of these species.

The highly synchronised breeding season of the Eastern quoll occurs with predictable regularity in Tasmania. One experiment was conducted to test whether the reproductive activity of the male quoll was sensitive to a change in daylength and therefore whether daylength was a possible cue influencing the timing of the breeding season. The findings of this study are presented in Chapter 5.

1.3.2 Social Factors

While some species regulate their breeding activity using proximate photoperiod cues, the actual stimulus for mating may be triggered by additional factors. In two populations of *A. minimus maritimus* living in close proximity to each other, the timing of the breeding season differed by up to one month in some years (Wilson & Bourne 1984). The lag was attributed to the different rainfall pattern at the two sites which controlled the time of the spring food supply. *Antechinus stuartii* and *A. swainsonii* living sympatrically show a difference of one to four months in the timing of breeding between populations at high and low altitudes (Dickman 1982). A difference of three to four weeks in the timing of breeding in *Dasyurus hallucatus* has been reported to occur between inland and coastal populations (Schmitt *et al.* 1988). Larger mammals such as the African elephant, *Loxodonta africana*, show a seasonal peak in breeding that is related closely to rainfall. This ensures that

the calves are born at the beginning of the rains when a new flush of grass and leaves appear (Hall-Martin 1987). These differences cannot be accounted for by photoperiod change and suggest that local factors involving the microclimate or relating to social structure of the species can also influence the timing of breeding.

Social factors, including pheromones, are known to play a significant part in the regulation of reproduction in many eutherian species (Whitten 1956; Epple 1973; Mykytowycz 1973; Hall-Martin 1987 among others). Odours are considered important in regulating the degree of aggressive interactions during the breeding period and also in territory marking and establishing dominance and hierarchies within the social group. Many marsupials have specific sites on their body that are used primarily for olfactory transmission and reception. For example the Brushtail and Ringtail possum and the Sugar glider have obvious and well developed scent glands that are important in marking territories and in advertising sexual receptiveness during the breeding period (Biggins 1984). Chemical communication is thought to play an important role in the social structure and breeding activity of these species (Biggins 1984; Kratzing 1984; Russell 1984)

Although most species of Dasyuridae are nocturnal and solitary they still display a wide repertoire of auditory and olfactory responses (Croft 1982; Russell 1984). Dasyurids mark the substrate using techniques such as cloacal dragging, urine dripping, rubbing the chin and sternum, saliva deposition as well as grooming behaviour to distribute scent over the body (Ewer 1968; Aslin 1980; Buchmann & Guiler 1977; Eisenberg & Golani 1977; Croft 1982; Godsell 1983). Mouth sniffing, naso-nasal sniffing and touching and cloacal sniffing are other frequent behaviours which help to redistribute scents between individuals (Croft 1982).

The interest in pheromones and the role of olfaction in marsupial reproduction have witnessed a rapid expansion since the refinement of RIA techniques has enabled endocrinological studies to complement olfactory work. Although the specific role of olfaction and chemical communication has not been investigated in depth in many marsupials, males in species such as the Tammar wallaby (Catling & Sutherland 1980; Inns 1982; McDougall 1985), the Kowari (Fletcher 1983) and the Brown antechinus (Scott 1986) have been shown to respond to the presence of females in oestrus, with a marked

elevation in plasma testosterone levels. The androgen rise in the male tammar is thought to be linked to a pheromone associated with an increase in progesterone levels in the female (McConnell *et al.* 1984). Often these responses can be induced merely by presenting the male with bedding containing the droppings or urine from the female.

Some male mice produce a testosterone controlled odour that elicits aggression in other males with the dominant individual producing more of the pheromone than subordinates (Archer 1979a). This dominance pattern is also true of other eutherians including Marmoset monkeys and the African elephant, *Loxodonta africana* (Epple 1973; Dunbar 1985; Hall-Martin 1987). In addition, the role of stimuli such as physical contact may act in conjunction with pheromones to increase the response to mate. In the vole, *Microtus agrestis,* for example, the stimulus to breed is provided by a pheromone from the sexually mature male but the mating response is increased and hightened by tactile contact with the female (Spears & Clarke 1986). Similarly, an absence of breeding in captive wild rabbits, *Orytctolagus cuniculus,* can be due to a lack of libido in the male resulting from inadequate interplay of visual, tactile, acoustic and olfactory stimulation (Mykytowycz 1973).

This study conducted a number of experiments to determine whether social factors influence the reproductive activity of the male Eastern quoll. Social factors such as physical contact and proximity to the female were examined and their significance related to the natural pattern of reproduction in the wild. These experiments are described in Chapter 6.

1.3.3 Stress Factors

In addition to environmental and social factors, stressors (i.e. stress producing agents) may adversly affect reproductive activity. 'Stress' is a broad term used to describe a wide variety of reactions displayed by animals responding to unfavourable stimuli. Selye (1946) viewed stress as a specific syndrome of adaptive responses involving many systems of the body including the adrenal cortex, thymus and the digestive system (reviewed by Lee & McDonald 1985). These responses are controlled by the central and sympathetic nervous systems and may be expressed physiologically by, for example, a loss of body weight, an increased cardiac output, a contraction of the spleen (causing a change in the proportion of blood components), liver glycogenolysis and an inhibition of the immuno-inflamatory response (Ganong

1965; Archer 1979a; Kelley 1985; Roth 1985). In the male mammal stress may affect reproduction either directly by impairing spermatogenesis or indirectly via effects on the condition and well-being of the animal.

Selye (1946) drew particular attention to the increased activity of the pituitary gland and adrenal cortex in times of stress. When a mammal experiences unfavourable stimuli, generally there is some increase in ACTH (adrenocorticotrophic hormone) secretion and increase in glucocorticoid levels, particularly cortisol and corticosterone (Ganong 1965; Turner & Bagnara 1976). Measuring corticosteroid concentrations, particularly free cortisol, provides a direct index of adrenal activity and to date is one of the most widely used parameters of an animal's response to stress (Ballard 1979; Roth 1985). The hormonal systems most affected by stress include the pituitary-adrenal and pituitary-gonadal systems and catecholamines (Levine 1985).

An increase in corticosteroids such as free cortisol, however, does not necessarily imply that an animal is suffering from stress. Stress must be considered in relation to the animal's ability to maintain its internal milieu, the metabolic clearance rate of cortisol and a series of other complex endocrine and physiological changes (Barnett 1973; Moberg 1985a). One consequence of elevated corticosteroid is an impairement of immunosuppression (Kelley 1985; Roth 1985) and the blood profiles of many marsupials and monotremes have been shown to be naturally of low resistance to disease and latent infection (Parsons *et al.* 1971a,b; Arundel *et al.* 1977; Cheal *et al.* 1976; Wintrobe *et al.* 1981; Canfield & Whittington 1983; Melrose *et al.* 1987). Measurement of differential white cell counts, plasma glucose and protein concentration as well as a variety of other electrolytic and haematological parameters may provide a further, yet traditional, physiological assessment of the level of stress.

A good example of the importance of all these factors is found in the phenomenon of post mating mortality in some male *Antechinus* and *Phascogale* species. Male mortality results from a corticosteroid imbalance (Lee *et al.* 1977) that is coincidental with a significant rise in plasma androgens near mating time (Bradley *et al.* 1980; McDonald *et al.* 1981; Lee & McDonald 1985). The phenomenon manifests itself in 'stress related' changes in the adrenal cortex, spleen and digestive tract, with additional symptoms

such as anaemia and spermatogenic failure (Cheal *et al.* 1976; Inns 1976; Barker *et al.* 1978; Kerr & Hedger 1983). Changes in the blood parameters of *A. stuartii* show that most males are anaemic immediately prior to death and suffer from marked lymphopenias and neutrophilias characteristic of severe stress (Cheal *et al.* 1976; Barker *et al.* 1978; Bradley *et al.* 1980).

Concentrations of total and free cortisol were measured in the male Eastern quoll in this study. This information is considered to be particularly important in determining whether the male experiences any levels indicative of stress during the breeding period. In addition, some workers have also suggested that physical restraint or examination of any kind may interfere with the mechanisms regulating hormone secretion (Lincoln 1978; Curlewis & Stone 1985a; McDonald 1977; Gemmell *et al.* 1987; McFarlane & Carrick 1987). and have used the measurement of cortisol concentration to interpret the effects of capture, handling, surgical intervention and anaesthesia on marsupials (Weiss & Richards 1971; Than & McDonald 1973; Bradley *et al.* 1980; Bradley 1985). It was important that this study ascertained whether stress factors were influencing the behaviour of male quolls in captivity and consequently producing abnormal endocrine profiles. Blood corticosteroid levels (total cortisol and bound and free components) were analysed and the general haematological profile and blood-plasma chemistry of the species examined. The results of this investigation are presented in Chapter 7.

1.4 GENERAL DESCRIPTION OF THE EASTERN QUOLL

The Eastern quoll, *Dasyurus viverrinus*, is one of six species of the genus *Dasyurus*. Other members include the Northern quoll, *D. hallucatus,* Western quoll, *D. geoffroii*, Tiger quoll, *D. maculatus*, New Guinea quoll, *D. albopunctatus,* and the Bronze quoll, *D. spartacus.* Comparative life history information on the Australian species has been presented in a number of publications, the most recent being that by Lee and Cockburn (1985) and Tyndale-Biscoe and Renfree (1987).

The Eastern quoll is similar in size and appearance to a domestic cat but has spotted fur; hence the common name native cat or spotted native cat. The white spots cover the body but do not extend onto the tail, a feature

that distinguishes the Eastern from the Tiger quoll. Two colours occur within the species: fawn-to-grey with white spots, or black with white spots (Plate 1a,b).

The Eastern quoll was once distributed over much of southeastern Victoria and southern Queensland (Archer 1979b; Mahoney & Ride 1984) but today is found only on the island of Tasmania. It occupies a wide variety of habitats ranging from dry sclerophyll forest to rainforest (Green 1967; Fletcher 1977; Godsell 1983; Hocking & Guiler 1983; Taylor *et al.* 1985) and flourishes in areas of cultivation where there is a bush-pasture interface. At present the Eastern quoll is considered 'vulnerable to extinction' (Archer 1979b) and classified as a 'wholly protected species' by the Tasmanian Department of Lands, Parks and Wildlife.

Quolls are solitary animals although in some areas high densities occur which result in considerable home range overlap. Godsell (1983) categorised males as being either resident or transient in their movements with home ranges of up to 44 hectares. Females tended to be more stable with smaller home ranges of up to 35 hectares. The quoll is preyed upon by feral cats, dogs, owls and possibly snakes but predation levels are low and restricted largely to the young.

Eastern quoll hunt and scavenge for food with predatory capabilities matching other placental carnivores of similar size (Pellis & Nelson 1984). Insects such as the Southern army worm, *Persectania ewingii,* and Corbie grub, *Oncopera intricata,* form their staple diet but birds, small mammals, berry fruits and carrion are eaten when available (Green 1967; Blackhall 1980; Godsell 1983). The quoll is sexually dimorphic in size and weight. Mature males weigh between 1.1 and 1.9 kg. The females are smaller and less variable in weight, ranging from 0.7 to 1.1 kg (this study). A seasonal cycle of poor condition is characterised by a partial moult from July to August and a high ecto- and endo-parasite load, particularly ticks and larvae of the flea, *Uropsylla tasmanica* (Pearse 1981).

In the wild, Eastern quoll have a short breeding season and mating occurs between late May and June each year (Godsell 1983 and this study). The female gives birth approximately 20 days after mating and up to 6 young can be accomodated in the pouch (Plate 2a). The young remain in the pouch for two months after birth (Plate 2b) and then are left in a nest or den (Plate 3a).

Plate 1b : Brown female quoll, *Dasyurus viverrinus* with infant young soon to be weaned.

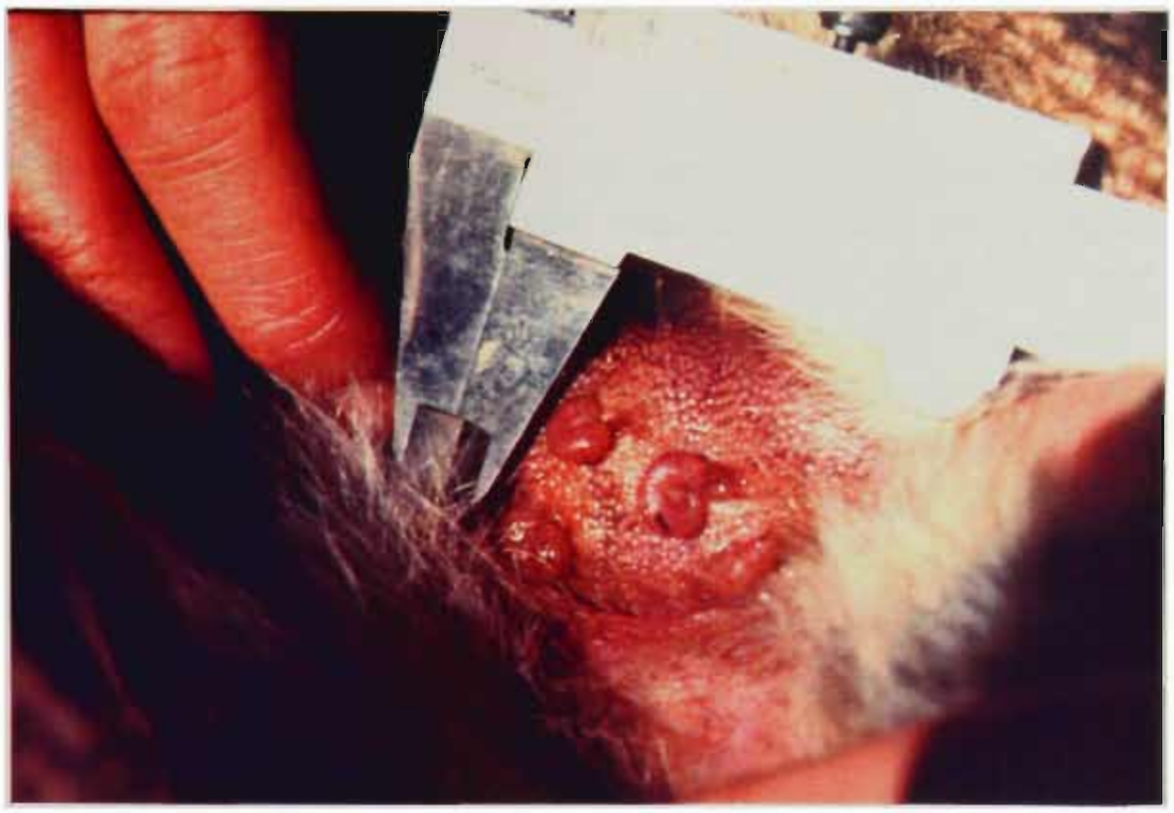

Plate 2a : One week old pouch young. Note the glandular interior of the pouch.

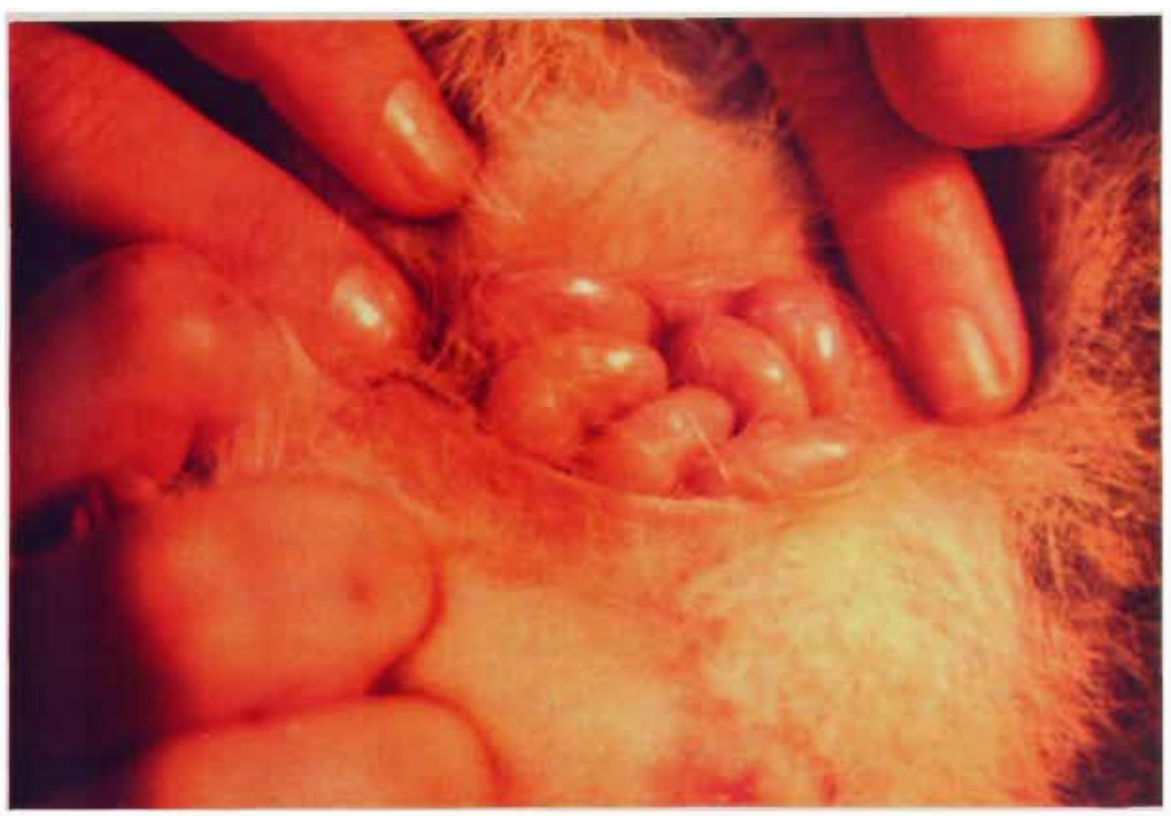

Plate 2b : Pouch young aged 30 days. The pouch has to be opened to expose the young.

The mother is then free to forage for food unhampered by rapidly growing young (Plate 3b). Denning coincides with the time the young relinquish the mother's teat and when significant changes in milk constituents and plasma prolactin concentrations occur (Hinds & Merchant 1986; Green *et al.* 1987). The young are weaned at approximately 5 to 6 months of age and during this time females may be captured with a pouch that typifies heavy lactational demands (Plate 4a). During the latter stages of weaning the young cling to their mother's back on hunting trips (Plate 4b) before emerging and dispersing into the population as juveniles. Male and female quoll reach sexual maturity in their first year and longevity in the wild is estimated to be 3 to 4 years (Godsell 1983 and this study).

Early observations on the female reproductive cycle and on embryology were made by Hill (1900) and Sandes (1903). Hill described the species as being monoestrus and, on the basis of histological changes in the uterine epithelium, estimated the oestrous cycle length to be 34 days with a gestation length of between 8 to 14 days (Hill & O'Donoghue 1913). The gestation length appeared short when compared to similar sized Dasyuridae such as the Tiger quoll (21 days, Fleay 1940; Conway 1988), Western quoll (16 to 23 days, Arnold & Sheild 1970), Kowari (28 to 36 days, Fletcher 1983) and the Tasmanian devil (21 to 31 days, reviewed by Hughes 1982). More recent studies have found the female to be polyoestrus rather than monoestrus with a gestation length of between 19 and 24 days (Fletcher 1977; Green & Eberhard 1983). The female quoll can return to oestrus and produce a second litter in the same season if the young are lost early in lactation. It has been observed that females in captivity undergo several oestrous cycles per year although this probably occurs infrequently in the wild (J. Merchant pers. comm. in Tyndale-Biscoe 1984). Figure 1.1 outlines the reproductive cycle of the female quoll in relation to the timing of the breeding season in Tasmania.

Godsell (1983) found the quoll to be a "...promiscuous breeder...and both sexes mated with more than one individual." Some other dasyurid females are known to copulate with a number of males and also are capable of storing sperm early in the mating period (Hill & O'Donoghue 1913; Woolley 1966; reviewed by Lee & Cockburn 1985). Hill and O'Donoghue (1913) reported that copulation in the quoll preceded ovulation by about 5 days and that spermatozoa could be stored at the entrance of the fallopian tubes for up to 14 days. More than 20 embryos were recovered from the uteri of some females

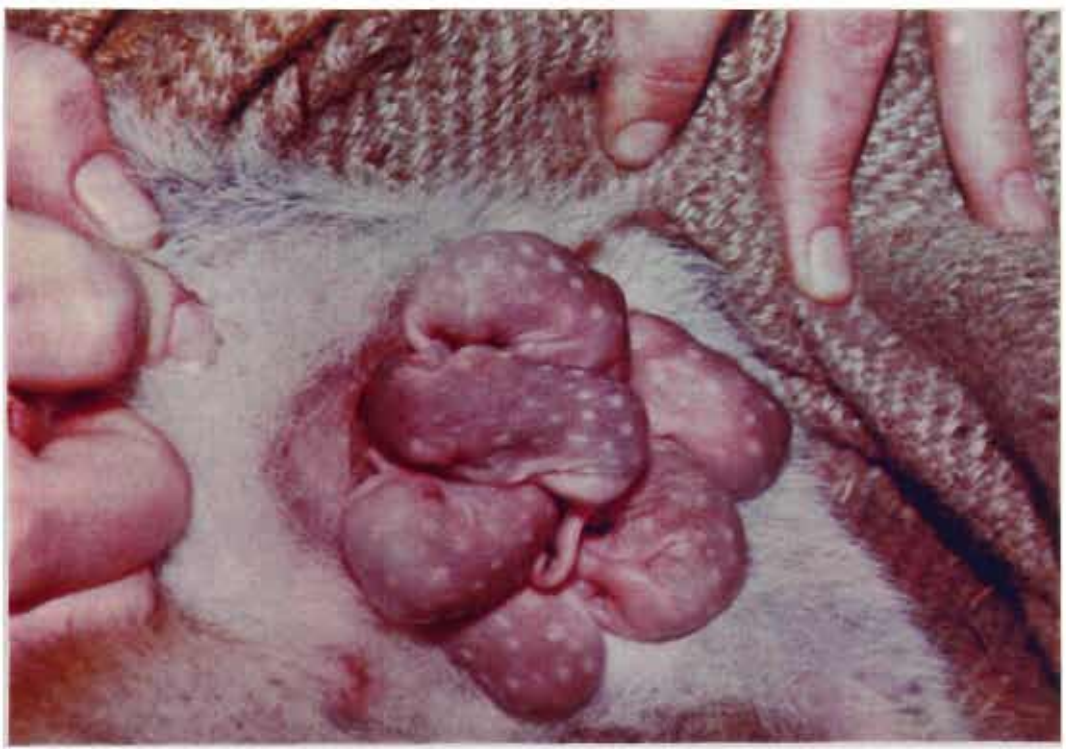

Plate 3a : Young at 60 days of age soon to be left in a nest in the den.

Plate 3b : 75 day old young soon to open their eyes. They are restless in the nest and actively suckle from different teats.

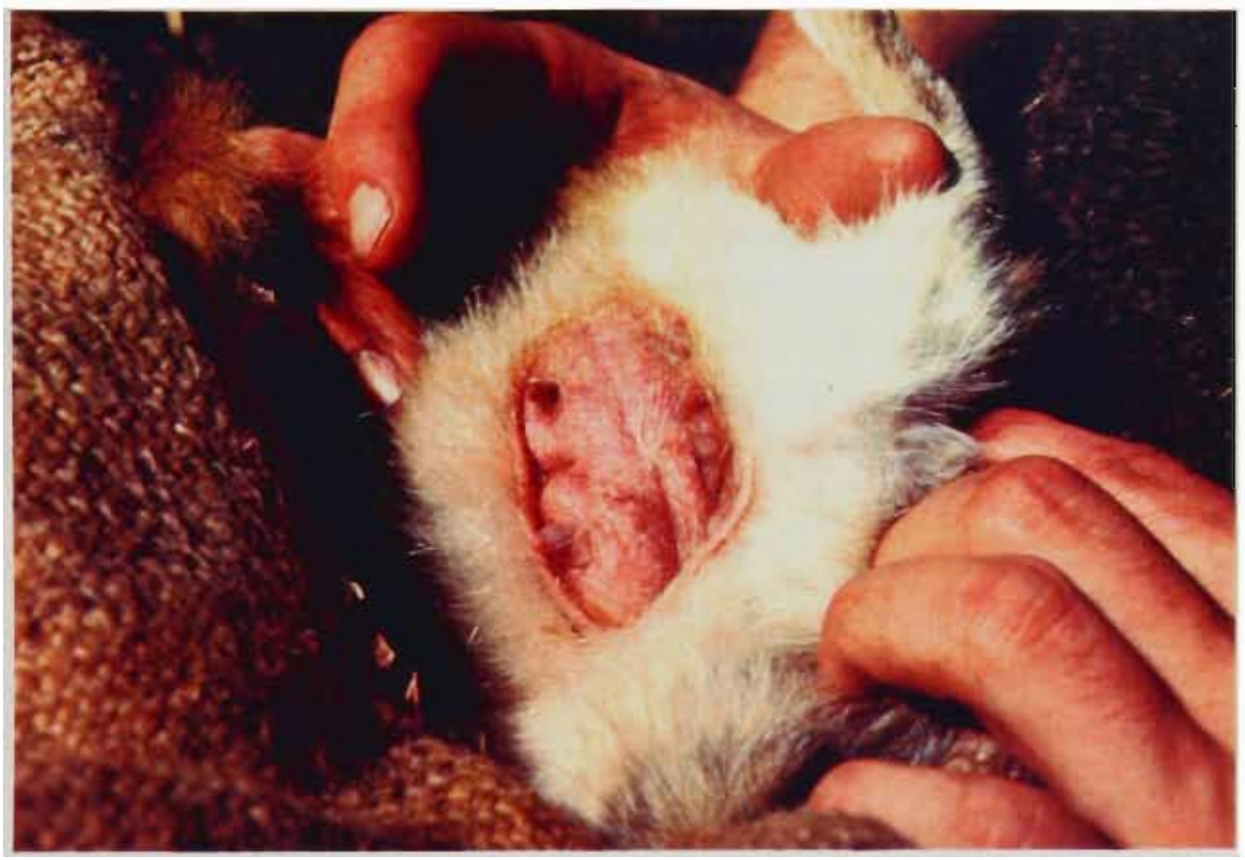

Plate 4a : Pouch typical of late lactation with enlarged mammary glands and elongated teats. Observed from November to January.

Plate 4b : Infants aged 5 1/2 months clinging to their mother's back. This occurs just prior to weaning.

and Hill and O'Donoghue suggested that multiple young were born but only those that could be accommodated on the teats survived. Reports of multiple births and of the number of eggs shed or embryos developed exceeding the number of teats have been made for other species (*Phascogale tapoatafa,* Cuttle 1982; *Sarcophilus harrisii,* Hughes 1982; *Antechinus swainsonii,* Williams & Williams 1982; *A. stuartii,* Selwood 1983; *Dasyurus hallucatus,* Schmitt *et al.* 1988).

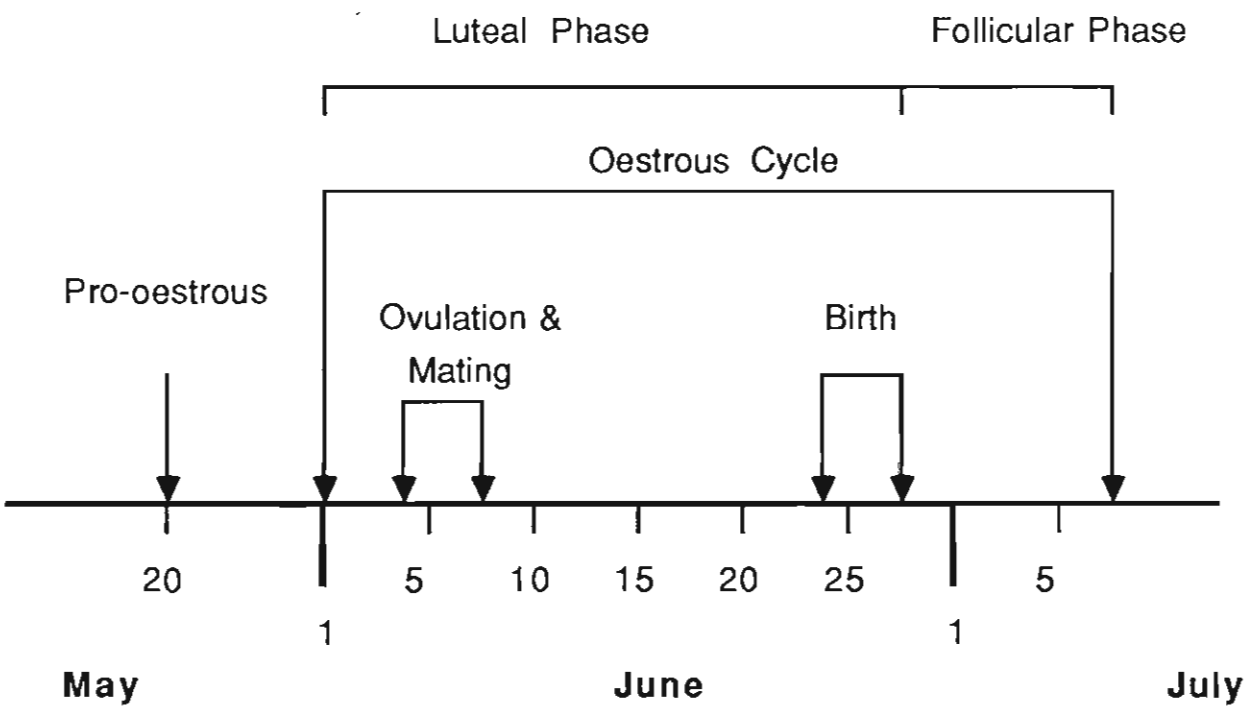

Fig. 1.1 Oestrous cycle of the female Eastern quoll *Dasyurus viverrinus* in the wild. The timing of breeding is highly synchronised each year in Tasmania although the dates may vary slightly. The female has a short pro-oestrous phase followed by a period of up to 5 days before spontaneous ovulation. The follicular phase of the oestrous cycle is suppressed by lactation. (Based on information from Hill & O'Donoghue 1913; Fletcher 1985; this study)

General observations on the species, with notes on the breeding activity and growth rates of the young, have been made by a number of workers (Fleay 1935; Nelson & Smith 1971; Weber 1975; Merchant *et al.* 1984). Green (1967) presented information on body weight, body size, diet and the reproductive status of Eastern quoll collected at sites in north-eastern Tasmania. He observed that invariably females had 6 teats and that during the breeding period they usually carried a full complement of young.

The only comprehensive study conducted on the Eastern quoll has been an examination of the population ecology by Godsell (1983). Godsell conducted a three year field program investigating the seasonal movements and ecology of the quoll. Fletcher (1977; 1985) investigated a number of reproductive parameters in quoll shortly after their capture and subsequent maintenance in captivity. He collected urine samples from males and described the microscopic structure of sperm. He also collected testicular material and tissue from accessory organs including the prostate, Cowper's glands and glans penis for histological examination. Fletcher's work represents one of the few reports to date on the reproductive physiology of the male Eastern quoll.

1.5 AIMS OF THE STUDY

The aim of this thesis is to present an endocrinological account of the changes associated with breeding in the Eastern quoll, *Dasyurus viverrinus,* and to investigate some of the factors that may influence breeding activity. The specific aims of the project are:

(a) to outline the seasonal pattern of reproduction as it occurs in the wild, highlighting the endocrine changes associated with life history events such as mating and weaning of the young

(b) to determine whether the endocrine and physiological system of the male quoll is sensitive to a change in light and therefore likely to be influenced by photoperiod

(c) to investigate a number of social conditions including physical contact and proximity to the female quoll to assess the importance of social factors on the timing of breeding activity

(d) and to determine whether stress plays any part in the breeding strategy of the Eastern quoll.

The project focuses primarily on the male of the species but presents new information on the female.

The aims of the project have been addressed by measuring the hormones controlling reproductive activity and by monitoring the physiological cycles that accompany endocrine changes. Information was obtained through hormonal analysis of LH, testosterone, cortisol and cortisol partitioning in the male and LH, progesterone and prolactin in the female. This information was combined with details on changes in body weight, testes size, the oestrous cycle and pouch development as well as changes in haematological and biochemical parameters. A number of experiments were conducted to determine whether the mechanisms controlling endocrine secretion in this species were similar to those of other mammals and hence the foundations for interpreting endocrinological and physiological findings were sound.

Hormone measurement is regarded throughout this study as a biological tool in the sense that the:

> "endocrine evolution of hormonal control has involved not so much the nature of hormones secreted as the uses to which they are put" (Medawar 1953, cited in McDonald & Waring 1979, p 873).

It is hoped that the information on reproductive cycles obtained from this study may lead to a better understanding of the breeding strategy of the Eastern quoll in its natural environment.

CHAPTER 2

GENERAL METHODS

2.1 FIELD WORK

Field work was conducted at two sites. The southern site at Cradoc was established to monitor seasonal changes in a wild population of quolls and was surveyed on a regular basis. Animals were not removed from this area. Animals required for captive experiments were collected in the north of Tasmania at Ringarooma. This site also enabled a comparison of the reproductive condition of animals living at different locations. Trapping at other sites was on single occasions for collection or survey purposes only. Quolls were obtained under permit from the Tasmanian Department of Lands, Parks and Wildlife.

2.1.1 Site Descriptions

Cradoc is a small town, situated 50 km south of Hobart in the Huon valley. The area surrounding the site was cultivated as apple orchards but bordered by paddocks cleared for grazing and by large areas of bush land (Plate 5a). The bush comprised dry sclerophyll forest predominantly of Stringy bark, *Eucalyptus obliqua*, Peppermint gum, *E. viminalis,* and Tasmanian blue gum, *E. globulus*. Other tree species in the area included Native cherry, *Exocarpos cupressiformis*, Silver wattle, *Acacia dealbata* and various *Banksia* and *Melaluca* species. Trees, soil and debris had been bulldozed at various intervals to form copses. Bracken, ferns, sag and cutting grass formed a light ground cover and fallen logs and rocky outcrops provided ample refuge sites for quolls.

The second site was in the north-east of Tasmania near Ringarooma. The property 'Gum Flat' comprised approximately 250 acres of pasture, bordered by 500 acres of wet sclerophyll forest, merging into denser temperate rainforest vegetation. Myrtle, *Nothofagus cunninghamii*, Blackwood, *Acacia melanoxylon,* and Sassafrass, *Atherosperma moschatum*, were the dominant tree species in this area.

Plate 5a : Field site at Cradoc. The trap line followed the bush-pasture interface.

Plate 5b : Quolls were caught in wire Mascot traps hidden in the vegetation.

2.1.2 Trapping

Trapping at Cradoc commenced in May 1983 and proceeded monthly until January 1985. Two additional trips were made in December 1985 and January 1986 to monitor juvenile dispersal. Twenty wire traps (Mascot brand), baited with dead cockerel chickens, were placed at intervals along a two kilometer bush-pasture interface or in the undergrowth (Plate 5b). Using a trap line rather than a grid enabled more potential den sites to be investigated (Begg 1981a,b). Traps were provided with bark and leaves for nesting material and protected externally from the prevailing weather. Covering the traps served to minimise the stress of capture which in turn facilitated easier handling and collection of blood samples. Traps were opened for four consecutive nights during the first week of every month and inspection commenced at daybreak. The trapping method was based on capture, mark and release.

Trapping at Ringarooma was irregular and centred around the need to collect animals for captive experiments, to observe pouch young or for specific blood sampling. Traps were placed along the bush-pasture interface or around the homesite, hay barns and other outbuildings.

Other species that were captured during this study included: the Tasmanian devil, *Sarcophilus harrisii,* Tiger quoll, *Dasyurus maculatus,* Brushtail possum, *Trichosurus vulpecula,* feral cats, *Felis domesticus* and the Swamp rat, *Rattus lutreolus.*

2.2 CAPTIVE COLONY

Animals were maintained with permission from the Tasmanian Division of Lands, Parks and Wildlife and the University of Tasmania's Committee on the Ethical Aspects of Research involving Animals. Additional information on this colony is supplied in Bryant (1988).

2.2.1 Enclosure Design and Housing

A variety of enclosures were used throughout this study. The two largest external enclosures (13m x 16m x 12m, Plate 6a, and 8m x 5m x 2m) were used to maintain colonies of animals over several breeding seasons. They were built of reinforced wire set into concrete. They contained some bush

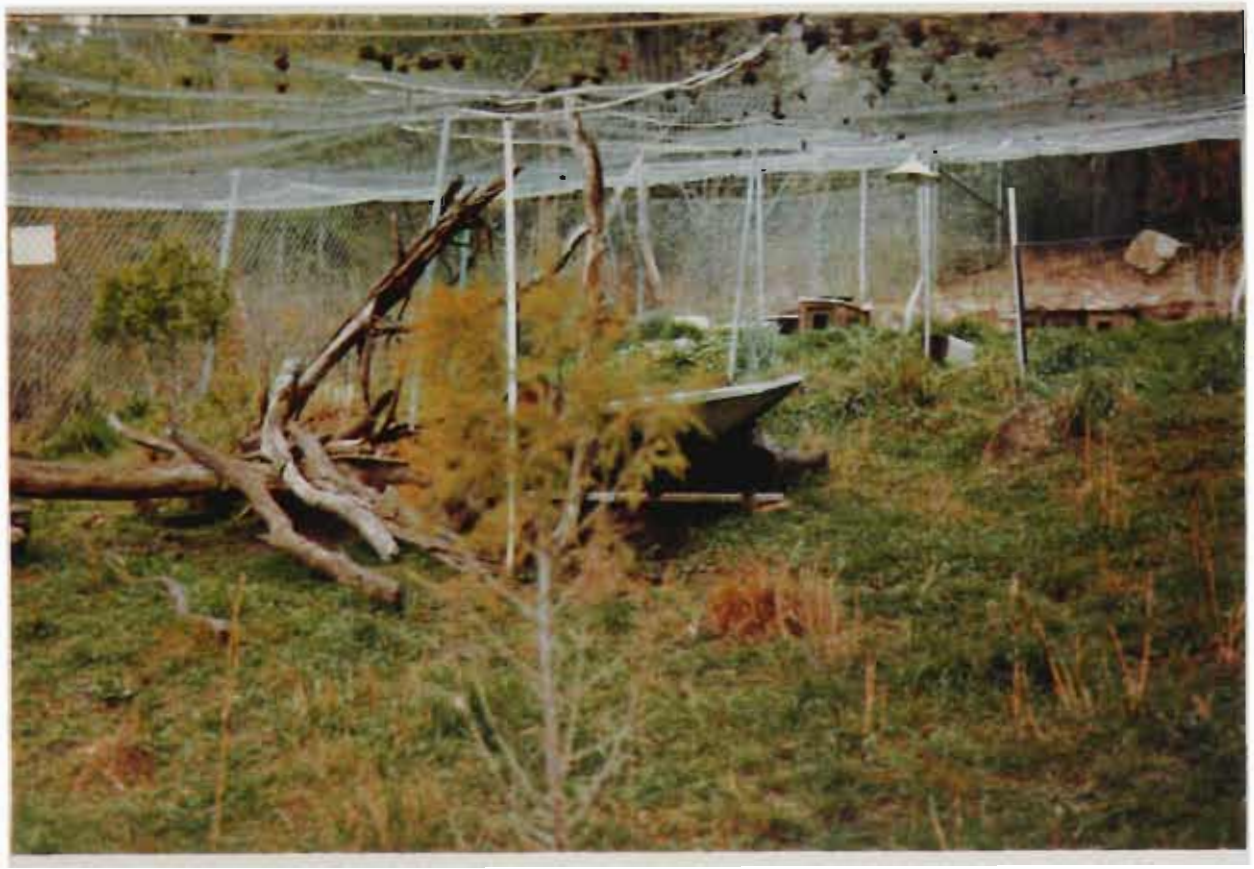

Plate 6a : The largest external enclosure housing a captive colony over the breeding season.

Plate 6b : Smaller wire cages provided individual housing. These were used during the Photoperiod experiment.

but were also provided with nest boxes, logs and vegetation suitable for nest sites. A third external pen (measuring 1m x 3m x 3m) had a concrete floor and tin roof and housed one pair of animals over a breeding season. These three enclosures were situated approximately 400 meters from the main zoology department building and were isolated from campus facilities.

Smaller groups of animals or individual quoll were housed in various sized wire cages as described in Chapter 5 and Chapter 6. The cages were lined with straw and provided with a nest box and, where possible, climbing apparatus (Plate 6b). The quolls often constructed nests either on top of the straw or by tunnelling beneath it and over the short term appeared to maintain good physical condition.

Godsell (1983) reported that quolls in the wild only occasionally share nests or dens. In this study captive males and females appeared compatible when housed together and often one male and several females would share the same nest box causing crowded conditions. During the mating period, aggressive displays of chasing, biting and interlocking mouths were observed but these resulted in only minor injuries. Females received wounds to the back and neck during copulation and occasionally these injuries required treatment. In addition, females were separated from the colony a few weeks after giving birth because crowding in nest boxes usually resulted in injury or insufficient room for growing young to suckle. Females with large pouch young also competed for nest sites which often resulted in dislodgment and loss of young.

2.2.2 Diet

Adults and larvae of *Persectania ewingii* and *Oncopera intricata* (Southern army worms and Corbie grubs) form the bulk of the Eastern quoll's diet in the wild (Green 1967; Blackhall 1980). In captivity, animals were maintained on commercially prepared dried dog food (Pal ®, *ad libitum*), supplemented three times per week with fresh meat including dead cockerel chickens, diced lamb or beef heart, mice or rats. Fresh meat was important not only to maintain the animals in good condition but also to provide variety in the diet. Water was freely available.

2.3 GENERAL METHODS

2.3.1 Blood Sampling

A blood sample was collected from a peripheral ear vein immediately the animal was restrained in a hessian sack. The perimeter of the ear was shaved using a scalpel blade and disinfected. The vessels were dilated by massaging with a mixture of xylene and alcohol (1:1). A marginal blood vessel was cut and the blood scooped into a heparinised collecting vial (Plate 7a). Blood samples from juveniles and small females were collected into blood-plasma separator vials (Microtainers ®). These contained heparinised beads or a gel plug which separated the red cells and plasma after centrifuging. The ear was wiped continuously to enable a pool of blood to collect. Bleeding was stopped by applying pressure to the cut vessel.

These simple techniques were found the most effective and safest for obtaining a blood sample without assistance. Usually between 0.5 ml and 4.0 ml of blood could be collected depending on the size of the blood vessel and the behaviour of the animal. Animals sampled repeatedly over two to three years showed little scarring or damage to the ear in any way.

The blood samples were stored in a cooled vacuum flask until returned to the laboratory and centrifuged (usually within 6 hours). Samples collected from captive animals were spun immediately. The plasma was stored in 200µl aliquots at -20°C until assayed.

Blood films were prepared by dropping a small amount of blood on a glass slide and smearing it at approximately 45 degrees with the edge of a second slide. The slides were air dried and later fixed in methanol. They were stained with Leishams and Wright's stain (1:1) and mounted using Depex mounting medium. Haematocrits were prepared by drawing blood into heparinised capillary tubes and then fitting with critocaps. The samples were centrifuged and the red cell percentage determined using a MSE Micro Haematocrit Reader.

2.3.2 Identification and Body Measurements

Every quoll (except pouch young) was tattooed with a number inside the right ear lobe. This was performed using metal forceps fitted with spiked numbers coated with indelible black ink. Tattooing was found the most effective means of identification and numbers were clearly legible after three

Plate 7a: Blood samples were collected from a marginal ear vein.

Plate 7b: Body measurements were obtained from small young without removing them from the pouch.

years.

Body weight and pes length were recorded and the width and height of the male's scrotum was measured using vernier calipers. Width measured across the two testes in the scrotum and height measured the height (or length) of one testis including the epididymis.

The female's pouch was classified according to Begg's (1981b) descriptions for the Northern quoll, *Dasyurus hallucatus*. Elongated teats or lactating mammary glands were measured and their position in the pouch noted. Pouch young were measured according to the criteria described by Sharman *et al.* (1964) with crown-rump, head, pes, manus, ear and tail length measured where possible. Young were not removed from the teat to obtain a body weight (Plate 7b).

2.3.3 Detection of Oestrus

The onset of oestrus was detected by behavioural changes in the female and these were similar to those described by Ganslosser and Meissner (1984) for *Dasyuroides byrnei* and by Croft (1982) for other Dasyurid species. They included pacing, tail lifting, guttural calls, increased agitation, vulva distention and the presence of vaginal mucus. The stage of the female's pouch also corresponded to her reproductive condition (see Chapter 4) and signs that the female had mated included: loss of fur on the neck and back, neck wounds, hair matting, blood in the urine and a mucous discharge.

2.3.4 Veterinary Care and Anaesthetics.

Quolls in the wild have been reported to carry high ecto- and endo-parasite loads, particularly ticks and fleas (Pearse 1981). The flea larvae burrow into the soft parts of the body, especially the ears, tail and feet and can cause extensive damage due to infection and gangrene. Any embedded larvae were removed from animals brought into captivity and serious infections were treated successfully with penicillin (0.3 ml per kg), antibiotic powder and saline bathing.

A mixture of Fluothane (Halothane B.P.) and oxygen, administered via a portable anaesthetic machine, was effective in inducing the deep anaesthesia necessary for surgical procedures. Ketalar (Ketamine Hydrochloride, Parke-Davis) was administered as a pre-medical muscle relaxant but this caused adverse muscle spasms and was used infrequently.

2.3.5 Statistics

Statistical procedures are based on the methods outlined by Zar (1984) and Fowler and Cohen (1987). The t-tests are unpaired unless stated otherwise and the test for analysis of variance is abbreviated to 'ANOVA'. Percentage data and testes index have been analysed using the non-parametric Mann-Whitney U test. This test converts observations to ranks and may be used for variables measured on the ordinal or interval/ratio scales. Normally the 5% level (0.05) or below (0.01, 0.001) has been accepted as significant.

CHAPTER 3

RADIOIMMUNOASSAY TECHNIQUES AND VALIDATIONS

3.1 INTRODUCTION

Radioimmunoassay (RIA) is a technique used for measuring the concentration of a particular hormone or other biological compound in body fluids. Its principles are based on the body's immune-defence system whereby antibodies are produced in response to foreign substances or antigens. The specificity of the antibody to the antigen and the binding of the two together provides the basis of RIA (Chard 1982). Only recently have data become available on the endocrine control of reproduction in marsupials. This has been possible through the modification and refinement of RIA techniques enabling the low concentration of some marsupial hormones to be measured (Hearn 1972; Sutherland *et al.* 1980; Stewart *et al.* 1981). These measurements have led not only to an assessment of the animal's immediate hormonal milieu, but, when combined with other data, provide a more detailed history of the marsupial reproductive strategy.

This chapter describes the RIA techniques used throughout this study to measure endocrine levels in the Eastern quoll. LH, testosterone and cortisol were measured in the male quoll and LH, progesterone and prolactin were monitored in the female. The assays for testosterone, LH and cortisol required validation for this species and the details are provided in the relevant sections. The following abbreviations have been used: ng, nanogram; pg, picogram; cpm, counts per minute; ovx, ovariectomy; DHT, dihydrotestosterone; ♂, male; ♀, female.

3.2 RIA FOR TESTOSTERONE

3.2.1 Introduction

Testosterone (17ß-hydroxy-4-androsten-3-one) is a androgen from the C_{19} group of steroids and is the principal secretory product of the testes (Gower 1979). Testosterone is produced primarily by the Leydig cells of the interstitial tissue of the testes but also in small amounts by the adrenal glands and the female ovary. In most mammals the secretion of testosterone is controlled via a negative feedback mechanism with pituitary LH (luteinising hormone) modulated via the hypothalamus (deKretser 1984). Testosterone and 5α-DHT are responsible for many facets of the development and maturity of the testes and epididymides as well as accessory gland secretions and secondary characteristics such as aggression and muscle development (Ganong 1965; Gower 1979). Testosterone is usually transported in the blood bound to a specific protein or sex hormone binding globulin (SHBG) and while bound it is rendered biologically inactive. In the Dasyuridae androgens are not bound to SHBG but instead are bound to albumin with a low affinity but high capacity (Sernia 1978; Bradley 1982). At certain times of the year, for example during the breeding period, some dasyurid males may be exposed to high levels of biologically active steroid.

The RIA technique used to measure testosterone in this study was based on the protocol of Fletcher (1983) but modified to use ^{125}Iodine tracer and antisera AS 6050. Other modifications were made to account for a plasma effect occurring at low concentrations of testosterone. Comparisons between the use of iodinated and tritiated tracers for steroid measurement have been presented by a number of workers, all of whom agree that the choice is a matter of convenience (Jeffcoate *et al.* 1973; Hammond *et al.* 1977; Chard 1982; Corrie *et al.* 1982).

3.2.2 Materials and Methods

3.2.2.1 Radioiodinated Testosterone

Initially, testosterone was iodinated using histamine, sodium (^{125}I) iodide and a chloramine-T method (Appendix A.1). However, it became more practical to purchase commercially prepared testosterone from Amersham International (Surrey Hills, NSW.).

Testosterone-3-(0-carboxymethyl)-oximino-(2-(^{125}I) iodohistamine) had a specific activity of ~2000Ci per mmol (~74 TBq) and a radioactive concentration of 100μCi per ml (3.7 MBq) at the activity reference date. Radioactive purity was reported to be more than 90% with less than 5% free ^{125}I present after reverse phase HPLC purification. The tracer was not used beyond its activity reference date and was stored in lead containers at 4°C.

3.2.2.2 Antisera

Antiserum As 6050 was donated by Dr. Ron Cox, Hormone Development Group, CSIRO, Sydney and was raised in sheep against the antigen Testosterone-3-CMO-BSA. It showed 100% cross reaction with testosterone (17ß-hydroxy-4-androsten-3-one), 31.0% with 5α-DHT, 30.0% with 4-androsten-3ß,17ß-diol-3-one, 3.5% with 4-androsten-17ß,19-diol-3-one, 1.3% with 4-androsten-3,17-dione and a cross reactivity of between 0.1% and 0.004% with a range of other steroids tested.

The antiserum was prepared in assay buffer and stored in aliquots of 100μl of 1:100 at -20°C. It was used at a working dilution of 1:10,800 and a final dilution of 1:76,000.

3.2.2.3 Testosterone Standards

A stock solution of 1μg per ml testosterone (4-androsten-17ß-01-3-one, Sigma St. Louis, USA) was prepared in redistilled absolute ethanol and stored at -20°C. The stock was diluted with ethanol to produce standards ranging from 2 to 2,000 pg per 50μl. The standards were stored at 4°C and were used in every assay.

3.2.2.4 Steroid Assay Buffer

A phosphate buffered saline (0.2 M Na_2PO_4 + 0.1% gelatin, pH 7.0) was prepared by adding 6.10 g NaH_2PO_4, 8.66 g Na_2HPO_4 , 9.0 g NaCl, 1.0 g Na azide and 0.1g gelatin (warmed to dissolve) to 1.0 litre of deionized water. All chemicals were analytical reagent grade (May & Baker, Aust. Pty. Ltd.) except for laboratory grade sodium azide. The assay buffer was stored at 4°C for up to 3 months.

3.2.2.5 Charcoal

Norit A charcoal powder (6.25g, activated and untreated) was washed several times in distilled water and the fines decanted after each wash. The final slurry was oven dried and a 0.625% suspension prepared in assay buffer. A 200µl charcoal suspension was used to separate the bound from the free steroid fraction in the assay. The addition of a dextran coating was found to make little difference to the separation procedure and was not applied to the charcoal.

3.2.2.6 Stripped (steroid free) Plasma

Normal sheep plasma (50mls) was mixed overnight with 5mls of 6.25% charcoal and 200µl of ^{125}I-testosterone containing 200,000 counts. The preparation was centrifuged at 3,000 rpm to remove the bulk of the charcoal then re-spun 3 to 4 times in an Eppendorf Micro Centrifuge to remove any remaining fine sediment. This process effectively removed the steroid from the sheep plasma as only background radiation counts were detected when the plasma was assayed. The stripped plasma was stored in volumes of 5mls at -20°C. Smaller quantities of pooled quoll plasma were stripped of steroid using the same method.

3.2.2.7 Internal Standard (Quality Control)

An internal standard or quality control was included in each assay to monitor variation at the minimum sensitivity range. Medium and high assay variation were calculated using the testosterone standards. Two nanograms of testosterone standard were mixed with 8 mls of steroid free sheep plasma and stored as 200µl aliquots containing 50pg of testosterone. After assaying, the

internal standard contained 18.5pg testosterone per assay tube.

3.2.2.8 Extraction Solvent

Diethyl ether (Ajax Chemicals, AR grade) was used to extract testosterone from the plasma. The ether was redistilled by heating in a water bath and then cooling the evaporation over a chilled rotating coil under vacuum. The ether was used within a few days of distilling to eliminate any high solvent blanks.

3.2.2.9 Glassware and Test Tubes

All solutions were prepared and stored in glass and glass stoppered bottles. Borosilicate disposable glass test-tubes with caps (Meteor or Corning pyrex) were used for extraction (10ml) and assaying (5ml) and disposable polypropylene serology tubes (James Hardie, Melbourne) were used to count the radioactive samples.

Glassware, assay tubes and pipette tips (except those radioactively contaminated) were soaked overnight in Pyroneg® detergent, washed with Decon® and rinsed several times in distilled water and ethanol.

3.2.3 Procedure

Fifty microlitres of each testosterone standard and ethanol zero were air dried. They were reconstituted in 200µl of stripped sheep plasma and left for 2 hours at room temperature. Quoll plasmas were aliquoted as either 200, 150 or 100µl duplicates or as 100 or 50µl singletons. Quoll plasmas, testosterone standards, internal standards and solvent blanks were extracted by rigorous vortex with 2.5 mls of solvent for 3 x 20 seconds. The tubes were let stand for several minutes to allow separation of the phases then placed in a bath of dry ice to freeze the aqueous phase. The supernatant (solvent phase) was decanted into fresh tubes and the pellet (aqueous phase) thawed in a warm water bath and re-extracted (double extraction) using fresh solvent. The supernatants were pooled and dried under a filtered air stream at 25°C in a fume cabinet. A quantity of of assay buffer (1.2 mls or 500µl buffer for singletons) was added to each tube and the assay left to equilibrate at room temperature for at least 2 hours (with occasional mixing).

Each duplicate assay tube received 500µl of extracted sample, 100µl of ^{125}I-testosterone containing approximately 20,000 cpm and 100µl of (1:10,800) AS 6050. Total counts and non-specific binding tubes had their volumes adjusted with assay buffer. The assay was vortexed and left overnight at 4°C in an ice bath.

The next day 200µl of a 0.625% charcoal suspension was added to every tube except total counts. The tubes were vortexed and left for 30 minutes at 4°C. They were centrifuged at 2,500 rpm for 15 minutes then 800µl of supernatant was counted for 1 minute in an automatic gamma counting machine (MR 252, Roche). The cpms were converted into the percentage bound and free and a standard curve was plotted on 4 cycle log paper using the equation :

$$\%\, B / B_0 = \frac{\text{cpm of X - cpm (NSB - BG)}}{\text{cpm Zero standard - cpm (NSB - BG)}} \times \frac{100}{1}$$

where

B = the amount of tracer bound

B_0 = the amount of tracer bound at zero steroid

X = unknown or standard

NSB = non-specific binding

BG = background counts

3.2.4 Validations

3.2.4.1 The Need for Extraction

Experiment 1. Two standard curves were prepared and assayed without any prior extraction. The first curve had testosterone standards equilibrated in buffer and the second curve in stripped quoll plasma (tested as steroid free). Figure 3.1 shows that these two curves did not superimpose and instead the plasma curve was displaced to the right and slightly skewed.

Experiment 2. Duplicates of three male quoll plasmas were either extracted or not extracted then compared on a standard curve. The testosterone concentration was much higher in the plasmas that were not extracted than in those doubly extracted prior to assay (not extracted 10.2, 10.0, 6.4 ng per ml vs

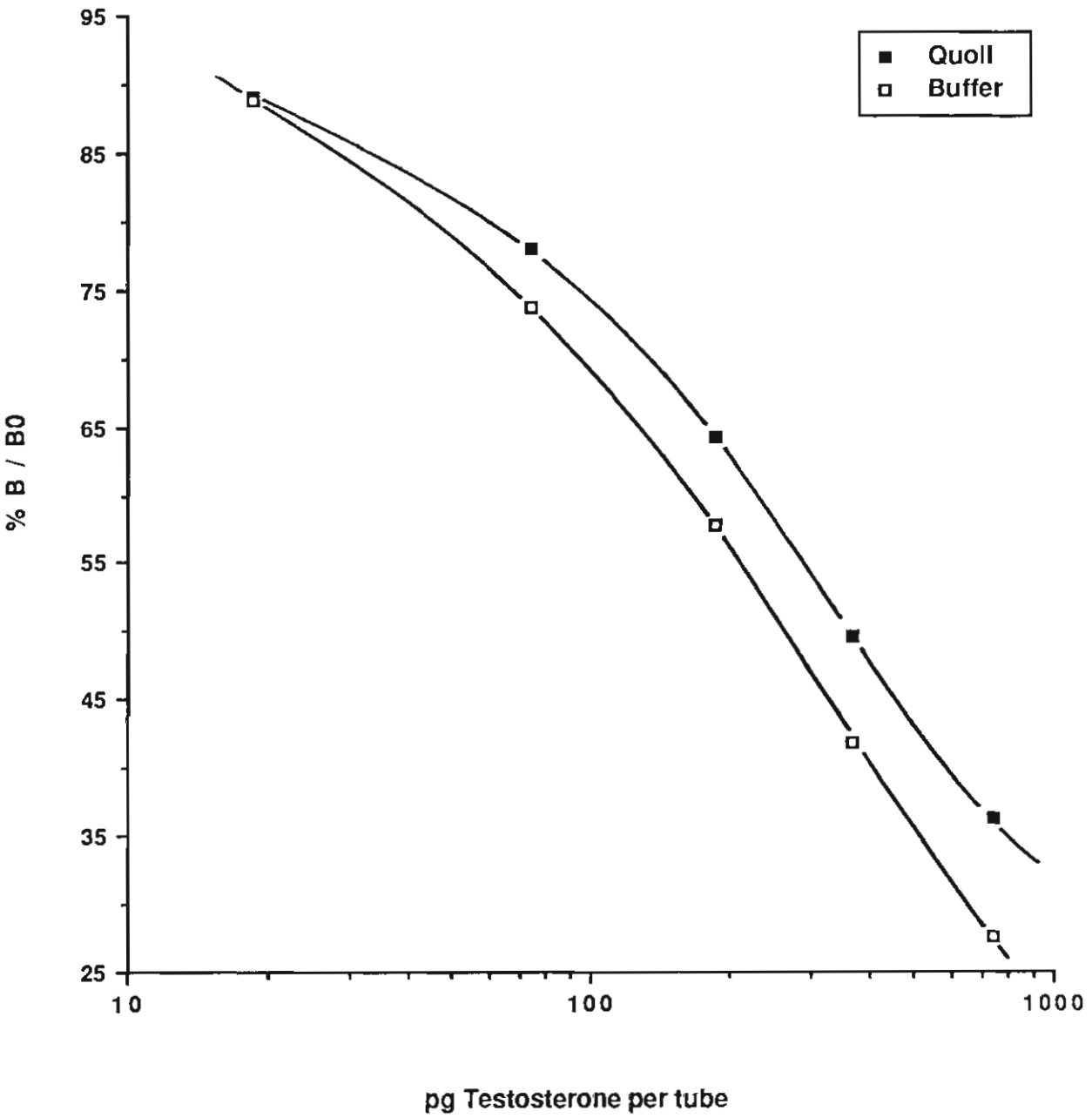

Fig. 3.1 Comparison between standard curves prepared in buffer and steroid free quoll plasma. The quoll curve is displaced to the right and skews at increasing concentrations of testosterone.

extracted 2.4, 1.6, 1.9 ng per ml).

The results of Experiment 1 and 2 indicated the need for an extraction step in the assay procedure.

3.2.4.2 Effect of Plasma on the Standard Curve and Recovery

Three experiments were conducted to investigate the effect of plasma components and buffer salts on the standard curve.

Experiment 1 Four standard curves were prepared by drying ethanol testosterone standards and equilibrating them in either assay buffer or steroid free quoll plasma and either extracting or not extracting them prior to assay.

Figure 3.2 shows that there was little difference between the two buffer curves, indicating that the extraction process does not alter the binding of antibodies in buffer salts. The two curves equilibrated in steroid free quoll plasma were displaced to the right and this displacement increased further after applying correction factors to the curve. These results indicate that some components in the quoll plasma may be interfering with the binding of the labelled steroid and antibody and that an extraction process is necessary to eliminate erratic binding.

Experiment 2 Three curves were prepared to examine the effect of plasma components and recovery on the standard curve. The curves used ethanol testosterone standards that were air dried and equilibrated in either (1) assay buffer, (2) pooled male quoll plasma or (3) plasma from castrated male quolls. The 3 curves were equilibrated overnight and extracted twice.

The 3 curves were not superimposed and the plasma curves were displaced to the right (Fig. 3.3). The position of the pooled male plasma curve suggests that endogenous testosterone in the plasma may be competing for binding sites with the standard and labelled testosterone. The position of the castrate curve also suggests there was some competition for sites on the antibody and it may be that testosterone of adrenal origin is present in the castrate samples. However, parallelism was closely approximated and this suggests a good recovery of cold testosterone.

These two experiments have shown that testosterone standards extracted in buffer salts differ from standards extracted in quoll plasma. A chromatographic step would be required to determine the precise nature of the difference. It may be that the nature and size of the radioactively labelled iodine

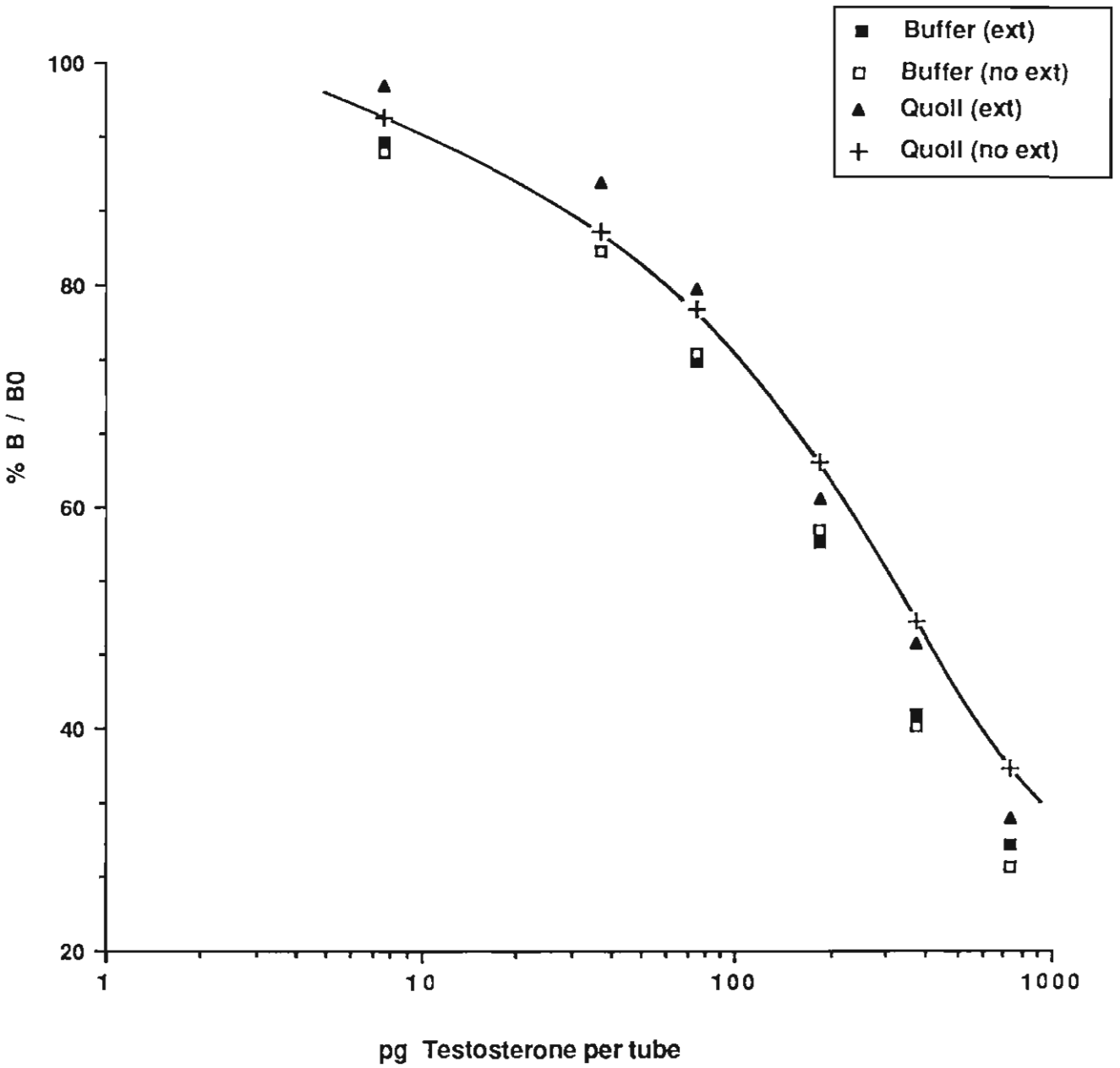

Fig. 3.2 Effect of stripped quoll plasma (extracted and non extracted) on the buffer standard curve. Quoll plasma components cross reacted with the antibody therefore extraction is required.

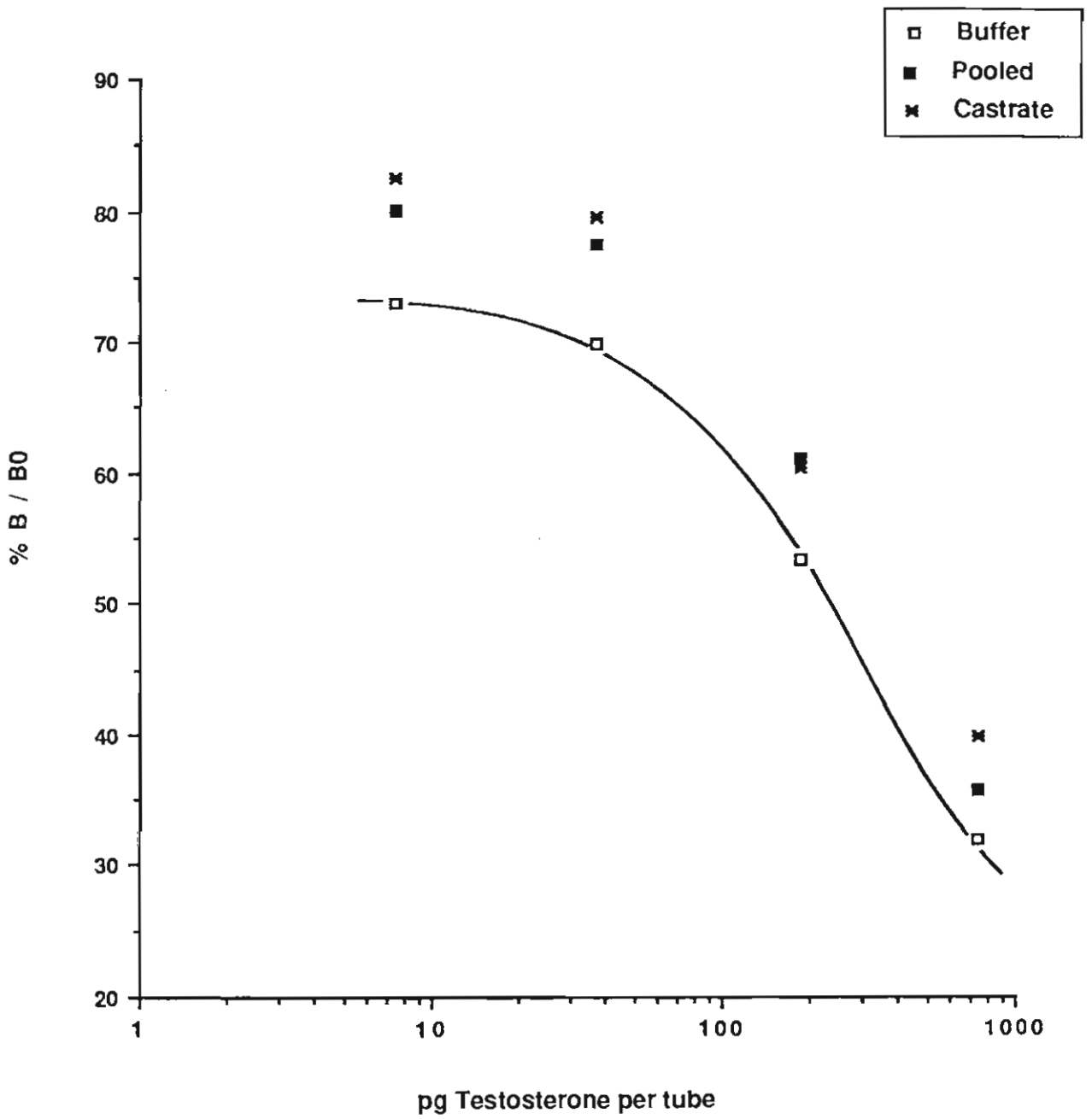

Fig. 3.3 Effect of quoll serum components on the binding of testosterone to the antibody. Quoll curves are displaced to the right.

reacts differently with the plasma or buffer than previously used tritiated labels.

Experiment 3 To alleviate the differences between buffer and plasma, testosterone standards were equilibrated in stripped sheep plasma. Figure 3.4 shows the similarity between the stripped sheep and quoll curve and the difference between these and the buffer curve. The extraction of testosterone standards in sheep plasma therefore produced a curve which was comparable with a quoll standard curve and that, provided the assay validations did not produce anomalies, this assay would be very accurate in measuring testosterone.

3.2.4.3 Type and Volume of Solvent

The following solvents were examined for their suitability in extracting testosterone: freshly distilled diethyl ether, diethyl ether (laboratory grade), anaesthetic ether (BP contains 0.002% w/v hydroquinone) and dichloromethane. Each solvent was tested separately and in combination with heat and a 1 molar sodium hydroxide solution.

All solvent curves were approximately parallel, however, skewing at either end of some curves and high solvent blanks suggested that freshly distilled diethyl ether was the most efficient extraction solvent. Heat and sodium hydroxide were detrimental in some cases and did not improve extraction efficiency consistently. Dichloromethane was ineffective as an extraction solvent because it congealed upon contact with the plasma making it difficult to vortex and air dry.

3.2.4.4 Extraction Efficiency

The efficiency of extracting testosterone was determined in pooled quoll plasma, stripped sheep plasma, Tasmanian devil plasma (see Appendix B) and assay buffer. Four sets of ethanol testosterone standards containing 100µl of ^{125}I-testosterone were air dried and equilibrated in 200µl of species plasma or buffer. The tubes were double extracted and each extraction dried down separately. Samples were equilibrated in 800µl of assay buffer and their radioactivity expressed as a percentage of the total added.

Table 3.1 shows that a single extraction with ether removed between 77.7% and 92.8% of testosterone in quoll and sheep plasma and in assay buffer. A second extraction improved efficiency to between 88.1% and 97.7%. Only a

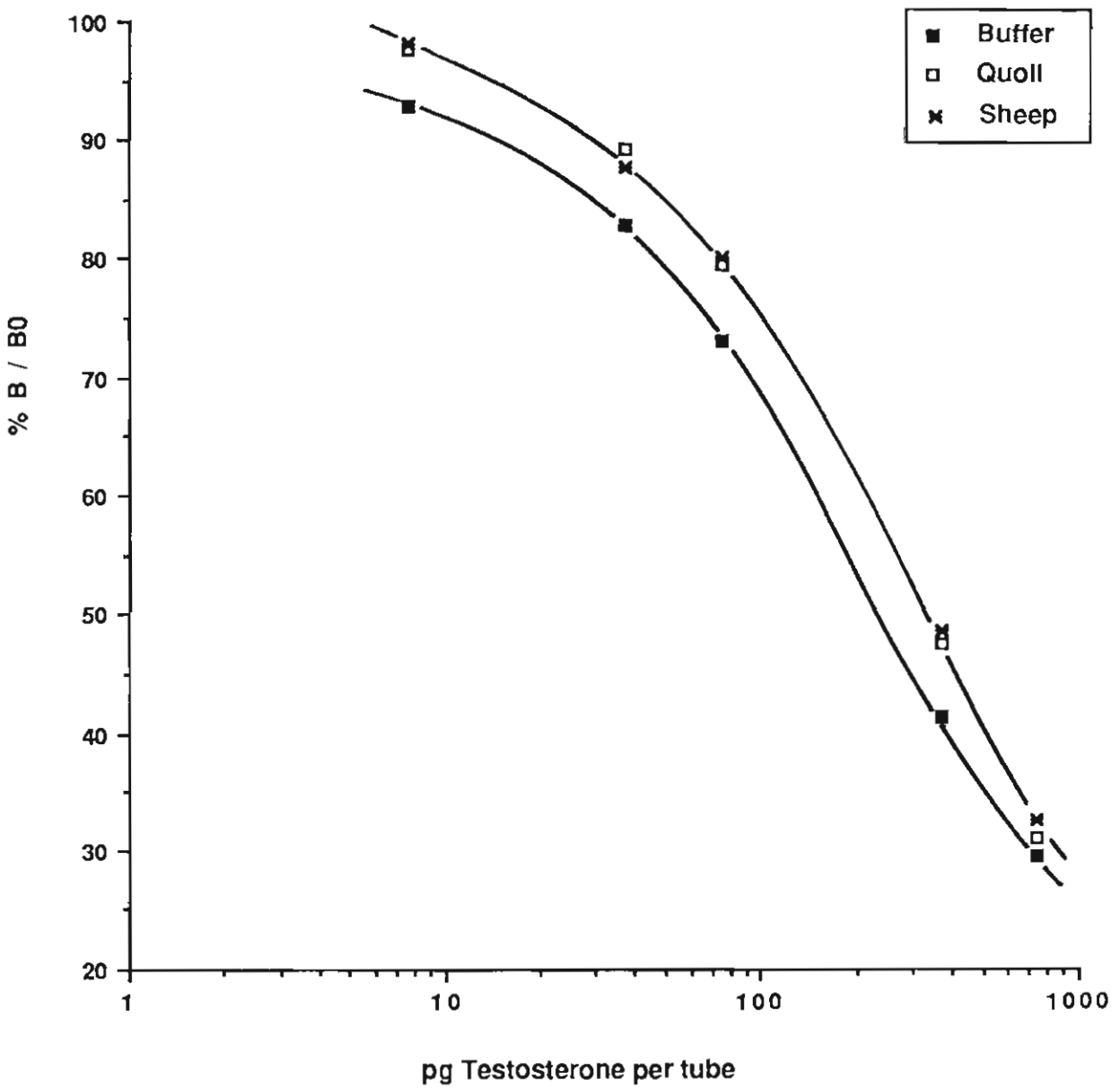

Fig. 3.4 Standard curves prepared in assay buffer, steroid free quoll and sheep plasma. Note the sheep and quoll curves are superimposed.

single extraction was required for devil plasma. No correction factor was applied to the standard curve extracted through sheep plasma (94.4 to 100% efficiency) but as only approximately 90% of testosterone was extracted from the quoll plasma, a 10% correction factor was applied to the unknowns.

Table 3.1 Efficiency of Extraction of ^{125}I-testosterone in quoll plasma, devil plasma, steroid free sheep plasma and assay buffer when added to non-labelled standard (expressed as % of total added).

Standard	Extraction	Quoll	Devil	Sheep	Buffer
Zero	Single	77.7	98.5	88.6	75.2
	Double	10.1	3.0	5.8	10.1
	% Total	87.8	100.0	94.4	85.3
2,000 pg	Single	74.3	99.5	93.0	84.3
	Double	10.2	3.2	5.4	10.9
	% Total	84.5	100.0	98.4	95.2
1,000 pg	Single	78.4	100.0	92.2	82.4
	Double	12.0	1.1	4.9	9.4
	% Total	90.4	100.0	97.1	91.8
500 pg	Single	81.7	97.5	92.9	85.0
	Double	9.5	2.7	4.7	10.6
	% Total	91.2	100.0	97.6	95.6
200 pg	Single	80.5	-	-	80.4
	Double	10.0	-	-	11.4
	% Total	90.5	-	-	91.8
100 pg	Single	75.2	98.4	93.9	82.5
	Double	11.6	5.6	4.8	9.3
	% Total	86.8	100.0	98.7	91.8
20 pg	Single	75.9	92.0	96.0	75.7
	Double	9.7	5.8	4.3	9.3
	% Total	85.6	97.8	100.0	85.0

3.2.4.5 Reproducibility and Precision

Variation in the RIA of testosterone was determined by (a) variation in testosterone standards, (b) variation in the internal standard (quality control) and (c) variation in duplicates of two male quoll plasma pools included in two assays (intra- and inter- assay variation).

Testosterone standards varied 0.9% to 8.4%, the largest variation occurring in the 2 ng standard (n=18 assays). The internal standard of 18.5 pg testosterone ranged between 19.2 ± 3.7 pg ($\bar{x}$ ± s.d., n= 11 assays). The inter- and intra- assay variations are presented in Table 3.2. Inter-assay variation ranged between 9.1 to 11.3% and intra-assay variation between 12.5 to 14.8% both of which fall within the standards outlined by Abraham (1980).

Table 3.2 Variation in measurement of testosterone in replicates from two quoll plasma pools compared in two assays.

Assay		Pool 1 pg per ml		Pool 2 pg per ml
No. 1		972, 945 972, 986 770, 1080 891, 789		1418, 1661 1256, 1553 1418, 1391 1782, 1431 1620, 1755
	n = 8	926 ± 104 c.v. = 11.3 %	n = 10	1529 ± 173 c.v. = 11.3%
No. 2		1215, 1026 1296, 1053 1080, 1283 1148, 1080		1526, 1580 1958, 1931 1566, 1499 1850, 1755 1796, 1350
	n = 8	1148 ± 105 c.v. = 9.1 %	n = 10	1681 ± 204 c.v. = 12.1 %
Variation	n = 16	1073 ± 153 c.v. = 14.8 %	n = 20	1605 ± 200 c.v. = 12.5 %

3.2.4.6 Dose Response

Serial dilutions of plasma from no.17♂ (30.4.84) and no. 44♂ (30.4.84) were extracted and measured on a standard curve (Fig. 3.5). Their resultant curves were parallel, although displaced from the buffer curve, and gave similar concentrations for serial dilutions when converted to pg per tube (Table 3.3).

Table 3.3 Conversion of serial dilutions of male quoll plasma.

Quoll	Volume	% B / B_0	pg per tube	pg per ml
17	25 µl	87.6	49	1,960
	50 µl	78.6	93	1,860
	75 µl	69.5	150	2,250
	100 µl	62.5	239	2,390
44	25 µl	92.8	28	1,120
	50 µl	83.5	68	1,360
	100 µl	72.2	131	1,310

3.2.4.7 Sensitivity

The sensitivity of the assays ranged between the lowest testosterone standard of 10pg and the internal standard of 18.5pg per tube (depending on the activity date of the ^{125}I-testosterone) or between 100 and 250pg testosterone per ml of plasma.

3.2.5 Standard Curve

An 'averaged' curve was constructed to monitor the variation in standards and to assess the deviation between assays. Although all standard curves were similar, especially at low concentrations of testosterone, the 'averaged' curve accounted for any standards that had inconsistent readings or faulty extraction and also for the variation in cpm, maximum binding and age

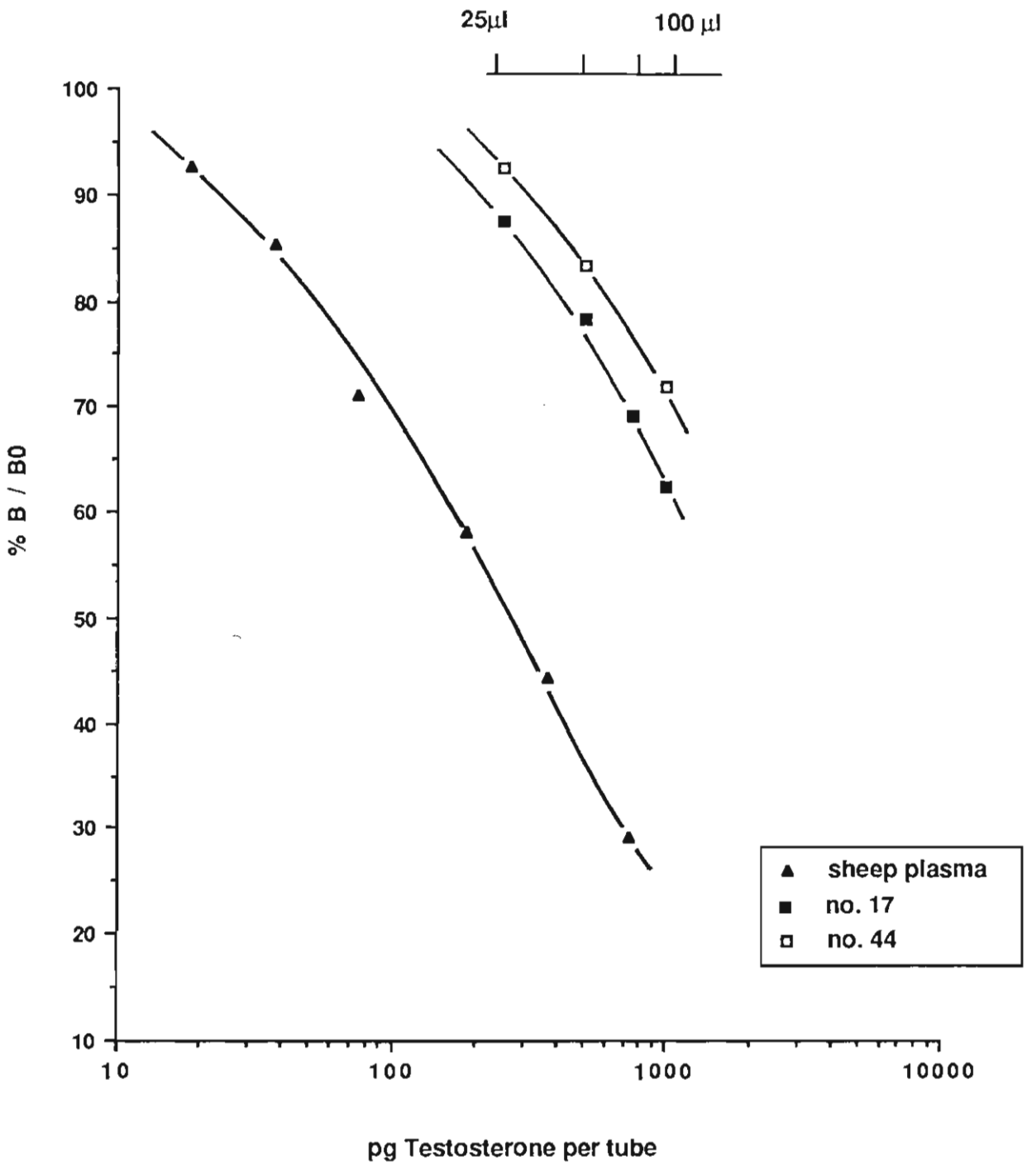

Fig. 3.5 Serial dilutions of two male quoll plasmas against a standard curve prepared in sheep plasma.

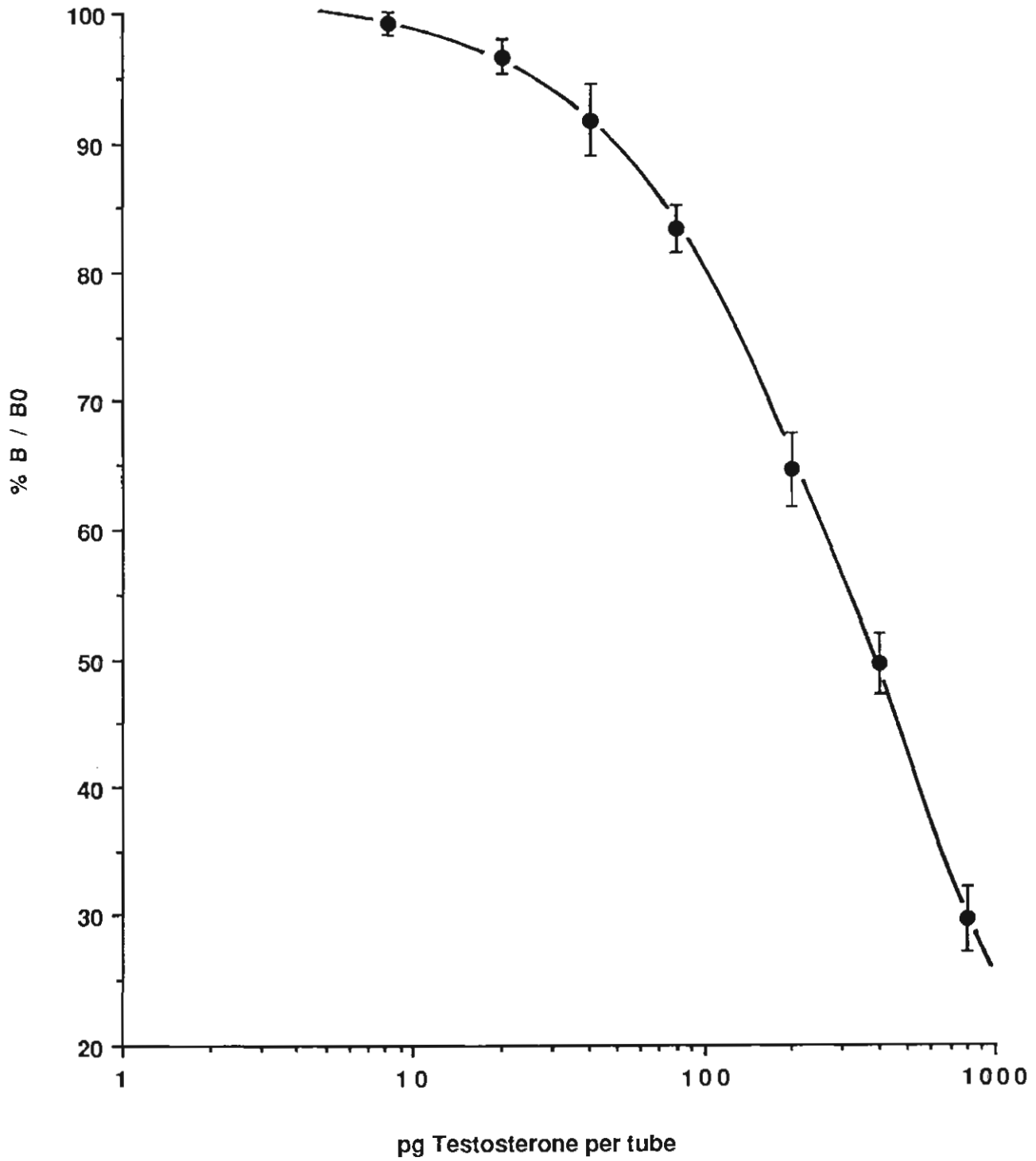

Fig. 3.6 Testosterone standard curve (± s.d., n=18).

of tracer. The 'averaged' curve shown in Fig. 3.6 represents the mean of 18 assays. The maximum binding was 49.9 ± 4.2% ($\bar{x}$ ± s.d., n=18) and the non-specific binding was 5.2 ± 1.5% ($\bar{x}$ ± s.d., n=18). All testosterone results were calculated on this curve using the $\%B/B_0$ equation. Seven assays performed at a later date also fell within the range of this curve.

3.3 RIA FOR LUTEINISING HORMONE (LH)

3.3.1 Introduction

Luteinising hormone (LH), or interstitial cell stimulating hormone (ICSH), is a gonadotrophic hormone secreted by the anterior pituitary gland under the influence of releasing factors from the hypothalamus. In the female mammal LH is usually responsible for follicular development and ovulation. In the male LH primarily stimulates the growth of the Leydig cells which contain the smooth endoplasmic reticulum, mitochondria and enzymes necessary for testosterone production and testicular steroidogenesis (deKretser 1984). In the majority of male mammals, a rise in LH concentration is followed shortly after by a rise in testosterone concentration. This in turn controls LH secretion via feedback from testosterone synthesis (Gower 1979).

The mechanisms of LH secretion and its role in marsupial reproduction have been investigated only recently. Specific and sensitive assays using, for example, the heterologous double antibody technique of Sutherland *et al.* (1980), have enabled very small concentrations of LH in non-oestrus macropodid females and larger peaks in pre-oestrus and oestrus females to be detected. This assay protocol is based on that of Sutherland *et al.* (1980) but has been used by Fletcher (1983) and Horn *et al.* (1985) on various marsupial species.

Most of the LH assays were performed using the facilities of the CSIRO, Rangelands, Canberra and Drs L. Hinds and K. Nicholas donated the first and second antibodies. Several LH assays were completed later in the Zoology Department of Tasmania using a second antisera (GAR 8), iodinated LH and standard (NIH-o-LH-S23) donated by Dr. T. Fletcher, Anatomy Department, Monash University.

3.3.2 Materials and Methods

3.3.2.1 Radioiodinated LH

Highly purified rat LH (NIADDK-rat-LH-I-6, National Hormone and Pituitary Program, Baltimore, USA) was iodinated using a gel column and chloramine-T method (Appendix A.2). ^{125} I-LH-I6 was diluted with PBS-BSA to approximately 20,000 cpm per 100µl per tube.

3.3.2.2 First and Second Antibody

Antibody 1, rabbit anti-ovine LH GDN-15 (200µl) was diluted with 20 mls PBS-EDTA (1/800) and 40µl normal rabbit serum and used at 200µl per assay tube.

The second antibody, sheep anti-rabbitγglobulin (ARγG) was raised in sheep no. 5968. After titration (6.2.86) the working concentration was 1:100 diluted with 0.2% PBS-BSA and used at 200µl per assay tube. A 1 ml aliquot of the second antibody GAR 8 was made up to 100 mls with PBS-BSA (1/100 dilution) and used at 200µl per assay tube.

3.3.2.3 LH Standards

LH standards were prepared by serial dilution of NIH-o-LH-S19 (100ng per 100µl) or NIH-o-LH-S23 with 0.2% PBS-BSA. Standards ranged from 0.0195ng per 200µl to 10.0ng per 200µl and were assayed in triplicate.

3.3.2.4 Assay Buffers

A stock PBS buffer (pH 7.4, 0.05 M) contained 5.68g Na_2HPO_4, 1.56g $NaH_2PO_4.2H_2O$, 8.18g NaCl in 1 litre distilled water. The assay buffer or 'diluent' (0.2%, pH 7.4) contained 1 litre of PBS stock, 2g BSA and 0.1g Na azide. The first antibody was diluted with PBS-EDTA (pH 7.0, 0.05 M) which contained 18.6g EDTA (m.w. 372) in 1 litre of PBS stock. Buffers were stored at 4°C.

3.3.2.5 Assay Tubes

Disposable plastic serology tubes DP6 (5ml, James Hardie, Melbourne) were used in the assay. Glassware was treated as in sect. 3.2.2.9.

3.3.3 Procedure

Day 1: Quoll plasmas were pipetted as 200µl or 150µl duplicates. The LH standards and non-specific binding tubes were prepared in triplicate (4 zero tubes). Samples received 300µl (or 350µl for 150µl duplicates) of diluent (PBS-BSA) and 200µl of antibody 1. They were vortexed and left overnight at 4°C. Non-specific binding tubes received 700µl of diluent but no first antibody.

Day 2: Samples received 100µl of ^{125}I-LH-I6 containing approximately 20,000 cpm and left overnight at 4°C.

Day 3: 200µl of antibody 2 was added to every tube except total counts and left at 4 °C.

Day 4: The assay was centrifuged at 3,000 rpm for 30 minutes. The supernatant was aspirated and the precipitated pellet counted in a gamma counter for 2.5 minutes.

Some modifications were made when using NIH-o-LH-S23. The second antibody, goat anti-rabbit (GAR 8), had a slightly higher affinity for the LH-anti-o-LH-antibody complex than did ARγG. This necessitated adding 200µl of stripped sheep plasma to the standard curve and non-specific binding tubes after the second antibody was added on day 3. Sample tubes also received 200µl of sheep plasma to keep incubation volumes constant. On day 3 the assay was left at 4°C for 2 hours and then centrifuged, thus completing the assay in three days.

The cpms were converted using the equation %B / B_0 (see section 3.2.3) and unknowns calculated from the standard curve. Corrections were made for smaller volumes of plasma.

3.3.4 Validations

Animal numbers and dates in the following section refer to quolls maintained at the CSIRO, Canberra.

3.3.4.1 Dose Response Curves

Plasmas from 5 quolls were titrated in triplicates of 50µl, 100µl, 200µl, 300µl and 400µl and their parallelism compared on a standard curve. The two male quolls were non-breeding samples (1♂, 8.9.83, 2♂, 6.9.83) but the 3 female

quolls had varied reproductive histories: B5♀, 15.9.83, old female with large tumour in kidney; L1♀, 15.9.83, old female with large cysts on her ovaries; L0♀, 15.9.83, old female with small reproductive tract.

All dose-response curves except for that of B5♀ were approximately parallel to the standard curve (Fig. 3.7). This indicates a relative lack of interference from non-specific factors in the quoll's plasma. The poor parallelism displayed by B5♀ could have been caused by secretory products from a kidney tumour while the low %B/ B_0 values for L1♀ could have been due to the large ovarian cyst secreting oestrogen.

3.3.4.2 Recovery

Two standard curves were prepared and 200µl of plasma from either B5♀, 15.9.83 or 1♂, 8.9.83 was added to each point on the curve. Parallelism, plotted on LOGIT Radioimmunoassay Sheets was satisfactory for the male plasma (Fig. 3.8) but the poor displacement observed for the female plasma may be accounted for by her reproductive history.

3.3.4.3 Pituitary Extracts

The anterior pituitary (ALP) and posterior pituitary (PLP) of castrate male no. 198 (21.1.86) were homogenized with 200 to 300µl PBS-BSA and made up to a 1 ml stock solution (1:1). Doubling dilutions of the stock from 1:1 to 1:512,000 were assayed in triplicate with a standard curve.

The ALP crude extract failed to show adequate displacement after the 1:2,000 dilution. The dose response curve for the more concentrated extracts did show displacement with standard NIH-o-LH-S19 (Fig. 3.9) but no additional pituitary material was available to repeat this validation. Fletcher (1983) presented an inhibition curve for pituitary homogenates for a female quoll using a similar assay in which good displacement was apparent.

3.3.4.4 Assay Sensitivity and Variance

The sensitivity of the assay when using NIH-o-LH-S19 or NIH-o-LH-S23 standards was between 150 to 250 pg per ml of plasma.

Inter-assay variation using standard NIH-o-LH-S19 and 3 plasma pools

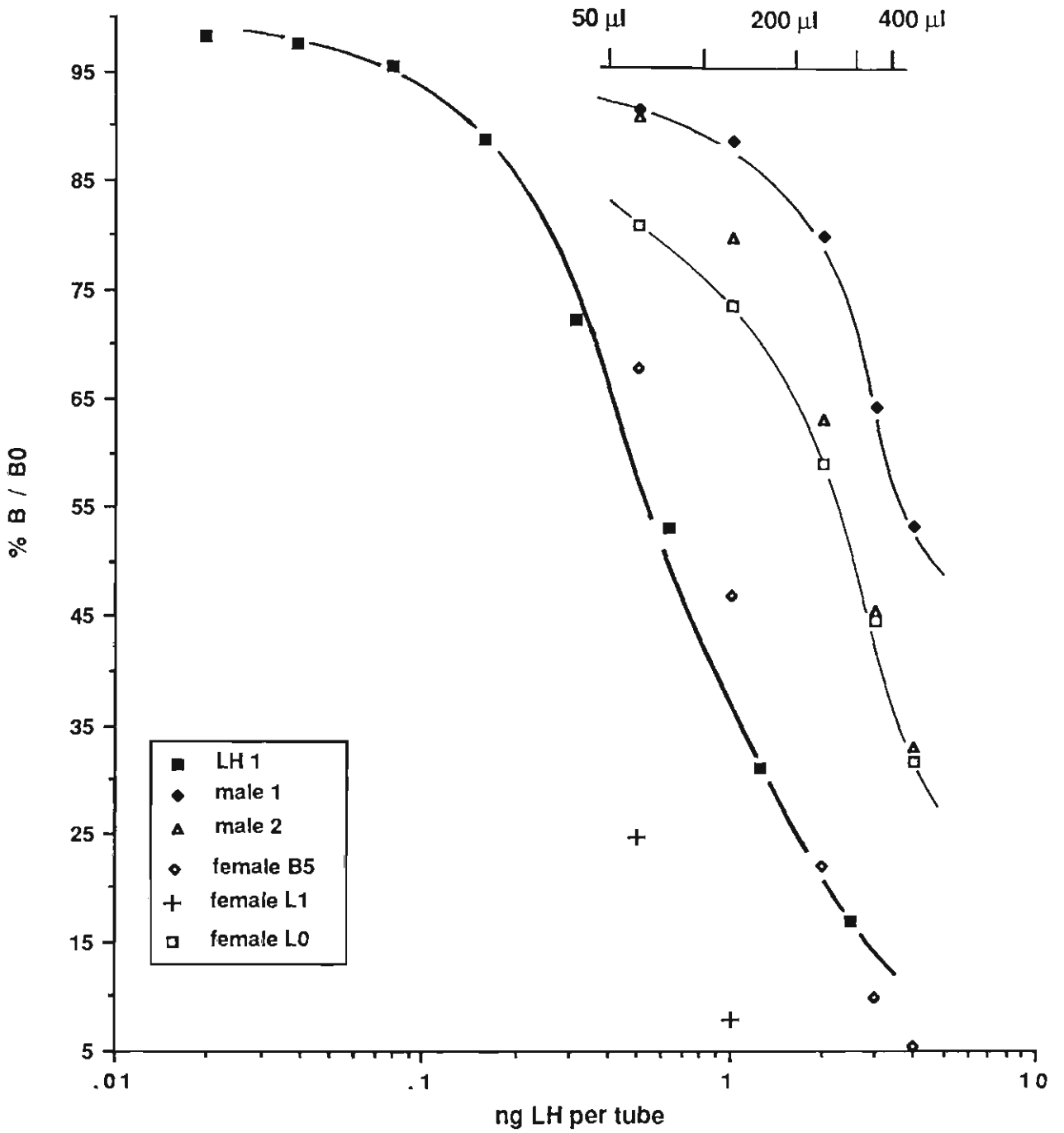

Fig. 3.7 Serial dilutions of 2 male and 3 female quoll plasma against LH standard curve. The response of females B5 and L1 may be explained by their reproductive histories.

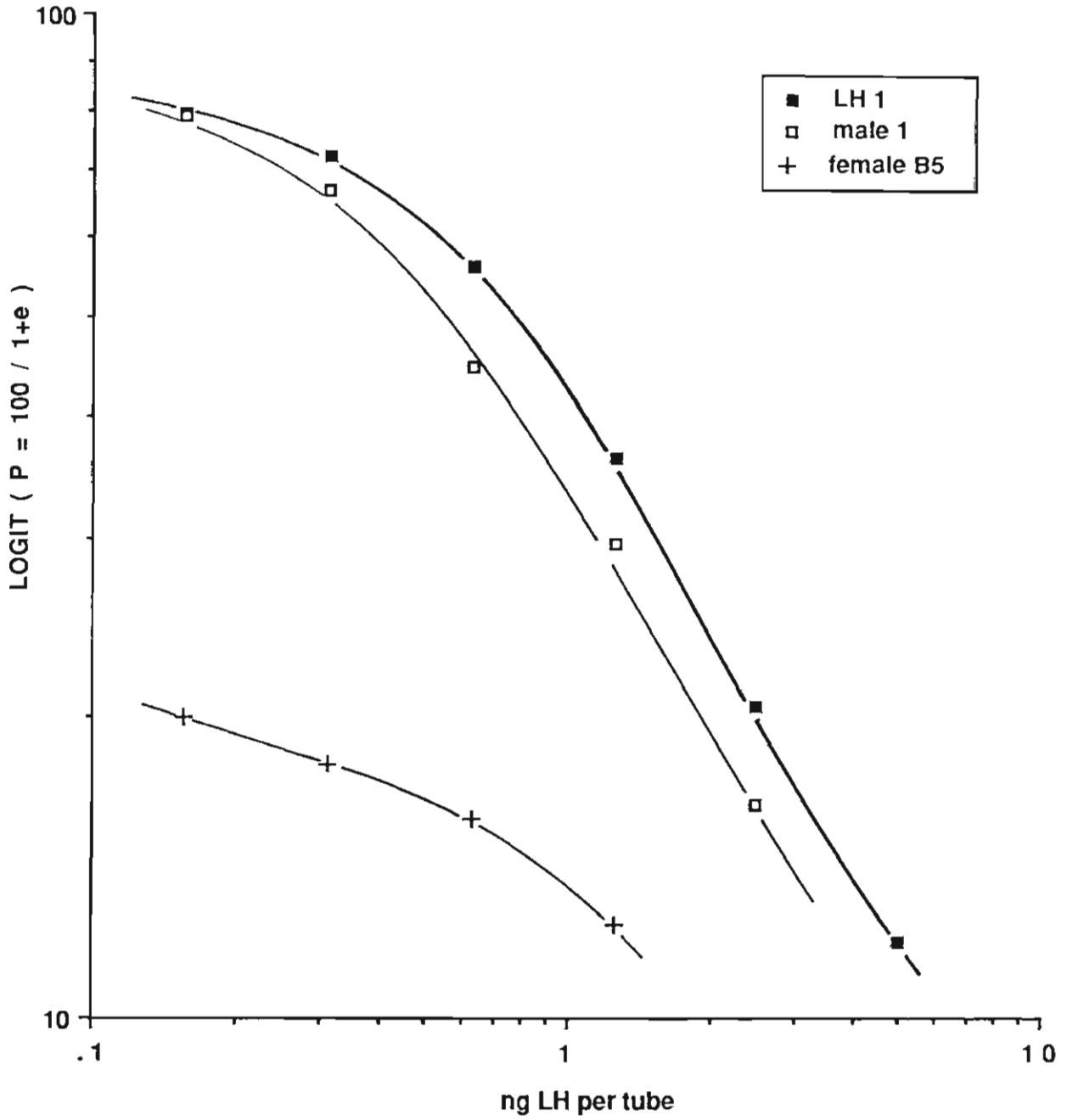

Fig. 3.8 Recovery of LH added to two pools of quoll plasma. Female B5 showed poor displacement.

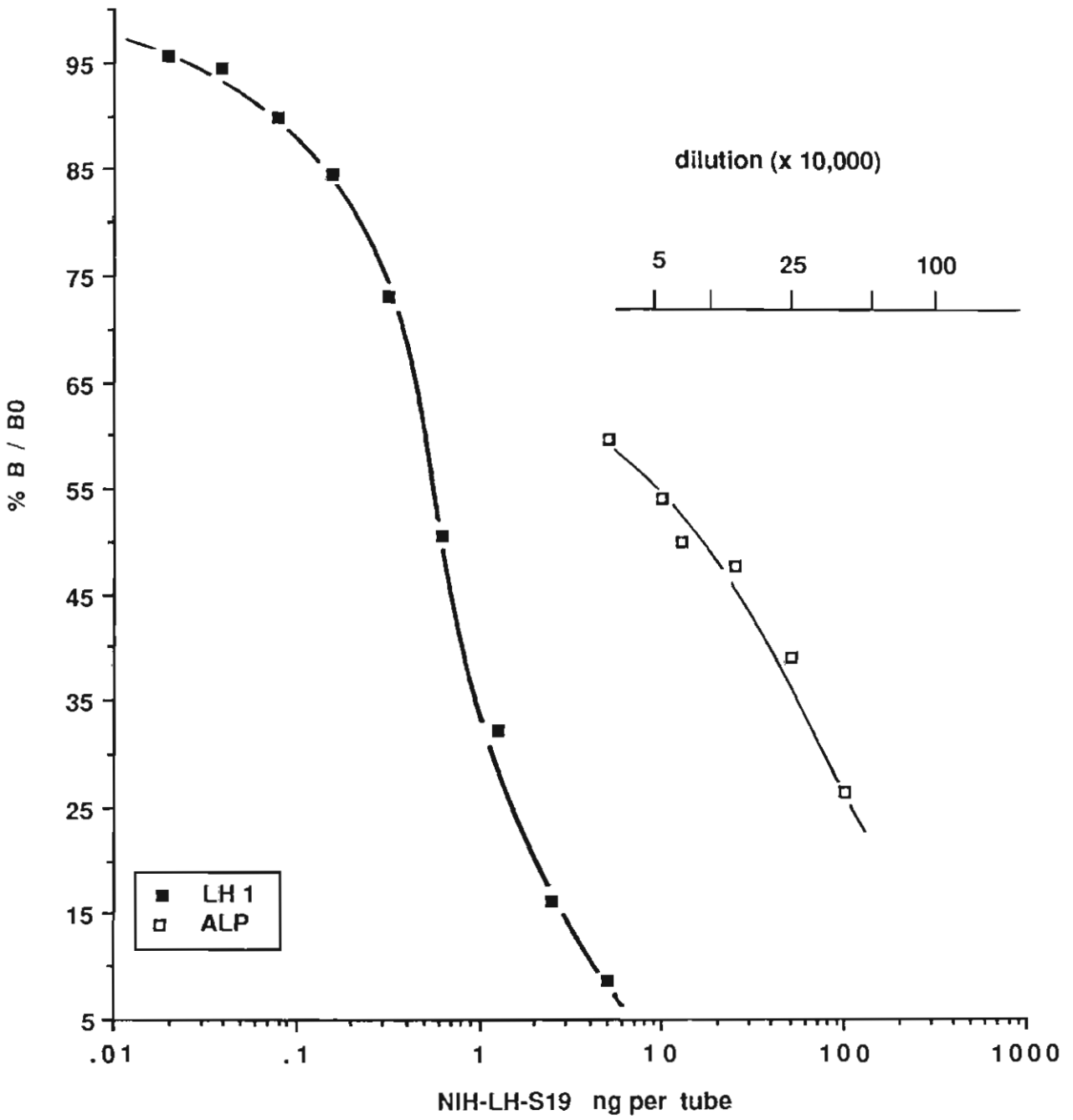

Fig. 3.9 Serial dilutions of anterior pituitary homogenate.

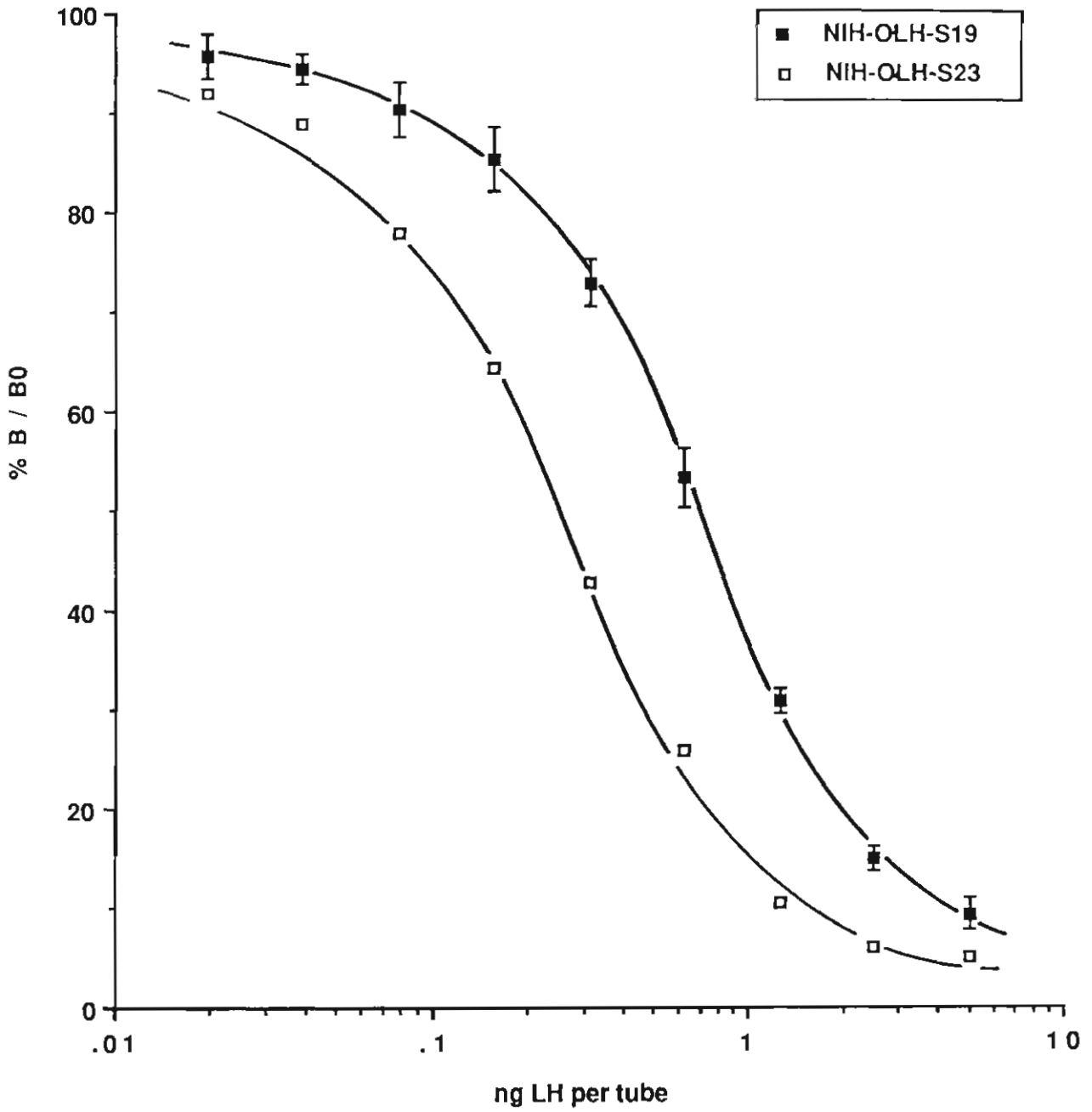

Fig. 3.10 LH standard curves using two different LH standards. NIH-OLH-S19 (± s.d., n=6).

included in every assay (ovx ♀, castrate ♂ and intact ♂) was between 8.2% and 19.0% (n=6). Only two LH assays were performed using standard NIH-o-LH-S23. Samples duplicated in these and previous assays showed a good correlation.

3.3.5 Standard Curve

Fig. 3.10 shows the standard curves when using NIH-o-LH-S19, NIH-o-LH-23 and GAR 8. Even though the $\%B/B_0$ for NIH-o-LH-S23 was lower than that of NIH-o-LH-S19, samples measured in both systems gave similar results and only a small conversion factor was applied.

3.4 RIA FOR TOTAL CORTISOL

3.4.1 Introduction

Cortisol is the major glucocorticoid found in the blood of most marsupial species although corticosterone and other corticosteroids have also been identified in significant concentrations (Oddie *et al.* 1976; McDonald 1977; Gower 1979; McDonald & Waring 1979; Sernia *et al.* 1979). The corticosteroids are formed in the adrenal cortex and are under the control of pituitary ACTH mediated by environmental influences and hormonal feedback (Turner & Bagnara 1976). In most mammals, cortisol is bound in the circulation to transcortin or corticosteroid binding globulin although small percentages may also be bound to albumin (Gower 1979).

There are a variety of methods available for measuring corticosteroids and corticosteroid binding proteins. Oddie *et al.* (1976) used double isotope dilution assays involving chromatography. With a changing emphasis on binding capacities and bound and free ratios of cortisol, techniques have since been employed using equilibrium dialysis and electrophoresis with steady-state polyacrylamide gels (Sernia 1978; Sernia *et al.* 1979; Bradley 1982).

In marsupial studies, blood corticosteroids are usually examined in relation to stress and breeding capacity (Weiss & McDonald 1967; Than & McDonald 1973; Janssens & Hinds 1981; Bradley 1982, 1987; Schmitt *et al.* 1988). Similarly, in this study the measure of cortisol was used to determine whether

levels suggestive of stress might occur in the Eastern quoll. Total plasma cortisol was measured using a RIA method based on Janssens and Hinds (1981). The assay procedure is similar to that for testosterone except that it uses a tritiated tracer and a slightly modified extraction procedure.

3.4.2 Materials and Methods

3.4.2.1 Tritiated Cortisol

Tritium-labelled cortisol Hydrocortisone [1,2,6,7-^{3}H(N)] or ^{3}H-C was obtained from New England Nuclear Research with a specific activity of 83.5Ci per mmol. The tracer was stored in a mixture of benzene and ethanol (9 : 1) in a lead container at -20°C. 25µl of ^{3}H-C containing approximately 10,000 cpm per 100µl was air-dried and reconstituted in 10.0 ml of assay buffer.

3.4.2.2 Antisera

The cortisol antibody (obtained from Dr. Ron Cox, Hormone Development group, Sydney, code no. 3368/ 09 08 78) was raised in sheep using the antigen 4-Pregnen-11,17α,21-triol-3, 20-dione-21-acetate, 3 CMO-BSA (Steraloids). A test dilution of 20,000x showed 100% cross reaction with cortisol and lower cross reactions with the following: cortisone (12.1%), 17 OH-progesterone (6.9%), corticosterone (1.5%), desoxy-corticosterone (0.8%) and progesterone (0.4%). The freeze dried preparation was equivalent to 1 ml of plasma and was made up to 10 mls with assay buffer and stored in aliquots of 100µl. The antibody (titrated on 14.2.86) was used at a 1: 20,000 working dilution and 1: 140,000 final dilution.

3.4.2.3 Cortisol Standard

Hydrocortisone (4-Pregnene-11ß,17α,21-triol-3,20-dione; F,Cortisol; mol. wt.362.5, anhydrous, 1gm Sigma) was diluted with absolute ethanol. Standards were prepared by serial dilution of a 20 ng per ml stock standard.

3.4.2.4 Assay Buffer and Charcoal

The assay buffer, 0.2 M Na_2PO_4 + 0.1% gelatin was prepared as described in sect. 3.2.2.4. The charcoal solution was the same as sect. 3.2.2.5 except that 0.625g of dextran was added (Dextran No. D-1390, Sigma).

3.4.2.5 Scintillation Fluid

A scintillation 'cocktail' was prepared by mixing 2 litres of toluene, 300 mls of dioxan and 84 mls liquifluor (Spectrofluor, Amersham Int.). Dioxan was added to facilitate the dissociation of labelled steroid. Samples were quenched in 10 mls of scintillant before counting.

3.4.3 Procedure

Quoll samples (100µl in duplicate) were extracted by rigorous vortexing with 400µl of absolute ethanol for 3 x 10 seconds. The samples were centrifuged at 3,000 rpm for 15 minutes at 4°C. The supernatant was poured into fresh tubes, the pellet resuspended in 400µl of ethanol and the extraction process repeated. The two supernatants were pooled (total vol. ~ 800µl) and a 600µl aliquot dried down under air at 50°C. The samples then received 500µl of assay buffer and were left to equilibrate at room temperature for 2 hours.

Standards (in triplicate) and quoll samples received 100µl of ^{3}H-C and 100µl of antibody (except total counts and non-specific binding tube where the incubation volume was made up to 700µl with assay buffer). The assay was left overnight to incubate in an iced water bath at 4°C.

Some charcoal suspension (200µl) was added to each tube (except total counts), vortexed and left at 4°C for 30 minutes. The samples were centrifuged at 3,000 rpm for 15 minutes at 4°C. 500µl of supernatant was mixed with 10mls of scintillation fluid and samples were left at room temperature for at least 2 hours before counting for 1 minute in a Beckman Scintillation Counter.

3.4.4 Validations

3.4.4.1 The Need for Extraction

Two standard curves were prepared; only one curve was extracted prior to assay but both were air dried and equilibrated in buffer. There was little

difference between the two curves and little or no interference from components in the buffer salts occurred (Fig. 3.11). The cortisol standards were therefore not extracted prior to assay.

3.4.4.2 Extraction Efficiency

The efficiency of extracting cortisol was measured using the same technique as for testosterone (sect. 3.2.4.4). A double extraction with ethanol returned 70.6% to 78.8% of the hormone added and a correction factor for 75.0% efficiency was applied.

3.4.4.3 Effect of Plasma on the Standard Curve and Recovery

Two standard curves using cortisol standards, equilibrated and extracted either through stripped quoll plasma or normal quoll plasma, were compared to a buffer standard curve (Fig. 3.12). The two quoll curves were not superimposed with the buffer curve but were displaced to the right. The extent of displacement was unlikely to be accounted for by endogenous cortisol and appeared to be a plasma effect similar to that which was noted in the testosterone and LH assays. Sheep plasma was not available as a substitute substrate and therefore this effect merely had to be noted and calculated for. The two quoll curves were parallel to the cortisol standard curve which indicates that the recovery of cortisol was good.

3.4.4.4 Dose Response

Three quoll plasmas, L0♀, 15.9.83, 1♂, 8.9.83 and L1♀, 15.9.83, were titrated in duplicates of 12.5µl, 25µl, 50µl and 100µl and compared on a standard curve (Fig. 3.13). The parallelisms were good although L0♀ showed relatively poor displacement. Volumes of 25µl or greater of plasma were required to achieve effecient extraction.

3.4.4.5 Sensitivity and Accuracy

The cortisol standard curve was sensitive to between 700 and 880 pg cortisol per ml of plasma. Intra- and inter-assay variations were not measured but the duplication between standards for 6 cortisol assays was very good and is reflected in the small deviation of the standard curve (see section 3.4.5).

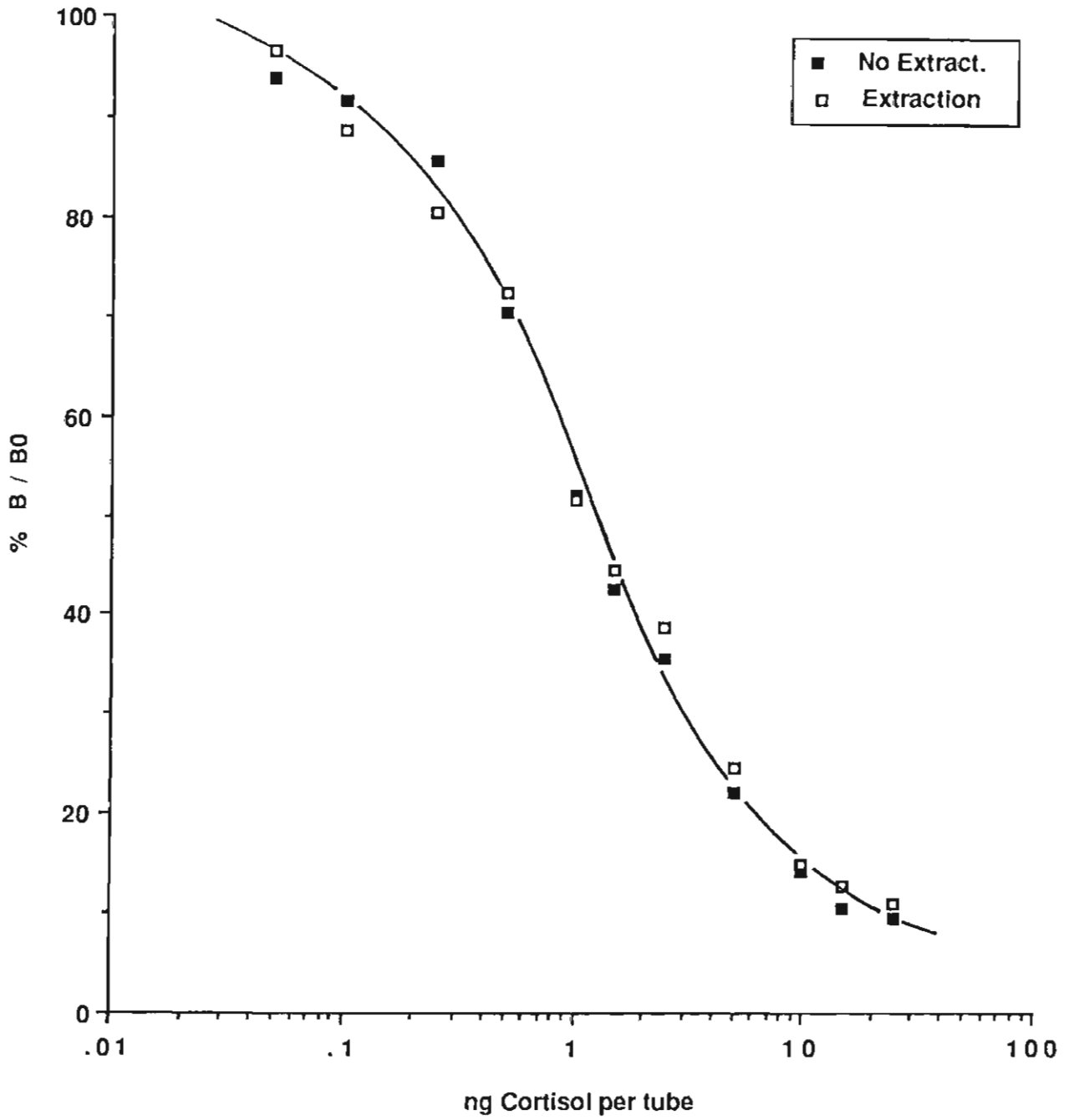

Fig. 3.11 Cortisol standards, extracted versus non extracted. Superimposition suggests extraction is not required.

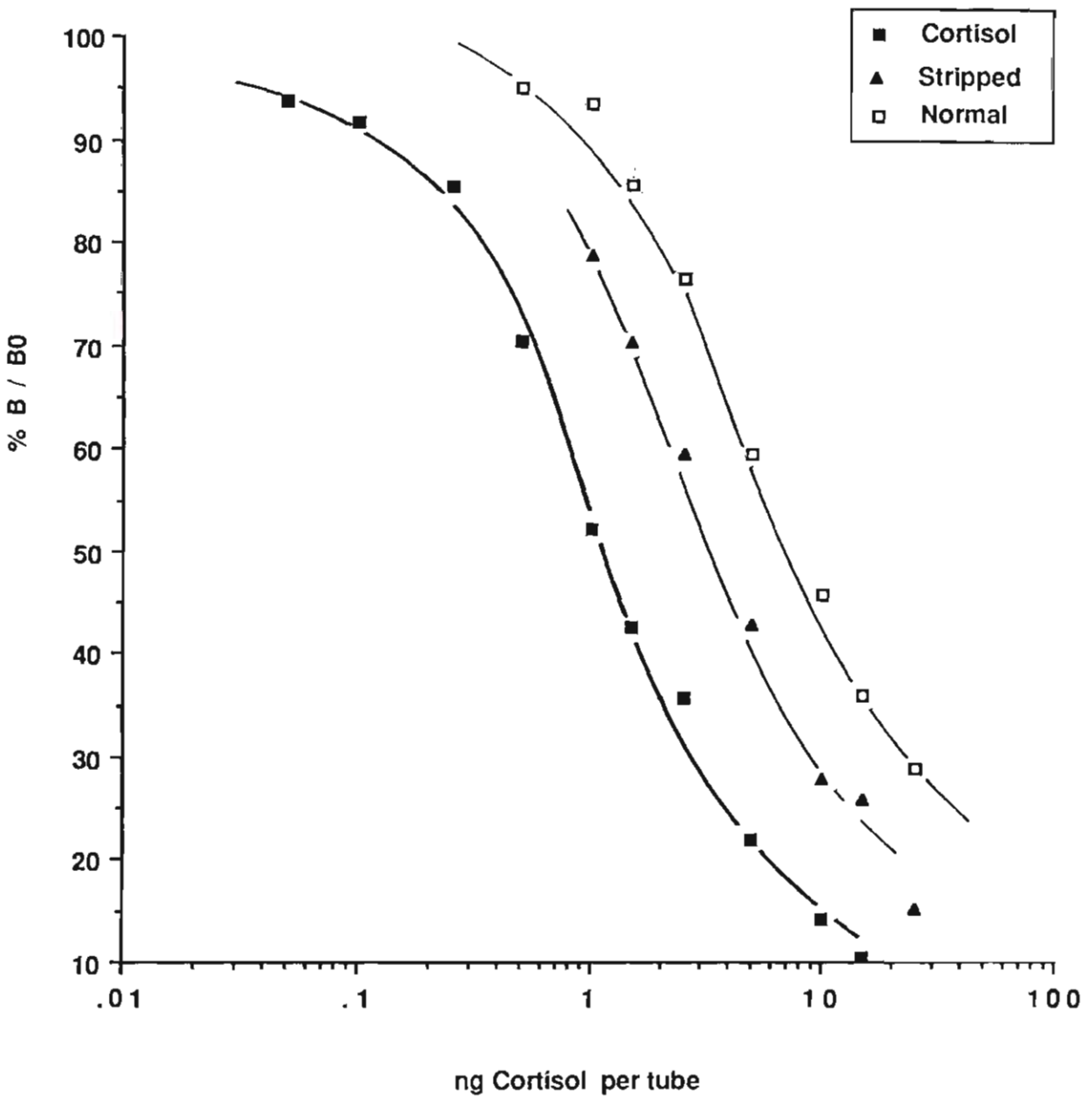

Fig. 3.12 The effect of quoll plasma on the cortisol standard curve. Good recovery but plasma effect noted.

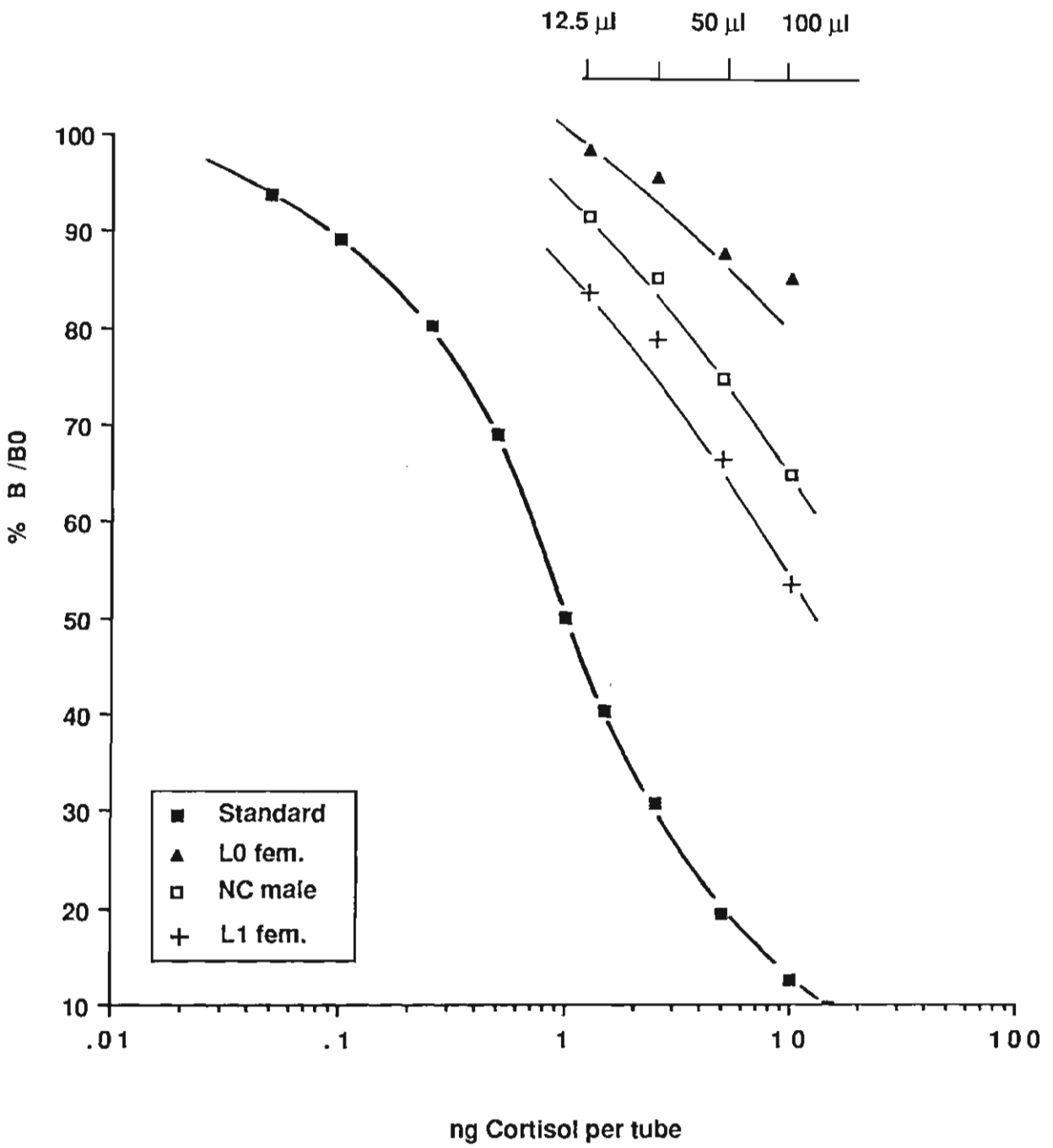

Fig. 3.13 Dose response curves for 3 quoll plasmas. Parallelisms were good although female L0 showed little displacement.

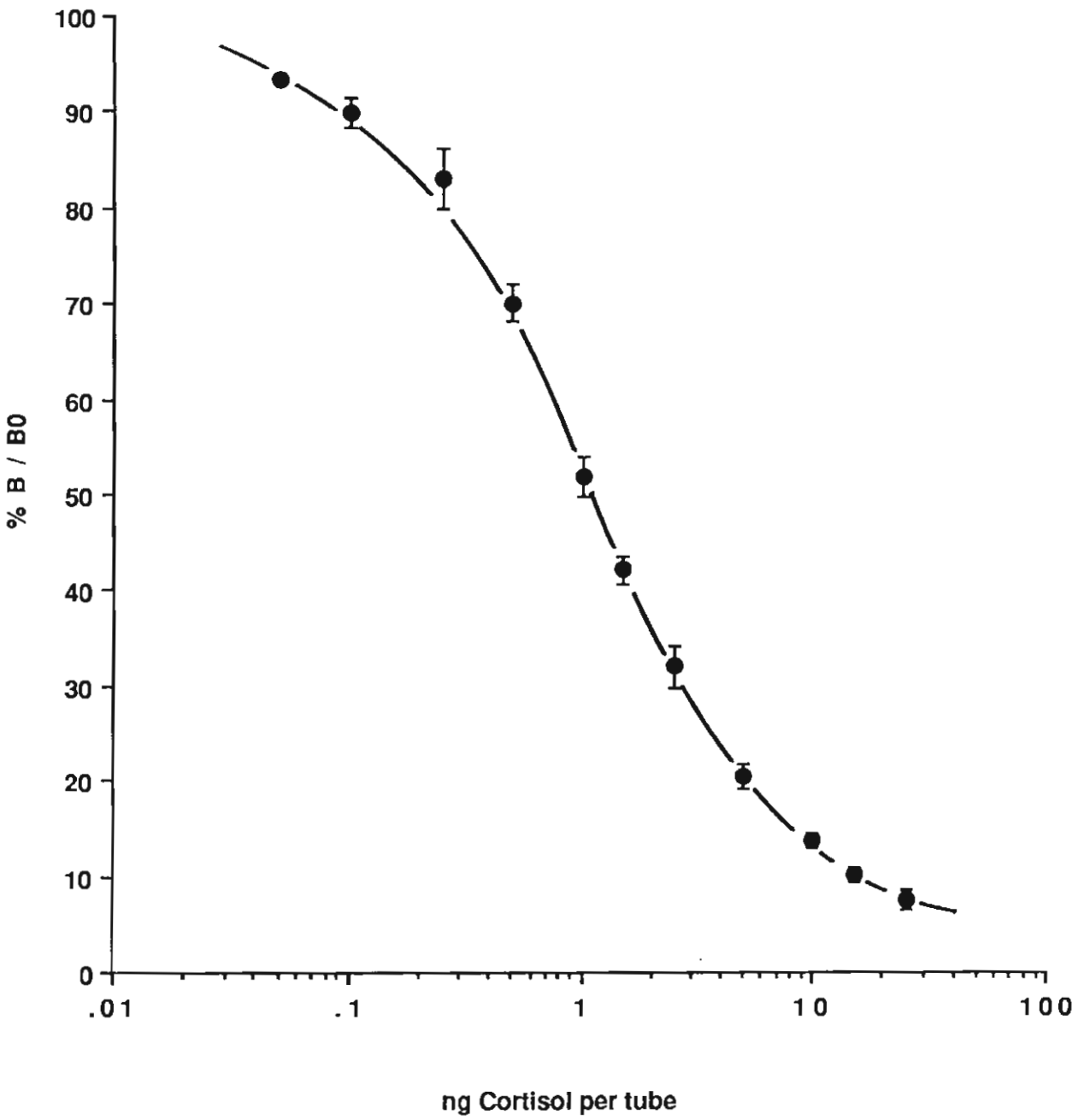

Fig. 3.14 Cortisol standard curve (± s.d., n=6).
Little variation occurred.

3.4.5 Standard Curve

Fig. 3.14 shows the standard curve representing the mean of 6 cortisol assays. The mean non-specific binding was 2.6 ± 0.8% ($\bar{x}$ ± s.d.) and the mean binding at zero standard was 56.8 ± 4.8% ($\bar{x}$ ± s.d.).

3.5 RIA FOR PROGESTERONE

3.5.1 Introduction

Progesterone is an ovarian steroid secreted by the corpus luteum usually under the influence of LH. Progesterone is responsible for the maintenance of pregnancy as it relaxes the smooth muscle of the uterus and reduces the excitability of the myometrium (Baird 1984). Progesterone has a particularly interesting role in the pregnancy of macropodid marsupials that exhibit embryonic diapause. Its concentration and effect during the oestrous cycle, gestation and diapause have been investigated by many workers (Lemon 1972; Sernia *et al.* 1980; Tyndale-Biscoe & Hinds 1984; Ward & Renfree 1984; Shaw & Renfree 1986). This study measured progesterone in wild and captive female quolls throughout the breeding and non-breeding period.

3.5.2 Materials and Methods

The methods used in the RIA for progesterone followed Hinds (1983) who was responsible for validating the assay for the Eastern quoll. The tracer, antibody (AS334) and standards also followed that of Hinds (1983). Only one assay was required to measure all the female samples in this study.

3.5.3 Standard Curve

The progesterone standard curve is shown in Fig. 3.15. The non-specific binding was 1.2% and the binding at zero standard was 32.0%. The hexane solvent blank was negligible and the sensitivity of the assay was 43.5 pg per ml as indicated by the lowest standard measured.

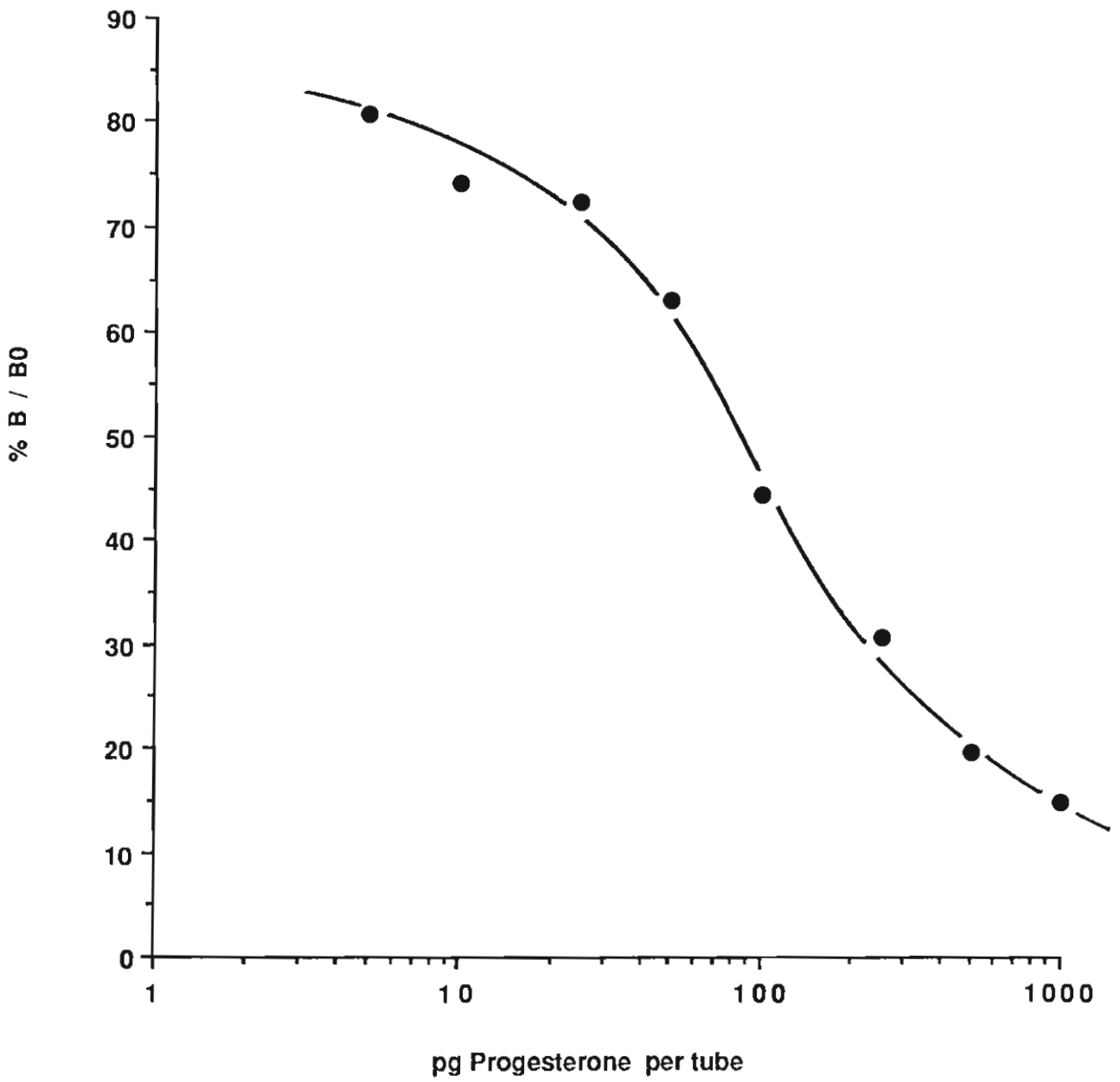

Fig. 3.15 Progesterone standard curve.

3.6 RIA FOR PROLACTIN

3.6.1 Introduction

Prolactin is secreted by the anterior pituitary gland and is associated with lactation and ovarian steroidogenesis in the female mammal. Although prolactin may be detected in the male, its functions are not well understood. In macropodid marsupials, prolactin acts to suppress the corpus luteum and progesterone secretion during embryonic diapause (Sernia *et al.* 1980; Stewart & Tyndale-Biscoe 1982; reviewed by Tyndale-Biscoe & Renfree 1987). In this project, prolactin was measured in wild and captive female quoll during late pregnancy and through the lactation period.

3.6.2 Materials and Methods

Prolactin NIH-OPRL-14 was iodinated as described in Appendix A.3. Antibody 1 was raised in a guinea pig to human prolactin and antibody 2 raised in a donkey and mixed with normal guinea pig serum. The assay used the same standard (OPRL-14) and buffers as Hinds (1983) and Hinds and Merchant (1986) and has been validated by these workers. One assay was sufficient to measure all the female samples.

3.6.3 Standard Curve

The prolactin standard curve is shown in Fig. 3.16. The non-specific binding was 2.4% and the zero standard had a maximum binding of 50.3%. Five quality controls used by Hinds (1983) were included in this assay (200 day lactating ♀, ovx ♀, intact ♂, castrate ♂ and ovx lactating ♀) and the results obtained in this study compared favourably with her values. The assay was sensitive to 2.2 ng (displacement of first standard) and NIH-PS-14 was converted to NIH-PS-12 equivalents (Hinds & Merchant 1986) by multiplying by 11.3.

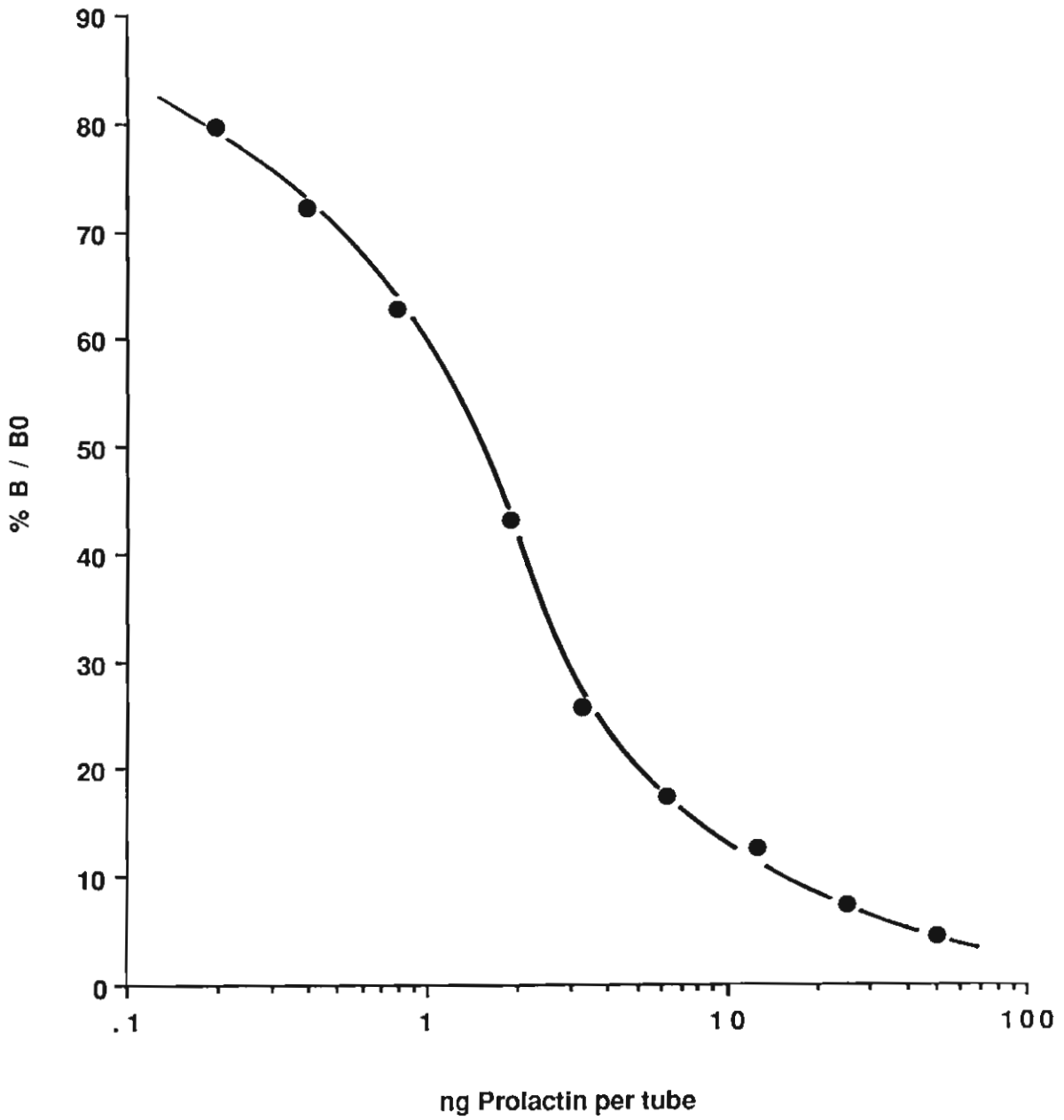

Fig. 3.16 Prolactin standard curve.

CHAPTER 4

SEASONAL CYCLES IN THE WILD

4.1 INTRODUCTION

The Eastern quoll has a well defined breeding season in Tasmania and mating occurs around late May to early June each year. The young are weaned and disperse into the population during the summer months from December to February.

Godsell (1982) reported that the density of quolls within her study area fluctuated seasonally and that the fluctuations were, in part, related to reproductive activity. She found the highest densities of animals occurred in summer when juveniles became trappable and the lowest population numbers in winter immediately following the breeding season. Dispersal of juvenile quolls and mortality, particularly female mortality, were considered the main factors responsible for the population decline in winter. Godsell reported that the influx of transient males into her study area during the breeding season was not accompanied by an increase in the number of females, therefore the ratio of males to females increased greatly at mating time. Godsell concluded that there were behavioural differences in the roles of the sexes in population regulation and that these behavioural differences resulted from differing requirements of each sex, namely "...mates for males and dens for females."

Godsell (1983) found that the body weight and scrotal width of adult male quoll fluctuated seasonally and that both were maximal prior to breeding. Fletcher (1985) noted that there was a seasonal variation in the size of the accessory glands of the quoll and that maximal weights of prostate and Cowper's glands occurred before breeding. During breeding these glands regressed in weight and showed dramatic histological deterioration in cell size and structure.

All male marsupials investigated so far show a rise in androgens in reponse to the breeding season although the duration and intensity of the rise appears species specific. Total androgens including plasma testosterone, have been measured in *Antechinus stuartii, A. swainsonii, A. flavipes, A. minimus,* and also in *Sminthopsis crassicaudata, Phascogale calura,*

Dasyuroides byrnei, and *Dasyurus hallucatus* (Bradley *et al.* 1980; McDonald *et al.* 1981; Fletcher 1983; Kerr & Hedger 1983; Wilson & Bourne 1984; Bradley 1987; Schmitt *et al.* 1988). Comparative information is available for males from other marsupial families, such as *Isoodon macrourus* (Gemmell *et al.* 1985), *Trichosurus vulpecula,* (Carrick & Cox 1977; Curlewis & Stone 1985a,b; Gemmell *et al.* 1986), *Didelphis virginiana,* (Harder & Fleming 1986) and some Macropodinae (Lincoln 1978; Catling & Sutherland 1980; Inns 1982).

Female marsupials show a number of physiological and endocrinological responses to breeding and in preparation for parturition. In the female Eastern quoll oestrus precedes ovulation by about 5 days with the luteal phase occupying about 60% of the oestrous cycle (Hill & O'Donoghue 1913). The gestation length according to Fletcher (1985) is 21 $\pm$ 2 days. Pregnancy occurs within the luteal phase and parturition coincides with regression of the corpora lutea. Lactation suppresses the subsequent follicular phase and the corpora lutea of pregnancy degenerate to corpora albicanta. The quoll shows a rise in plasma progesterone about 10 days after oestrus and about 10 days prior to birth (Hinds 1983). Birth occurs after a dramatic decline in progesterone concentration, a pattern that is similar to many other female marsupials. If the female quoll loses her young early in lactation she will return to oestrus and may re-mate to produce a second litter in the same season (Green & Eberhard 1983; Fletcher 1985).

The aim of this work is to monitor the endocrine and physiological changes associated with the natural breeding cycle of the Eastern quoll and to discuss the changes in relation to social events within the population. The results from this study can then provide the basis or control for analysing hormonal and body profiles of animals maintained under artificial breeding regimes in captivity (discussed in later chapters).

The reproductive status of males is determined by measuring plasma LH and plasma testosterone levels, body weight, scrotal size and pes length. Reproductive activity of the female is assessed from plasma LH, progesterone and prolactin levels, body weight, pes length, pouch development and pouch young measurements.

4.2 MATERIALS AND METHODS

General information regarding trap site, trapping method, blood collection and field measurements has been presented in Chapter 2.

4.2.1 Population Classification

The population classification used in this study was based on 4 successive nights trapping per month. The population classes were defined as: resident : animals caught in 3 or more consecutive months; neighbour : animals that were trapped throughout the year but not as consistently as resident animals (it is likely that neighbouring animals inhabit nearby areas and have some contact with the resident population); transients: animals caught during 1 or rarely 2 successive monthly sessions but not subsequently, and juveniles: young born and weaned during the current trapping season.

Animals of unknown status were those caught either solely in the first trapping month or during the final trapping month.

4.2.2 Identification of Age Classes

Animals were categorised as either adults or first year animals (first year includes juveniles). Adult animals were those entering at least their second breeding season (12 months or older). Juvenile and first year males (aged 11 months or younger) were distinguished from adult males on the basis of body weight, pes length and testes measurements. At first capture they had body weights between 600 and 900 grams, scrotal heights between 13.6 and 19.0 mm and scrotal widths between 16.5 and 19.5 mm. Juvenile females were identified by a virgin pouch and a body weight ranging between 400 and 700 grams.

Juveniles first appeared in the study area between December and February, those in February being larger and heavier than those in December. By April and May most first year animals had approached adult body size and pouch condition.

4.3 RESULTS

This chapter will discuss the trapping results from December 1983 until January 1985 as this represents consecutive trapping months surrounding one breeding season. Information obtained from either side of these dates will be mentioned to confirm results. Animals were measured on their first day of capture, those retrapped on subsequent nights were not remeasured.

4.3.1 Sequence of Reproductive Events

Mating and copulation occurred during the end of May and the first week of June. Newborn young were observed in the pouch by late June and early July. Young remained in the female's pouch during July and August but were no longer present by the trap session in September. Females had enlarged mammary glands from July until January but during December and January, pouches and mammary glands showed evidence of regression. Juvenile quolls entered the population from late November to February and probably were weaned during these months. Fig. 4.1 summarises these events.

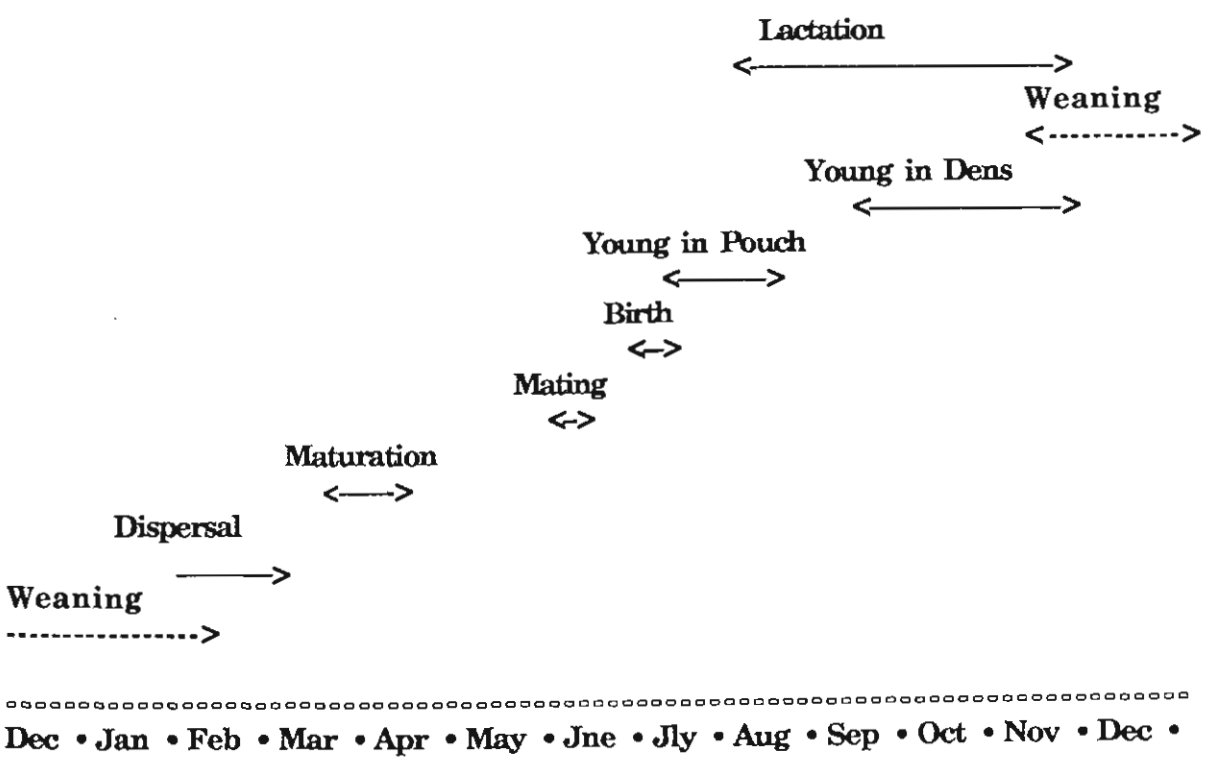

Fig. 4.1 Sequence of reproductive events for Eastern quoll at Cradoc.

4.3.2 Population Structure

From December 1983 until January 1985 samples were obtained from 223 quolls. This total comprised 53 individuals, 37 males and 16 females. Table 4.1 outlines the residency status for animals based on the frequency and regularity of captures over the 14 month period.

The number of individual males captured varied over the year but the number of resident males remained fairly constant. Of the 10 resident males, 2 adults (nos. 17 & 44) were captured in 12 and 13 of the trapping months. Four males (nos. 45, 46, 64 & 68) were trapped consistently in the area, initially as juveniles and then throughout the breeding period. Three neighbouring males were trapped as juveniles and 3 were adult males. Of the 16 transient males most were captured in only 1 trapping month. Seven were juveniles dispersing after weaning and 9 were adult males captured during either June or July.

Table 4.1 Residency of the quoll population at Cradoc (number of animals shown)

Status	Resident	Neighbour	Transient	Unknown
Adult males	6	3	9	0
Juvenile males	4	3	7	5
Total number of males	10	6	16	5
Adult females	4	0	4	0
Juvenile females	4	0	2	2
Total number of females	8	0	6	2

Eight female quoll were resident and maintained a continuous occupancy in the area. Six transient females were captured, 2 were juveniles dispersing after weaning and 4 were adults caught in December or January at

the end of the lactation period.

Five juvenile male and 2 juvenile female quolls were trapped during the last session in January 1985 and were of unknown status.

4.3.3 Movement of Animals

A core of about 15 animals were trapped consistently in the study area (Fig. 4.2a). The proportion of males to females was similar from December until March but the number of males increased surrounding the breeding season (Fig. 4.2b). Nine transient males (8 individuals) were trapped between June and July. By August, the population had returned to a core of residents with no new animals being trapped until the emergence of juveniles later in the year.

Eight resident females were trapped continuously over the study period but only 2 individuals were captured in June. While the trappability of females may have been reduced during the mating period, pregnancy, lactation and leaving the young in dens appeared to have had little effect on being trapped.

4.3.4 Males

4.3.4.1 Weight

Male quoll displayed a seasonal cycle in body weight (Fig. 4.3). Adult males showed an increase in body weight from December (mean 1.10 kg) to March (mean 1.48 kg), the largest increase occurring between February and March. Between March and May there was little change in body weight. Between May and June (mating period), every recaptured male lost between 75 and 175 grams in weight and this represented a reduction of 19% to 24% of total body weight. Males were significantly heavier in May than in December ($t=4.48$, d.f.10, $p<0.005$).

Juvenile quoll entered the population with body weights ranging between 600 and 900 grams (14 out of 19 juveniles). Three animals captured with body weights less than 600 grams were 'infants' and probably not yet weaned. Juveniles increased in body weight after weaning but in April were still significantly lighter than adult males ($t=2.90$, d.f.9, $0.005<p<0.01$). In May, juvenile weights were lighter but not significantly different from adult body

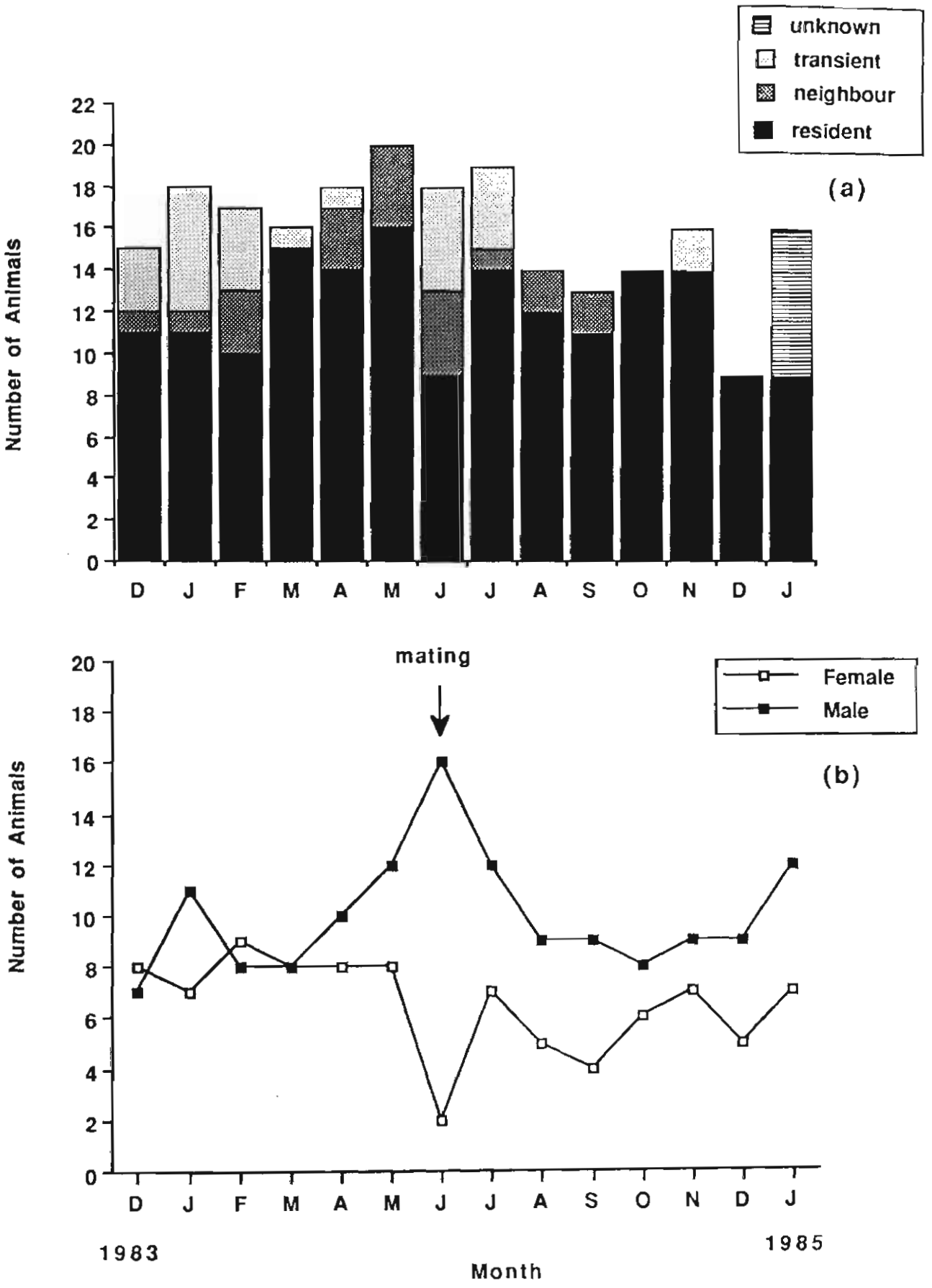

Fig. 4.2 Number of Eastern quoll captured each month according to residency status (a) and sex. (b)

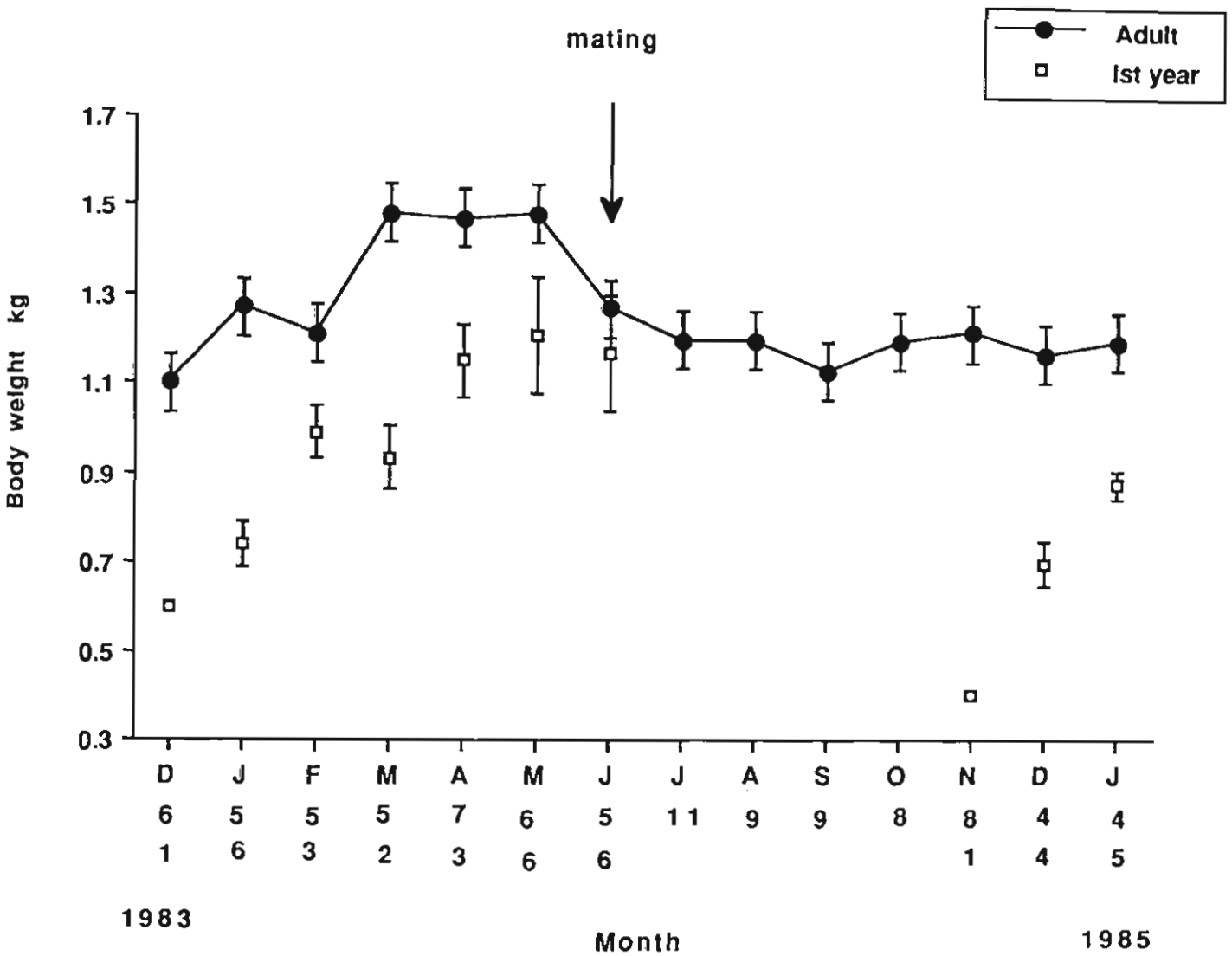

Fig. 4.3 Mean body weight for adult males and first year males (± s.e.). There was no difference in body weight between males in June. Transient males not included because their age was unknown. Adult sample size below month, 1st year males below adult.

weights ($t=1.81$, d.f.10, $0.05<p<0.1$).

Every first year male lost weight during the breeding season but, unlike older males, most showed an increase in weight after mating. This trend continued until the end of the year. One juvenile (no. 68) showed little gain in body weight over the study period and it is possible that this noticeably smaller male was the result of a second mating and pregnancy.

Transient males captured during June and July were significantly heavier than resident and neighbouring males. The mean body weight for transient males in June was 1.46 kg compared to 1.22 kg for resident and neighbouring animals ($t=1.88$, d.f. 14, $0.025<p<0.05$). In July, the difference was 1.32 kg compared to 1.05 kg ($t=2.11$, d.f. 10, $0.025<p<0.05$). In real terms, transient males were 20% to 25% heavier than resident and neighbouring males.

4.3.4.2 Scrotal Size

Scrotal size varied throughout the year with a cycle of development and regression similar to the cycle for body weight. Over the year scrotal size of adult males varied a total of 10.3 mm in width (49%) and 6.7 mm in height (38%) (Table 4.2). The variation in scrotal size is accounted for by monitoring the testes index calculated by:

$$\text{Testes index} = \frac{\text{scrotal width (mm) x scrotal height (mm)}}{100}$$

Testes index had a yearly range from 4.1 to 7.4 in adult males with the largest index occurring between April and May (Fig. 4.4). Testes index declined after May and continued to decline until November.

In December and January first year males had a testes index range between 2.2 and 4.3. Juvenile scrotums increased dramatically in width and height between March and April and it is likely that males attained sexual maturity during this time. Scrotal size declined after April and throughout the breeding season in a pattern that was similar to the decline seen in adult males.

Transient males tended to have larger scrotal dimensions than resident males but these differences were not significant during the breeding season (height & width, June, $0.05<p<0.1$, $p<0.4$; July, $p<0.375$, $p>0.4$).

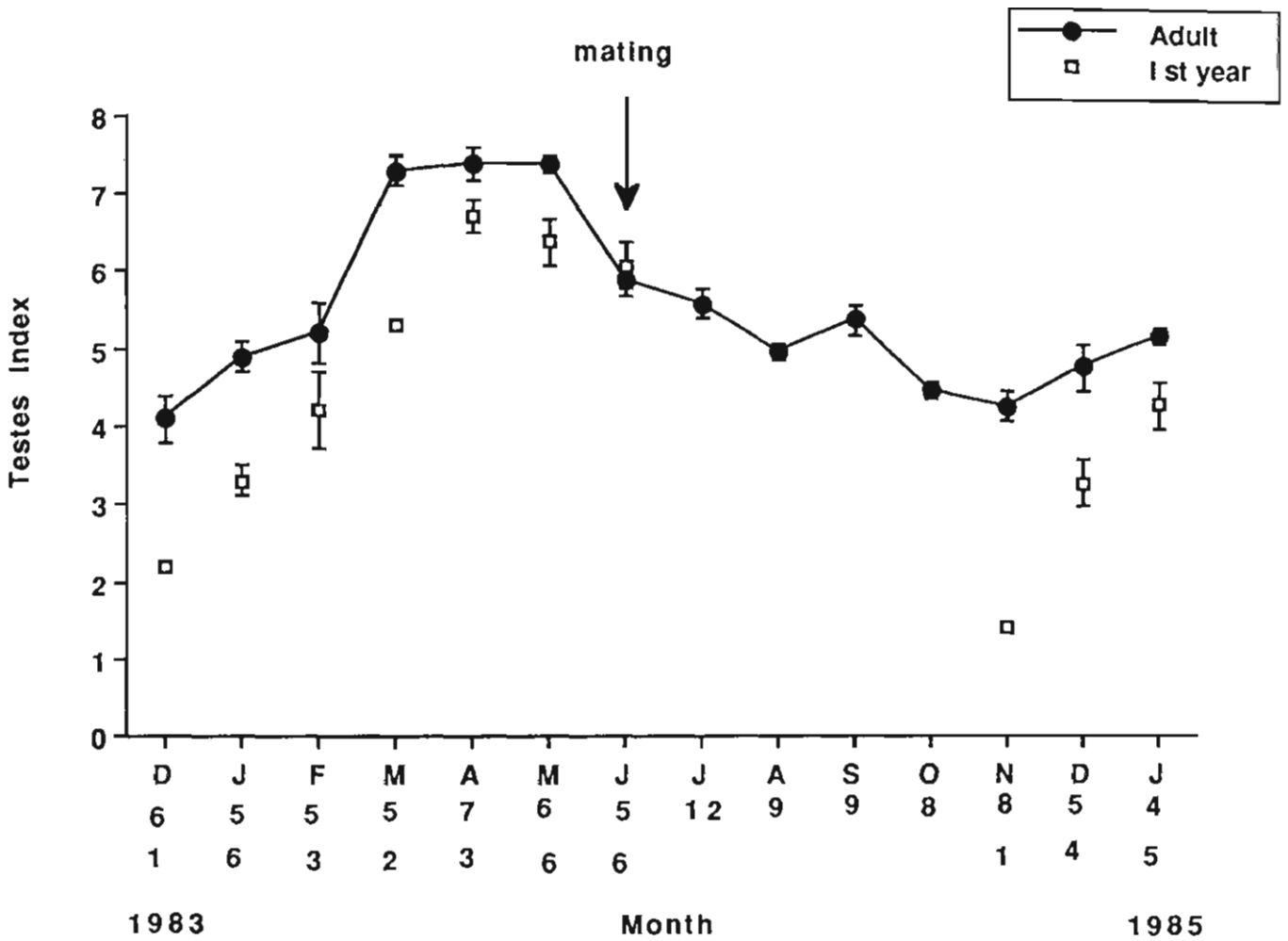

Fig. 4.4 Mean testes index for adult males and first year males (± s.e.). There was no difference in testes index in June between the two groups. Adult sample size below each month, first year males below adult.

Table 4.2 Change in scrotal height, width and index over the year in adult and first year breeding male Eastern quoll (mean + s.d).

	Adult Males				First Year Males		
Month	n	height mm	width mm	index	n	height mm	width mm	index
Dec.	6	20.0 ± 2.6	21.0 ± 2.2	4.1	1	13.6 -	16.5 -	2.2
Jan. 84	5	20.7 ± 1.4	23.5 ± 2.9	4.9	6	16.5 ± 2.5	19.9 ± 1.4	3.3
Feb.	5	21.1 ± 1.0	25.2 ± 1.3	5.2	2	18.9 ± 0.9	22.0 ± 2.4	4.2
Mar.	5	24.2 ± 0.9	30.3 ± 1.3	7.3	3	18.8 ± 2.3	24.0 ± 4.9	4.5
Apr.	7	24.5 ± 2.0	30.1 ± 0.8	7.4	4	22.6 ± 1.9	29.8 ± 0.8	6.7
May	6	23.5 ± 1.0	31.3 ± 0.5	7.4	6	22.0 ± 1.4	29.0 ± 1.7	6.4
June	5*	21.0 ± 0.5	27.8 ± 1.3	5.9	6	21.5 ± 1.2	28.4 ± 2.5	6.1
July	12	20.7 ± 2.4	26.9 ± 1.5	5.6	-	-	-	
Aug.	9	19.4 ± 0.9	25.9 ± 0.8	5.0	-	-	-	
Sep.	9	20.0 ± 1.5	26.7 ± 0.8	5.4	-	-	-	
Oct.	8	17.8 ± 1.3	25.0 ± 0.9	4.5	-	-	-	
Nov.	8	17.9 ± 1.2	24.1 ± 1.1	4.3	1	11.6 -	11.6 -	1.3
Dec.	5	18.4 ± 1.7	26.1 ± 1.6	4.8	4	15.7 ± 2.1	21.0 ± 2.5	3.3
Jan. 85	4	19.8 ± 0.5	26.3 ± 0.5	5.2	8	18.0 ± 1.5	23.8 ± 1.9	4.3

* transient males not included because age unknown

4.3.4.3 LH

The cycle of LH secretion in adult and juvenile male Eastern quoll is presented in Fig. 4.5. The LH values recorded earlier in 1983 and later in 1986, support this profile and confirm that LH secretion has an annual cycle with the highest levels occurring during the breeding period.

Adult males had basal LH concentrations of approximately 1.0 ng per ml during the non-breeding period. A significant increase in LH occurred between April and May ($t=2.92$, d.f.17, $p<0.005$) and between May and June

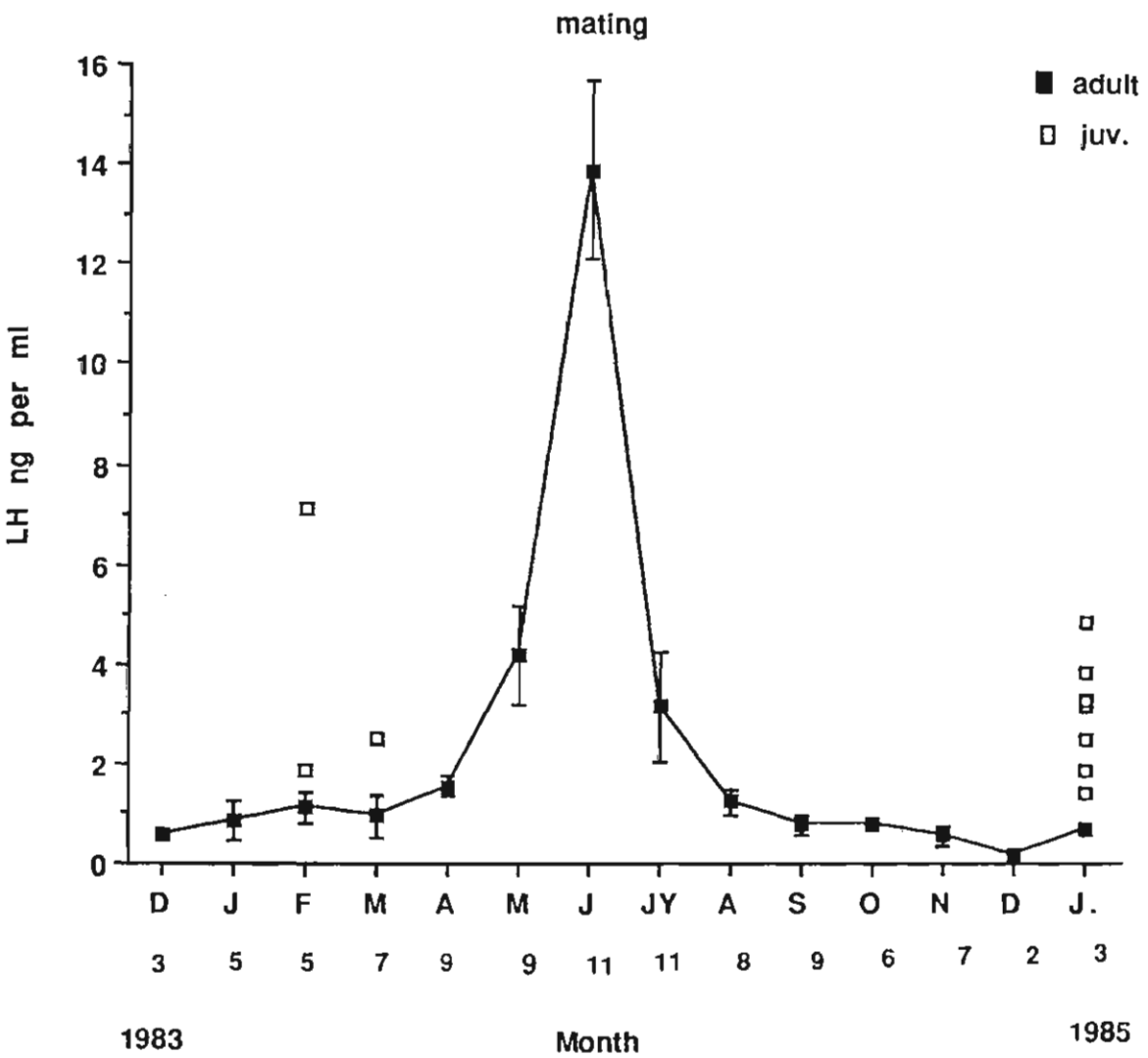

Fig. 4.5 Mean LH concentration (± s.e.) for adult male quoll. Individual juvenile levels are shown at time of first capture. Adult sample size below month.

(t=4.43, d.f.18, p<0.0005). The highest mean level of LH was 13.9 ±1.8 ng ($\bar{x}$ ± s.e., n=11) in June although one individual recorded a level of 23.4 ng per ml. LH levels decreased to a mean of 3.2 ng by July and by August had returned to basal levels.

Juvenile males had higher LH levels in their first month of capture than adults at that time. In January juveniles had a significantly higher mean LH level than adult males (t=4.07, d.f.12, p<0.005). Juveniles retrapped in the months following their first capture had LH values that were comparable to those of adults. For example, one juvenile male with a LH level of 7.1 ng in February had a level of 1.1 ng by April.

Transient males captured in June and July had significantly higher mean LH concentrations than resident and neighbouring males (June, t=3.35, d.f.8, p<0.01; July, t=3.37, d.f.10, p<0.005). These differences suggest transient males may sustain higher levels of LH for slightly longer periods than resident and neighbouring males.

4.3.4.4 Testosterone

Plasma testosterone concentration fluctuated around 1.0 ng per ml or lower in adult males for much of the year (Fig. 4.6). A significant increase in testosterone occurred between March and April (t=2.71, d.f. 17, p<0.01), April and May (t=1.81, d.f.21, 0.025<p<0.05) and between May and June (t=5.73, d.f.26, p<0.005). The highest testosterone levels were recorded during the mating period in June. The mean level was 5.0 ± 0.3 ng per ml ($\bar{x}$ ± s.e., n=15) with concentrations ranging between 2.5 and 7.6 ng. Testosterone levels decreased to a mean of 1.3 ng per ml by July and had reached basal levels by August. The levels recorded earlier in 1983 and later in 1986 highlight the annual cycle of testosterone secretion and confirm these ranges of concentrations throughout the year.

During December and January juvenile males had significantly higher testosterone levels than adult males (1984, t=3.49, d.f.16, p<0.0005; 1985, t=3.92, d.f.19, p<0.0005) (Fig. 4.6). Six juveniles retrapped in the months following weaning all had testosterone levels that were similar to adults. One male (no. 60) trapped in February had a body weight of 425 grams and a testosterone level of 0.65 ng per ml. When trapped again in March, the body weight had increased to 825 grams and the testosterone level was 2.1 ng per

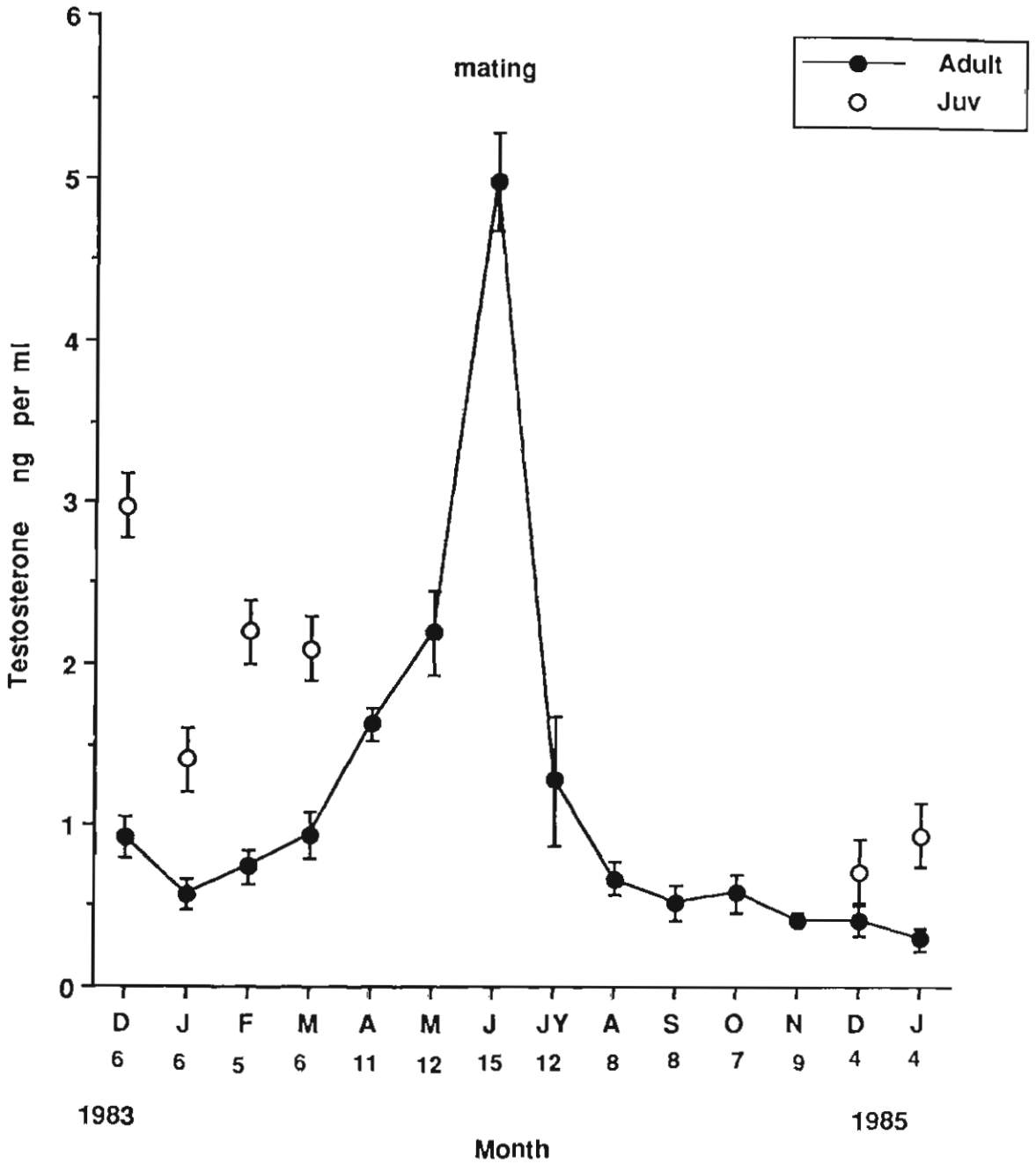

Fig. 4.6 Mean testosterone levels (± s.e.) for adult male quoll. Juvenile samples are for first capture only. Adult sample size below month, juveniles 3 to 5.

ml. It was likely that this animal was the result of a second pregnancy and subsequently weaned late in February.

Testosterone levels between transient males compared to resident and neighbouring males were not significantly different in June ($t=0.68$, d.f.14, $0.1<p<0.38$). During July, transient males had significantly higher levels of testosterone ($t=2.57$, d.f.10, $0.01<p<0.025$) compared to the basal levels measured in resident and neighbouring males (Fig. 4.7). This pattern of testosterone secretion is similar to and synchronised with the pattern described for LH secretion.

4.3.4.5 Profiles of Resident Male Quoll

Body and endocrine profiles are presented for three resident males trapped frequently over the study period. The LH and testosterone profiles for resident males no. 17 and 44 illustrate the sharp peak during breeding and the rapid return to basal levels by July (Fig. 4.8). Male no. 44 was significantly heavier than male no.17 (paired t-test, $t=9.63$, d.f.10 $p<0.0005$) but male no. 17 had significantly higher testosterone levels (paired t-test, $t=2.55$, d.f.10, $0.01<p<0.025$). There was no significant difference in testes index (Mann-Whitney, $U=53$, $p>0.05$) or LH levels (paired t-test, $t=0.29$, d.f.10, $0.375<p<0.4$) between the two males.

Juvenile male no. 45 increased in body weight after weaning to reach maximum body weight in May, several weeks before the breeding period (Fig. 4.9). The high level of testosterone in December coincides with the emergence into the population and the second high level with the mating period in June.

These individual profiles reflect the trends of the population in general and confirm the synchrony of the breeding cycle.

4.3.4.6 Synchrony of Male Cycles

The periodicity of the male cycles for body weight and testes index were similar (Fig. 4.10). Body weight and testes index increased after December and then stabilised between March and May. Both parameters gradually declined after May. In contrast, LH and testosterone levels gradually increased after March to show an obvious peak during the breeding season in June. Hormone levels rapidly returned to basal levels after July.

These results show that peaks in body weight and testes size do not

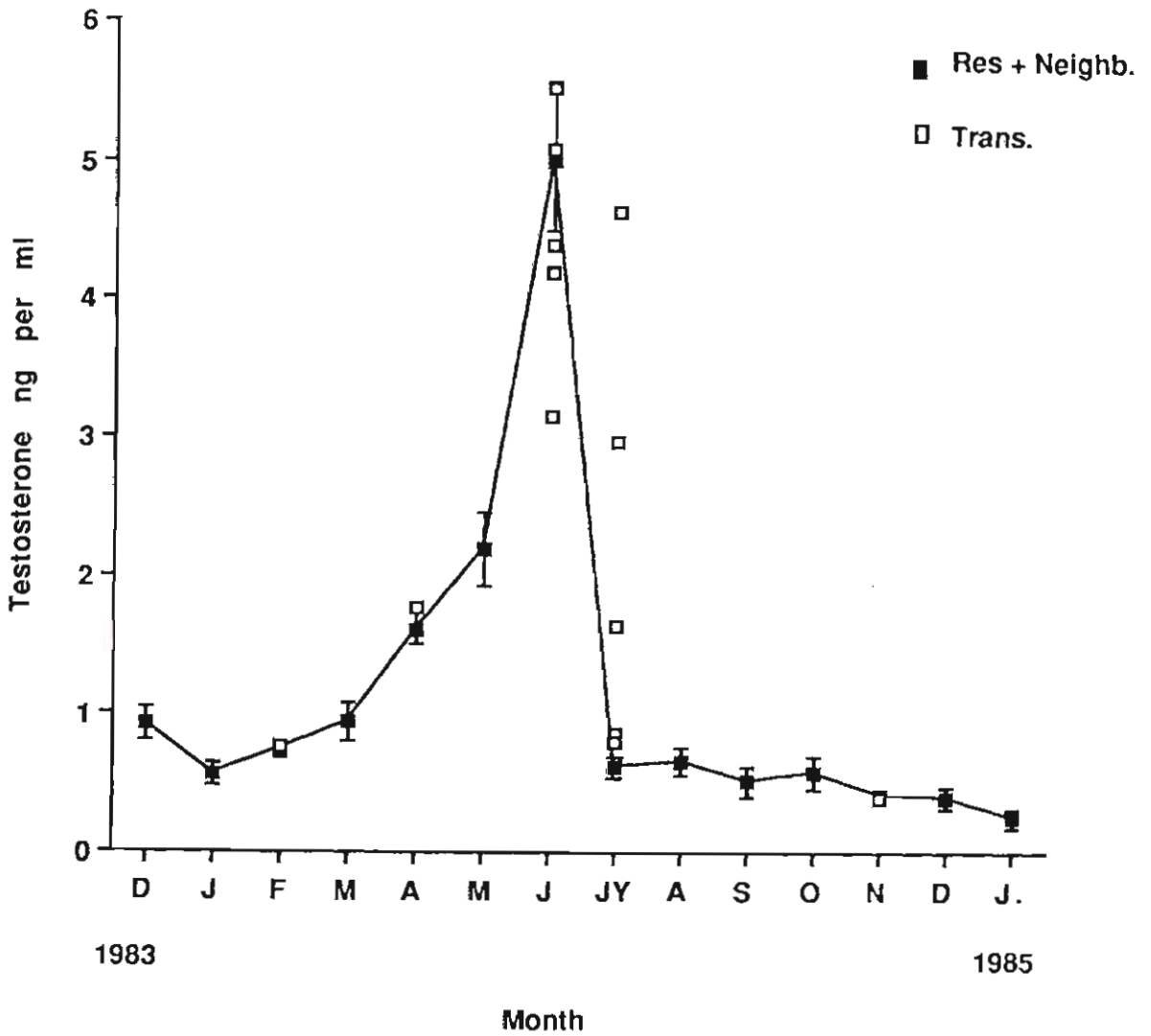

Fig. 4.7 Mean testosterone levels (± s.e.) in resident and neighbouring quolls. Individual transient males are shown. Prior to and during mating there was no difference between testosterone levels. In July, transient males had significantly higher testosterone levels. Sample number is the same as Fig. 4.6.

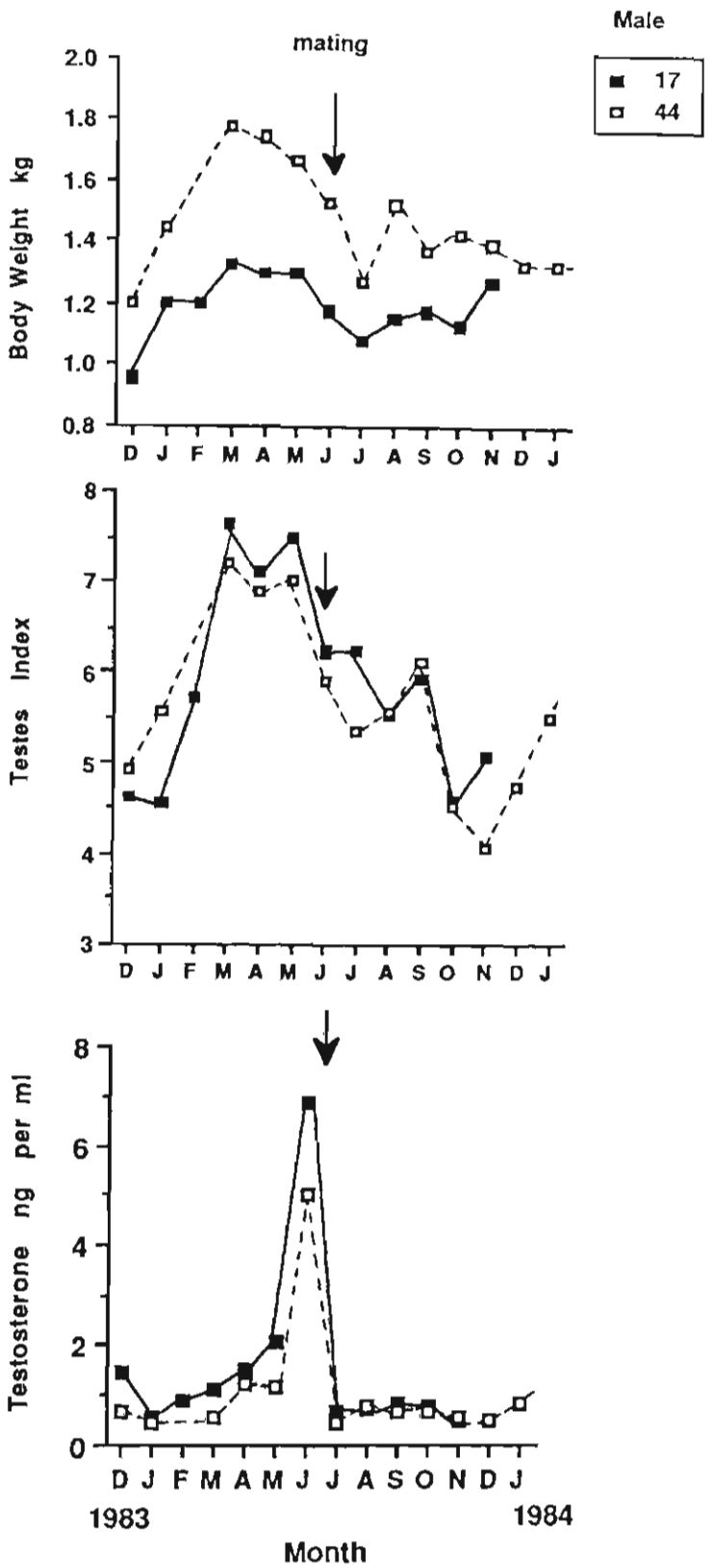

Fig. 4.8 Testosterone and body cycles for two adult resident males. Male no.44 was much larger than no. 17 but little difference occurred between their testes index or testosterone profile.

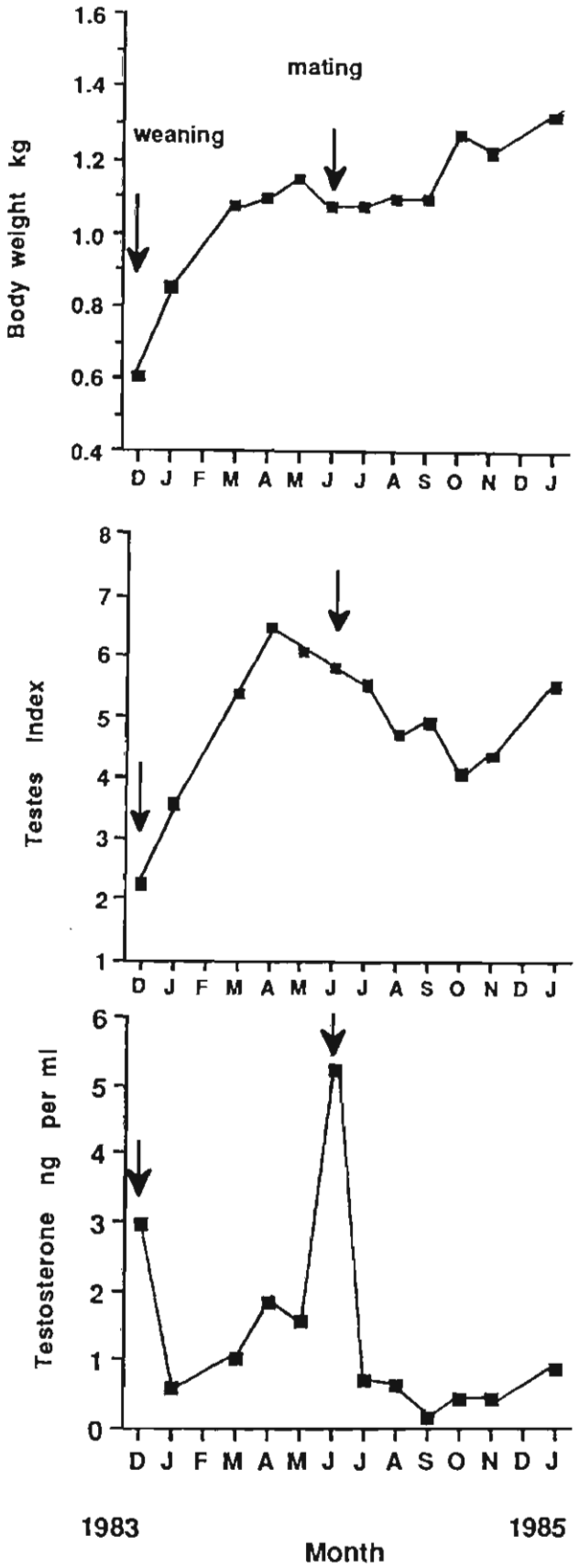

Fig. 4.9 Weight, testes index and testosterone cycles for male no. 45 that remained in the area after weaning. Note the high testosterone level in December.

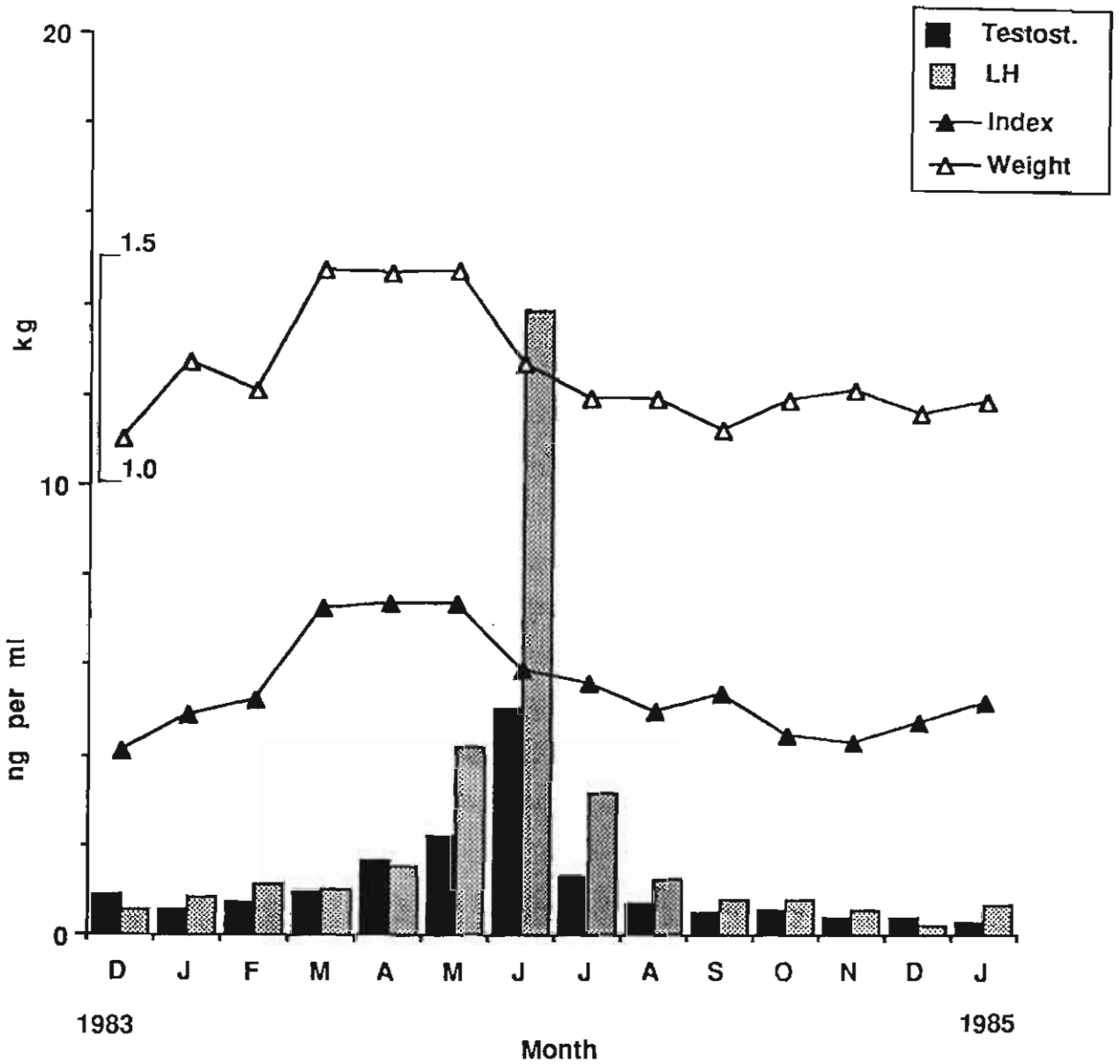

Fig. 4.10 Synchrony of mean body weight, testes index, LH and testosterone in adult male Eastern quoll. Body weight and testes size were maximal prior to hormonal peaks. Sample size as for earlier figures.

coincide with peaks in LH and testosterone concentration and that sexual maturity is attained independently of high levels of hormone.

4.3.7 Females

4.3.7.1 Weight

Adult female body weights ranged from 840 to 970 grams over the year (Fig. 4.11). Females increased in weight between January and May but the mean weight increase was small and represented a change of only 100 grams or 12% of total body weight over 5 months. The two females captured in June had both increased in weight since May therefore it is possible females continue to gain weight until June. Five females captured in May and recaptured in July had lost between 100 and 125 grams in weight (or 11.4% to 17.9% of total body weight). This represented a significant loss in weight using a t-test for matched pairs ($t=2.08$, d.f.6, $0.025<p<0.05$). Female weights fluctuated for the remainder of the year although a significant weight increase occurred between September and October($t=2.12$, d.f.8, $0.025<p<0.05$).

First year females gradually increased in weight after weaning. They were significantly lighter than adult females in May ($t=3.02$, d.f.6, $0.01<p<0.025$) but not in July ($t=1.81$, d.f.5, $0.05<p<0.1$). The pattern of weight change in juvenile females was similar to that of juvenile males, although considerable variation occurred. One juvenile female (no. 54) gained 225 grams between April and July (~40.9% increase) while another juvenile (no. 61) gained only 25 grams (~3.3% increase).

The mean weight of females in July carrying newborn young was 721 ± 90 grams ($\bar{x} \pm$ s.e.; n=6). The lightest weight of an animal with young was 575 grams.

4.3.7.2 Pouch Development

Female pouches began to increase in width and depth between March and May. A detailed description of the changes in pouch development surrounding birth is provided in Appendix C.

During the first two months of lactation (July and August) pouches increased in size and depth and tightly enveloped the young. Individual mammary glands were visible but not swollen. From August until December,

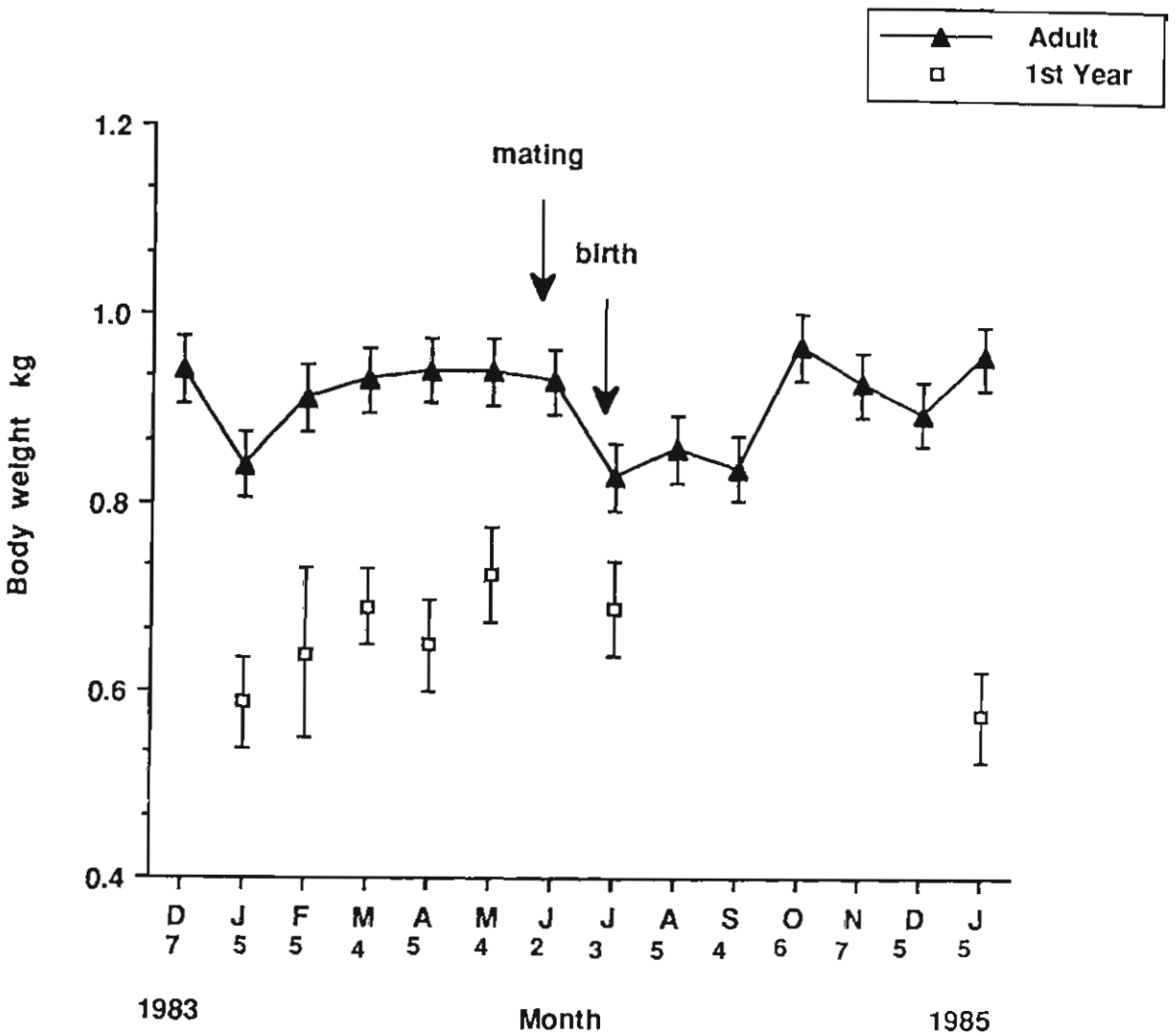

Fig. 4.11 Mean body weight for adult and first year female quoll (± s.e.). Adult sample size below month, first year females n= 2 - 5. First year females had lower weights in July than adults.

mammary glands increased in diameter, appeared swollen and protruded from the pouch. Non-lactating glands showed either little development or were flaccid with small teats.

Pouches and mammary glands regressed in size after December. Active teats, however, remained elongated and protruded from the tightening entrance of the pouch. By February, pouches were a shallow structure showing little obvious mammary gland development.

Two females that were not carrying young underwent a period of pouch development similar to that seen in parous females. Both animals were classified as old aged on the basis of body weight and date of first capture (adult in May 1983, last trapped in Jan. 1986).

4.3.7.3 Reproductive Success and Synchrony of Birth

During the study 10 out of a total of 12 females observed during a breeding season had young in the pouch (83% females breeding). From December 1983 to January 1985, 7 individual females were trapped consistently and 6 of these were observed with pouch young (86% of females breeding). Four of the females were breeding in their first year and all were carrying a full complement of young (6) in July. By August, 2 females had lost 1 pouch young each. The 2 older females were observed in July with 4 and 2 young each.

The size of pouch young provided a index of the female's date of mating and time of parturition. Young in the wild were aged using a growth curve constructed from young born in captivity and measured serially (curve provided in Appendix C). The date of mating was estimated by subtracting the age of the pouch young and the gestation length of 19 days, from the date of capture (Table 4.3). From this calculation it was possible that most females mated between the 3 and 9 of June (span of 7 days). This is consistent with the low capture rate of females at Cradoc between the 4 and 7 of June. One female carrying larger young probably mated some 7 to 9 days earlier than other females. These observations suggest that the mating period is probably confined to about 2 weeks of the year.

The survival rate of young was difficult to determine in the wild because they remained in a den after August. The number of young observed in the pouch in July was usually equivalent to the number of active mammary glands present throughout the lactation period. In one animal, however,

opposing mammary glands increased in size and in another animal the number of active glands increased. These discrepancies suggest that the young may utilise a number of teats and that the number of active glands is not an absolute guide to the number of young in the pouch.

Table 4.3 Estimated synchrony of mating using measurements of pouch young and a gestation period of 19 days.

Trapped 1984	Female no.	Pouch Young C. rump	Young Head length	Age days	Estimated date of birth	Estimated date of mating
1 July	52	7.5 mm	-	4 - 5	26 - 27 June	7 June - 8 June
1 July	42	15.2 mm	10.0 mm	20 - 21	11 - 12 June	23 May - 24 May
1 July	6	9.2 mm	6.0 mm	8 - 9	22 - 23 June	3 June - 4 June
1 July	54	9.2 mm	5.4 mm	7 - 9	22 - 24 June	3 June - 5 June
1 July	61*	5.2 mm	-	6 - 7	24 - 25 June	5 June - 6 June
4 July	7	8.8 mm	6.0 mm	7 - 8	27 - 28 June	8 June - 9 June

* signs of mating on 6 June

4.3.7.4 LH

Female LH levels were either undetectable or near the sensitivity range of the assay for most of the year (Fig. 4.12). Some fluctuations occurred between March and July during the onset of sexual maturity and ovarian development. The highest mean level of LH was 1.73 ng in June but this represented only two animals for which individual variation was high.

There was no apparent difference between mean LH levels in juvenile and adult females throughout the year. Juvenile females did not produce high levels of LH in January or February when emerging into the population and all levels were 0. 5 ng per ml, or less, during this time.

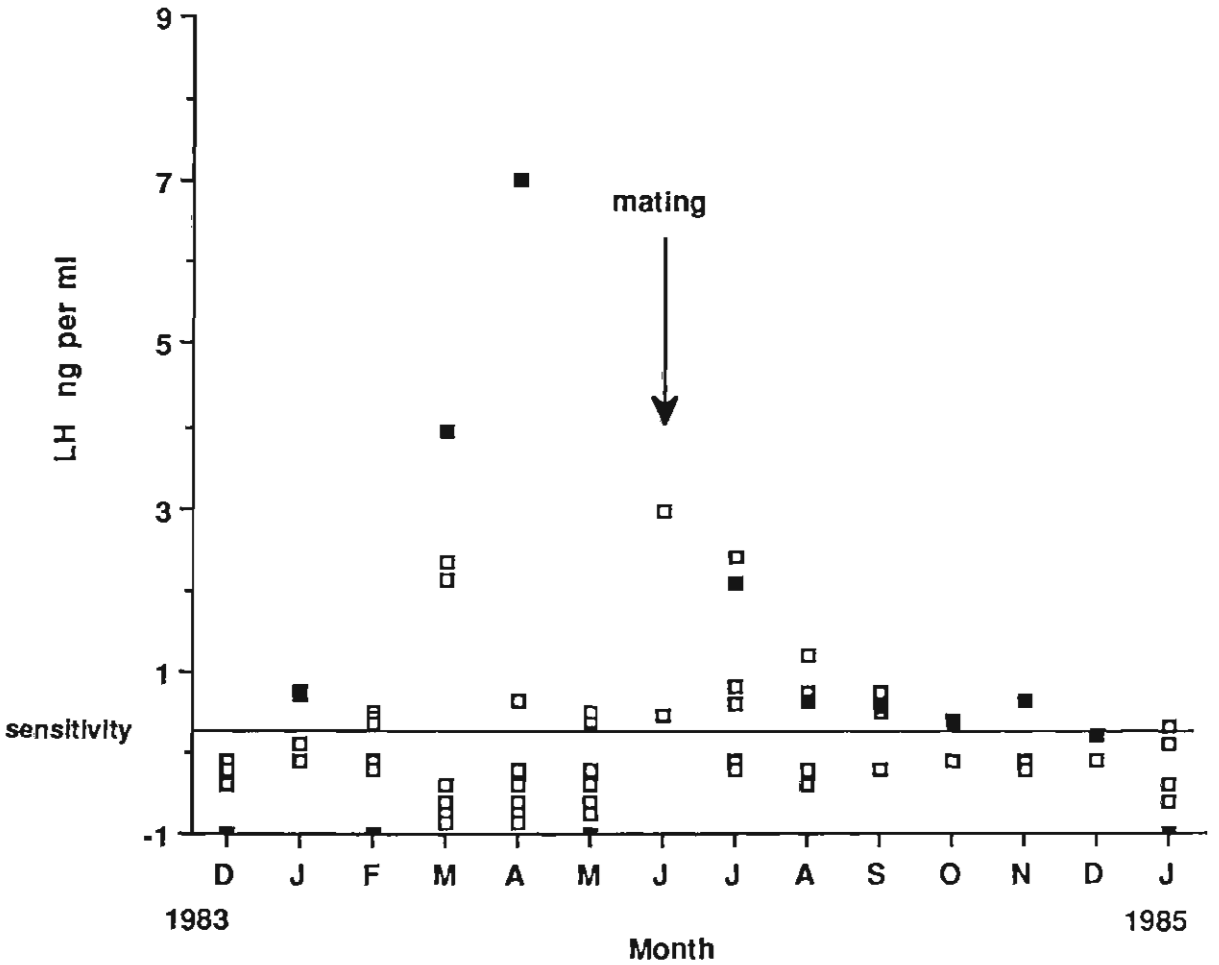

Fig. 4.12 Individual plasma LH levels in female quoll. Levels below 0.1 ng were below the sensitivity of the assay. Female no. 24 ■ was not observed with young after mating.

Figure 4.12 includes the LH levels for one female (no. 24) that did not appear with young. This animal had a profile that was similar to parous females and included the highest individual LH level of 7.1 ng in April.

4.3.7.5 Progesterone

Some sporadic increases in progesterone occurred in females prior to mating and parturition. The highest level of progesterone recorded was 9.4ng in one animal either one day before or after mating. Progesterone levels varied between 0.4 and 4.0 ng per ml in July in females carrying newborn young. Progesterone levels were 2.0 ng or lower throughout the lactation period (Fig. 4.13). The non-breeding resident female no. 24 produced a progesterone profile that was similar to parous females.

In January and February, 6 of 8 juvenile females had progesterone levels between 1.0 and 2.6 ng per ml compared to adult levels usually below 1.0 ng. These differences were not significant nor were there any differences between first year and older females during the breeding and lactation period.

4.3.7.6 Prolactin

Plasma prolactin was measured serially in 7 females with young and in 1 female (no. 24) that showed no evidence of producing young. Prolactin levels were also obtained from 2 juvenile and 3 lactating females on single occasions.

From February to June (the non-lactation period) prolactin levels ranged between 4.0 and 25.3 ng per ml. In July when females were carrying newborn young the range was between 9.7 and 21.0 ng per ml (Fig. 4.14). For most of the lactation period (August to January), prolactin levels fluctuated between 16.0 and 118.0 ng per ml. One female recorded a high level of 298.2 ng in November while suckling young 148 days of age.

The prolactin levels recorded from first year females appeared very similar to those occurring in older animals. In addition, the prolactin profile of female no. 24 was similar to that of lactating females (Fig. 4.14). While this animal underwent a sequence of pouch formation (Appendix C), the relatively high prolactin levels in October and November did not appear to coincide with any mammary gland development or reproductive activity.

The prolactin levels measured throughout the lactation period in this study were lower than those recorded by Hinds and Merchant (1986) for

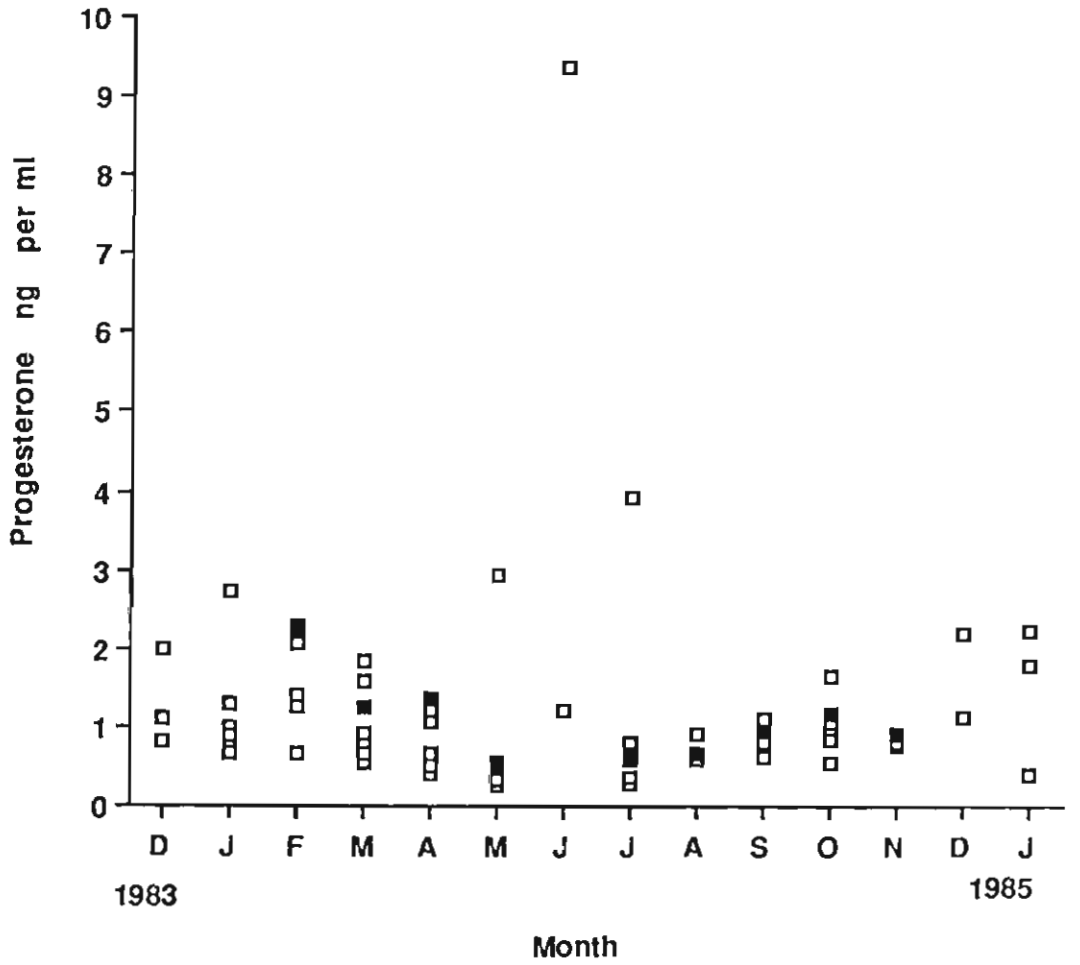

Fig. 4.13 Plasma progesterone levels in female quoll. Female no. 24 ■ did not appear with young. Only two females were captured during mating in June. Pouch young were aged ≥ 2 days by the July sample.

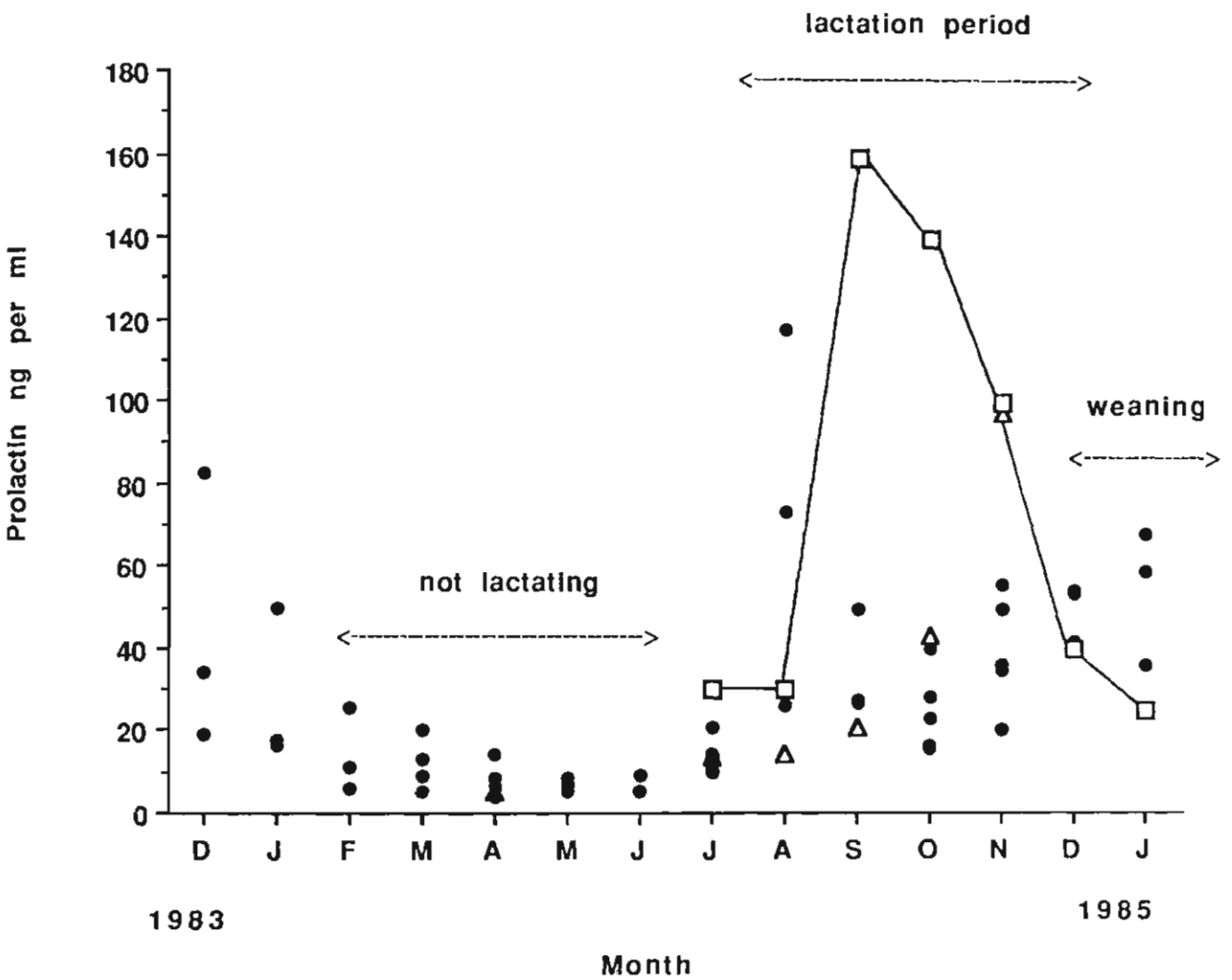

Fig. 4.14. Plasma prolactin levels in female Eastern quoll. Every female had ● young except female no. 24 △. One high value of 298 ng was recorded in November (not shown) from a female with young 148 days old. The mean prolactin levels obtained by Hinds & Merchant (1986) are also shown. ⊟

the quoll. Both sets of results show, however, that prolactin levels increase throughout the lactation period and decline during late lactation and the weaning period.

4.3.7.7 Synchrony of Resident Female Quoll

Figure 4.15 shows the synchrony between hormonal parameters and weight change for adult females over the 1984 breeding season. Although females experienced only a small change in body weight over the year, they lost weight over the mating period and increased in weight after the young were placed in dens. LH and progesterone levels appeared highest near the time of mating but only 2 females are represented by this sample. Prolactin levels were highest during the lactation period and declined after weaning.

4.3.8 Periodicity of Male and Female Cycles

Figure 4.16 presents a schematic diagram of the synchrony of weight and hormonal cycles between male and female quoll over the year. The diagram does not include changes in juvenile cycles.

Male and female body weights fluctuate throughout the year. The weight changes in the male show a significant annual cycle, however, both sexes lose body weight over the mating period.

The LH and testosterone cycles of the male quoll show a sharp peak during the mating period in June and the progesterone and LH profiles of the female quoll show a similar rise near this time. The cycle of prolactin secretion in the female does not appear to coincide with any male cycle.

4.3.9 Longevity

Two resident males were estimated to be at least 2 years and 6 months of age by January 1985 and were still in an apparently healthy condition. Two resident females (nos. 6 & 24) when first trapped in August 1983 were lactating and had enlarged mammary glands. They were therefore at least 1 year of age. Female no. 6 was last captured in January 1986 when at least 3 years and 6 months of age. She had two elongated nipples and a large, shallow pouch; evidence of recently weaned young. Female no. 24 was last trapped in January 1985 when at least 2 years and 6 months of age. This animal did not appear to be reproductively active.

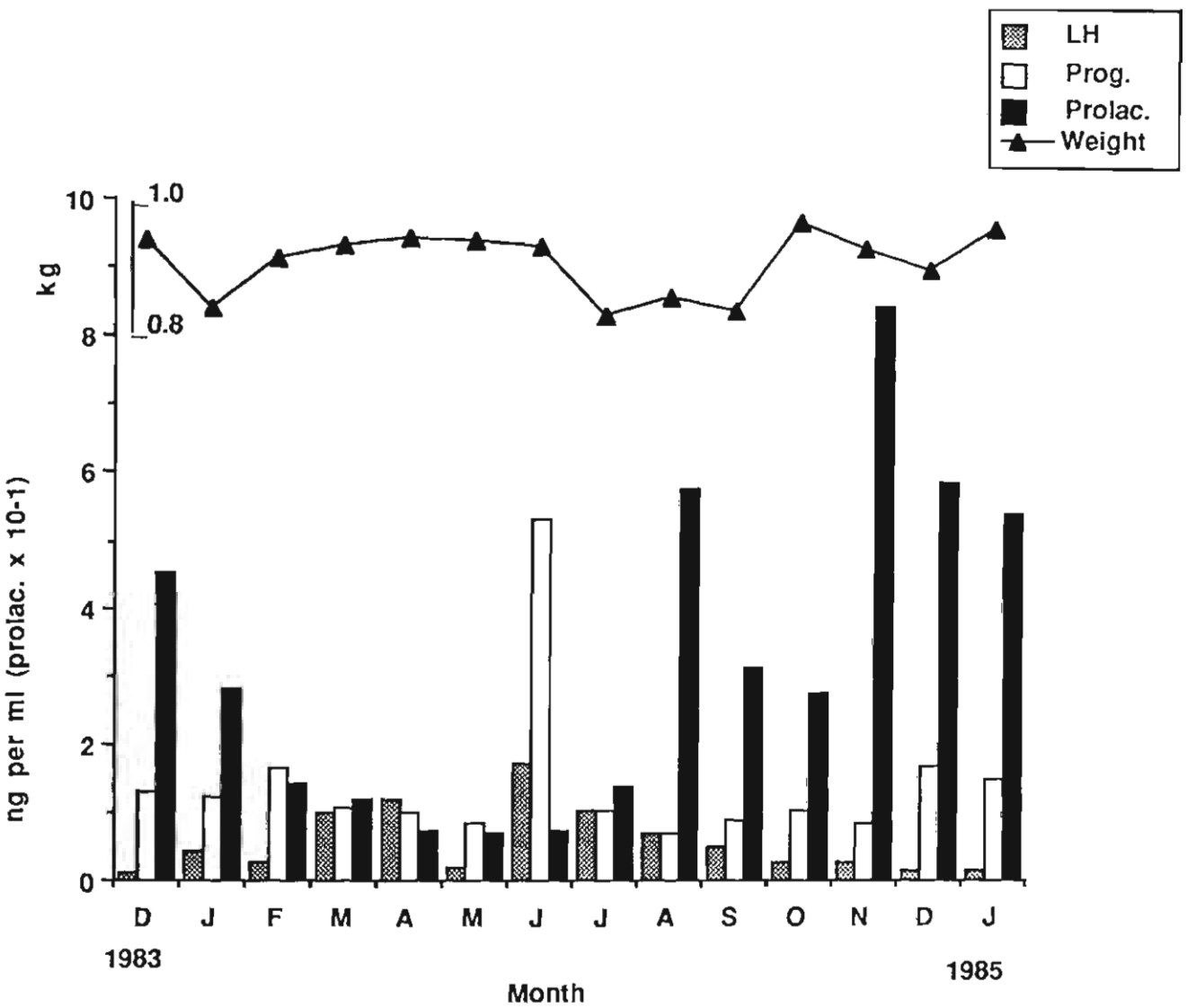

Fig. 4.15 Synchrony of mean body weight, LH, progesterone and prolactin in female Eastern quoll. Sample size as for previous figures.

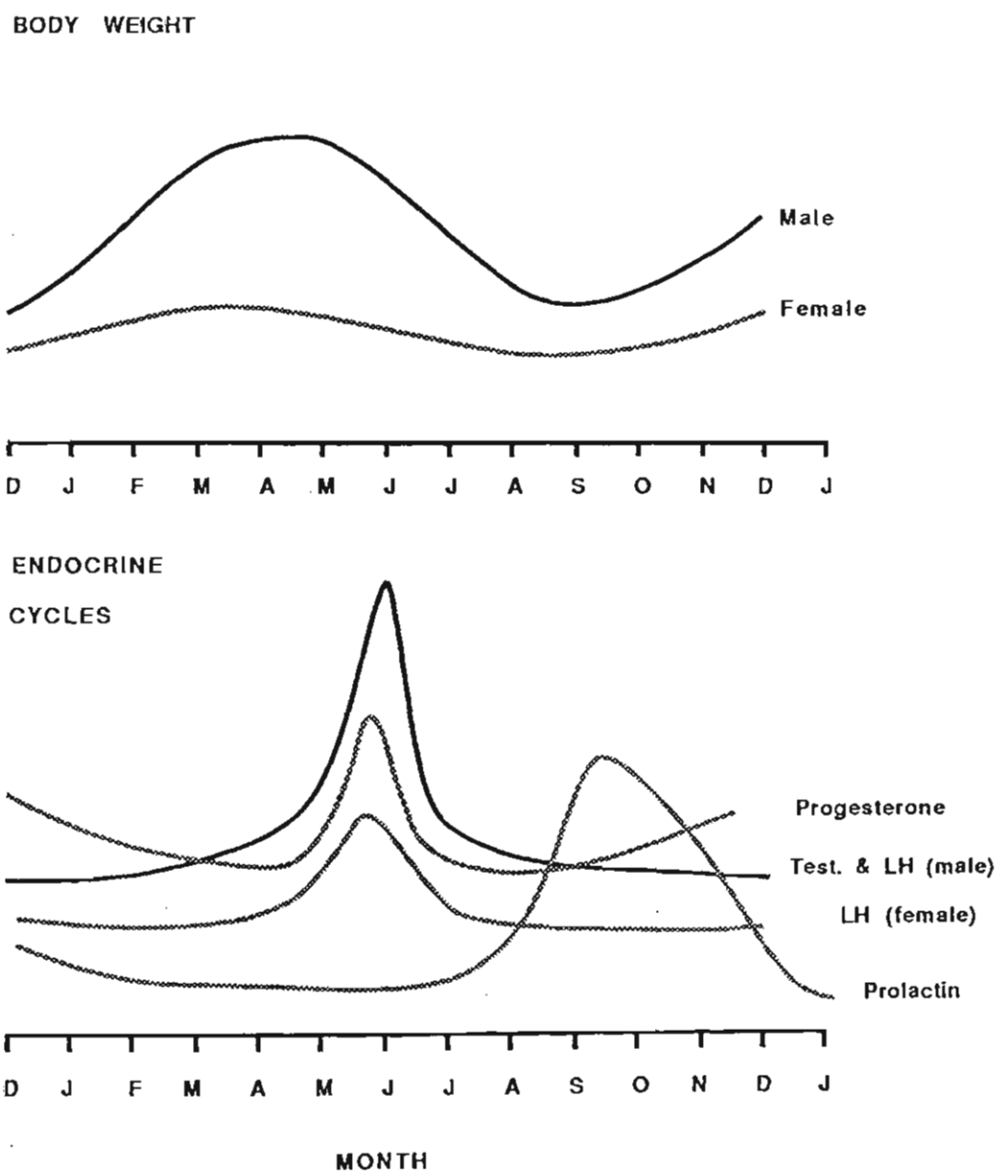

Fig. 4.16 Schematic diagram of the synchrony of weight and endocrine cycles of male ——— and female ~~~~~

Eastern quoll

It is likely that Eastern quoll survive for at least 3 to 4 years in the wild although their reproductive activity may be reduced by this time. This estimate of longevity agrees with Godsell (1983) and confirms that the Eastern quoll is one of the longer lived member of the Dasyuridae.

4.4 DISCUSSION

4.4.1 Population Events

Between December 1983 and January 1985 the population of Eastern quoll at Cradoc was influenced by two major events; both events being characterised by changes in population structure and endocrine cycles. The first event was the weaning and emergence of juveniles from December to February. Juvenile males dispersed quickly from the site and either were never retrapped or reappeared briefly as the breeding season approached. This dispersal behaviour is similar to that of other dasyurid males such as *Antechinus bilarni* and *Dasyurus hallucatus* (Begg 1981a,b). Juvenile female quoll tended to become resident in the area. The frequency with which females were retrapped and the absence of a neighbouring class, supports Godsell's (1983) estimates of smaller home ranges for females compared to males. Female Northern quoll, *Dasyurus hallucatus,* also appear more stable in their residency patterns than males (Begg 1981b).

The second major life history event centred around the mating period in June. The duration of the mating period was estimated to be about two weeks based on the size of the pouch young in July. Other seasonally breeding dasyurids, such as some *Antechinus, Sminthopsis* and *Planigale* species, experience mating periods of similar lengths or lasting up to six months of the year (reviewed by Lee *et al.* 1982; Read 1984).

The number of males in the study area increased between May and July. This was not accompanied by any increase in females so therefore at mating time the number of males exceeded the number of females. Male *Antechinus bilarni* and *A. stuartii* also increase their exploratory activities as the mating season approaches and male *A. flavipes* in captivity have been shown to increase their activity levels and metabolic rate in response to the

breeding season (Inns 1976; Begg 1981a; Moritz 1982). It is likely that male quoll were also increasing in activity and mobility as the breeding season approached. Begg (1981a,b) and Statham (1982) , however, have suggested that changing population numbers may also be due to a change in trappability of animals rather than immigration and this could also be a consideration.

Approximately 15 individuals were trapped continuously in the study area over a two year period. This core of animals may, in part, explain the high degree of synchrony in endocrine and body cycles within the population. The synchrony of events such as copulation, birth and weaning appear to be particularly characteristic of the family Dasyuridae (Dickman 1982; Read 1984; Wilson & Bourne 1984; Lee & Cockburn 1985) but also occurs in many other seasonally breeding mammal species (Farner & Follet 1979; Lincoln & Short 1980; Clarke 1981; Bronson 1985).

4.4.2 Male Body Cycles

4.4.2.1 Adult Males

Male Eastern quoll undergo a seasonal cycle in body weight and testes size. These results support observations made by previous workers on the quoll (Fletcher 1977; Godsell 1983) and describe a profile that occurs in other seasonally breeding male Dasyuridae (Woolley 1966; Taylor & Horner 1970; Inns 1976; Begg 1981a,b; Calaby & Taylor 1981; Cuttle 1982; Kerr & Hedger 1983; Woolley & Ahern 1983; Read 1984; Wilson & Bourne 1984; Kitchener *et al.* 1986; Bradley 1987).

Adult males show a large increase in body weight and scrotal size between February and March. While the food cycle may be responsible for some of this increase, adult males maintained maximum body weights for up to three months after the end of the insect flush. It has been suggested previously that the cycle of body weight not only coincides with the availability of insect larvae, but also with reproductive condition (Fletcher 1977; Godsell 1983; Green & Eberhard 1983) and that increased levels of plasma LH and testosterone may well be contributing to the increase in body weight and scrotal size after March.

The male quoll commences spermatogenesis well in advance of mating (Fletcher 1977) but by the time of mating, regression of the reproductive system is evident. Histology of the testes has revealed marked hyperplasia and

vacuolation of the Leydig cells as early as March with cellular deterioration continuing in the testes and epididymides until September (Fletcher 1977). On the basis of urine samples, sperm production also ceased near September. This process of degeneration in testicular tissue is similar to that described in a number of Dasyuridae (Taylor & Horner 1970; Kerr & Hedger 1983; Kitchener *et al.* 1986) and is consistent with the decrease in scrotal size occurring prior to breeding. Spermatogenesis is reported to cease either before or after breeding in *Dasyuroides byrnei,* (Fletcher 1983), *Sminthopsis leucopus,* (Woolley & Ahern 1983), *Phascogale tapoatafa,* (Cuttle 1982) and several *Antechinus* species (Begg 1981a; Calaby & Taylor 1981; Kerr & Hedger 1983; Wilson & Bourne 1984). This present study found that during the latter part of the year, the Eastern quoll displayed the morphological signs of reproductive regression such as scrotal baldness and flaccid penis.

Males maintain maximum body weight until the breeding period but may lose up to 20% of total body weight over the short mating period. This loss in weight may be caused by a number of factors, both behavioural and physiological, and suggests that the energy expended on reproduction may be relatively high. Food resources are naturally scarce during the winter months of June and July therefore food availability is one limiting factor. Weight loss may be the result of restricted feeding activity due to the pairing of individuals in a den over the copulatory period. In some smaller *Antechinus* species increased gluconeogenesis (the production of glucose from tissue protein) provides an alternative source of energy and reduces the male's need to feed during the mating period (Lee & Cockburn 1985). Studies reported in Chapter 7, however, found no evidence of male quoll experiencing gluconeogenesis during the mating period.

4.4.2.2. First Year Males

First year males show a steady increase in scrotal size and body weight after weaning. The largest increase in testes size occurred between March and April and this growth is likely to reflect an increase in Leydig cell number and size and development of the epididymis. Fletcher (1977) found that captive male quoll had numerous sperm in their urine by March and that the epididymis was packed with sperm from April until June. First year males probably produce fertile sperm by April therefore maturity is attained at a testes index of between 5.0 and 6.0.

The increase in body weight produced by juvenile males after weaning may be related to increasing age and attaining sexual maturity. The short lasting but high levels of LH and testosterone concentration recorded in juveniles near weaning probably stimulate the growth of the Leydig cells and exert numerous effects on the testes and accessory glands as they do in other male mammals (Turner & Bagnara 1976). Androgens are also known to have an anabolic effect on muscle protein which leads to an increase in weight and bone growth (Ganong 1965; Gower 1979). Some of the weight increase may also be related to food resources which are abundant at this time. Insect larvae and other invertebrates constitute the major proportion of the quoll's diet and are consumed in large quantities from late August until March (Blackhall 1980; Godsell 1983). However, as first year males continue to gain in weight after the end of the insect cycle, this suggests other factors are influencing body weight. In the male Brushtail possum, *Trichosurus vulpecula,* an increase in body weight prior to the start of the breeding season is thought to be a direct consequence of increased food intake stimulated by either an increase in social interaction, plasma androgens or environmental factors (Gemmell *et al.* 1986). An increase in androgens in the Eastern quoll may also signify increased activity levels that in turn enable the male to increase his food intake.

4.4.3 Male Endocrine Cycles

Few studies to date have measured LH and testosterone concentrations in male marsupials. This study found that the profile of LH secretion in the male Eastern quoll was very similar to that of testosterone. This finding supports the close relationship and regulation between LH and testosterone in the Eastern quoll as has been found in male mammals generally (deKretser 1984). LH and testosterone secretion are controlled, in part, by a negative feedback loop between the gonads and the hypothalamo-hypophyseal axis. This mechanism has been demonstrated experimentally in the quoll (Appendix E) and probably operates in a similar manner in other marsupials and eutherian mammals (Hearn 1975; Lincoln 1978; Catling & Sutherland 1980; Stewart *et al.* 1981; Inns 1982; Irby *et al.* 1984). Fletcher (1983) did not find a good correlation between LH and testosterone secretion in male *Dasyuroides byrnei,* but attributed this to insufficient sample

points rather than hormonal mechanisms. In the female marsupial LH and FSH secretion appear to be regulated by a similar negative feedback system but one involving the hypothalamo-hypophyseal axis and the ovary (Evans *et al.* 1980).

4.4.3.1. Adult Males

The basal and breeding concentrations of testosterone in the adult male quoll are similar to measurements from other marsupials and are within the range reported for many eutherian species (Table 4.4). Peripheral testosterone was maintained at a basal level of between 0.5 and 1.0 ng per ml during the non-breeding season. A steady increase in testosterone commenced one to two months prior to the breeding season and Fletcher (1977) found the weights of prostate and Cowper's glands in captive male quoll were also maximal at this time. It is likely that sperm production and the priming of the reproductive system, particularly the accessory glands, were a target of these increased levels. In the Tammar wallaby and Brushtail possum the prostate gland begins to increase in weight shortly before breeding and this has also been attributed to the accumulation and action of secretory products, particularly testosterone (Inns 1982; Curlewis & Stone 1985b).

A significant peak in LH and testosterone occurred during the mating period in June. LH ranged from 3.2 to 23.4 ng during breeding but most individuals had levels closer to the mean of 13.9 ng. This range in LH was wider and higher than the range of 3.0 to 5.0 ng reported for male tammars housed with oestrus females (Catling & Sutherland 1980) and suggests species variation may occur in LH secretion.

The mean testosterone level in male quoll during breeding was 5.0 ng per ml with some individuals as high as 7.6 ng. This study proposes that the peak in hormones during the breeding period are related more to male aggression than to testicular activity, which, as has been shown, declines before mating. The Dasyuridae, unlike most other marsupial families, lacks a sex hormone binding globulin in their blood (Sernia *et al.* 1979; Bradley 1982). This means that at times, such as during the breeding period, tissues may be exposed to high concentrations of testosterone. Males become aggressive and active during the breeding season with the aggression directly related to increased testosterone levels (Begg 1981b; Fletcher 1983; Kerr & Hedger 1983;

Table 4. 4 Range of plasma androgens during the breeding and non-breeding season in some male marsupial and eutherian mammals.

Species	Plasma Androgens (ng ml $^{-1}$) Non-breed.	Breeding	Source
Dasyuridae			
Antechinus stuartii	0.8 - 1.0	6.0 - 8.0	Bradley *et al.* 1980; Kerr & Hedger 1983
A. swainsonii	0.5 - 1.7	4.6 - 5.2	McDonald *et al.* 1981
A. flavipes	0.5 - 1.7	11.5 - 14.4	McDonald *et al.* 1981
A. minimus maritimus	0 - 0.5	3.0 - 5.0	Wilson & Bourne 1984
Phascogale calura	0.3 - 2.0	7.2 - 8.7	Bradley 1987
Sminthopsis crassicaudata	0.6 - 2.0	7.1 - 8.5	McDonald *et al.* 1981
Dasyuroides byrnei	0.5 - 1.5	2.5 - 4.5	Fletcher 1983
Dasyurus hallucatus	3.3-12.3	19.1-31.2	Schmitt *et al.* 1988
Sarcophilus harrisii	0.4-0.7	0.9-1.2	this study
Dasyurus viverrinus	0.5 - 1.0	2.0 - 5.5	this study
Other Marsupials			
Isoodon macrourus	0 - 2.0	2.0 ≥10.0	Gemmell *et al.* 1985; McFarlane *et al.* 1986b
Trichosurus vulpecula	1.0 - 4.0	10.0 - 20.0	Carrick & Cox 1977; Allen & Bradshaw 1980; Gemmell *et al.* 1986
Macropus eugenii	0 - 3.0	3.0 - 12.0	Catling & Sutherland 1980; Inns 1982
Didelphis virginiana	0.1 - 1.0	2.0 - 10.0	Harder & Fleming 1986
Eutherian Mammals			
Mustela vison	1.0 - 6.0	12.0 - 22.0	Sundquist *et al.* 1984
Rattus fuscipes	0.4 - 1.5	4.0 - 5.0	Kerr *et al.* 1980; Irby *et al.* 1984
Pteropus poliocephalus	1.4 - 2.7	29.0 - 32.0	McGuckin & Blackshaw 1987
Alopex lagopus	0.5 - 1.0	2.0 - 4.0	Smith *et al.* 1985
Ovis aries	0 - 5.0	10.0 - 18.0	Lincoln & Short 1980
Capreolus capreolus	0.1 - 1.0	2.0 - 7.0	Lincoln 1985
Odocoileus virginianus	0.1 - 2.0	4.0 - 14.0	Lincoln 1985
Loxodonta africana	0.32 - 14.4	19.7-81.1	Hall-Martin 1987

Wilson & Bourne 1984; reviewed by Lee & Cockburn 1985). Nelson and Smith (1971) observed bouts of aggression by captive male quolls during the females' oestrous cycles that resulted in dominant males killing subordinate animals. In this present study an increase in the number of males trapped around the mating period was likely to have increased social contact and competition for resources leading to aggressive encounters.

There were no differences in testosterone levels between first year and adult males or between resident, neighbouring or transient males in June. Transient males, however, had significantly higher LH concentrations in June and, during July, had higher LH and testosterone levels compared to other males. Any differences in hormone concentration that may have arisen due to the success of pairing with a female were not known. Although transient males were heavier with larger testes measurements than other males, there was no direct relationship between body weight and testosterone levels. One reason why transient males maintain elevated LH and testosterone levels after the breeding period could be for the acquisition of den sites and food resources. These may be acquired because of the greater mobility and increased aggression of the transient male compared to the resident male, and stimulated by increased hormone levels. As a consequence, transient males may also be successful in locating and competing for females that have lost their young, although this would not appear to be a common occurrence in the wild.

4.4.3.2 First Year Males

Juvenile quoll produced high LH and testosterone levels when entering the the population after weaning. These levels may be related to the onset of sexual maturity. Generally during puberty in male mammals, LH is responsible for the initiation of spermatogenesis. Testosterone, facilitated by LH and prolactin, stimulates the growth and secretory activity of the male accessory glands and duct system (Ramaley 1979). There is a sharp increase in the number of LH and FSH receptors in the Sertoli cells just before the onset of spermatogenesis and this is usually followed a short time later by an increase in testosterone concentration (Waites *et al.* 1985). Onset of sexual maturity in the male bandicoot, *Isoodon macrourus,* is identified by the first peak in plasma testosterone greater than 2.0 ng (Gemmell 1986) and testosterone levels from 1.0 to 2.0 ng may also have been sufficient to activate

this activity in the quoll.

High levels of androgens occur in the juvenile Australian Bush rat, *Rattus fuscipes*, despite the absence of mature Leydig cells. These are due to the activation of spermatogenesis and associated increase in Leydig cell volume (Kerr *et al.* 1980). The higher concentrations of testosterone in juvenile quolls could be in the form of 5α-dihydrotestosterone or androstanediol which are the primary steroids of the pre-pubertal testes and implicated as the likely active androgens in the prostate of the Brushtail possum (Curlewis & Stone 1985 a,b).

The high hormone levels produced by juvenile males after weaning may also have a behavioural role. Increased androgen levels may stimulate male aggression in the physically smaller juvenile quoll at a time when it is necessary to compete against adults for food resources and den sites.

4.4.4 Female Cycles

Adult female quoll showed no obvious seasonal cycle in body weight. Some females experienced a loss of 20% of body weight over the mating period but had recovered this by October. This suggests that body weight in females, as it is in males may be influenced by reproductive activity. Godsell (1983) found that females lost weight late in lactation, but this was not evident in captive or wild quoll in this study. Instead females gained weight in spring, coincident with the placing of young in the den and the seasonal flush of insects.

The cycle of pouch development and changes in the mammary glands of the Eastern quoll were similar to those found in other female dasyurids, particularly the larger species (*Antechinus* species, Woolley 1966; Begg 1981a; Williams & Williams 1982; *Dasyurus hallucatus,* Begg 1981b; *Dasyuroides byrnei,* Fletcher 1983; *Sarcophilus harrisii,* Guiler 1970). The functional properties of the quoll's pouch appeared similar to those of most other marsupials. The pouch enclosed the young completely for a proportion of the lactation period (birth to approx. 40 days of age) and inspection was via the small contracted pouch opening. This is also true of the pouch of the Tasmanian devil, *Sarcophilus harrisii,* which conceals the young for at least two of the eight month lactation period (pers. obs.).

Female quoll in this study were observed with 6 teats but previous workers have found females with between 6 and 8 teats (Hill & O'Donoghue 1913; Fleay 1935). Teat number is known to vary both within and between dasyurid species, for example, *S. crassicaudata* may have between 6 and 10 teats (Morton 1978) and *D. hallucatus* between 6 and 8 teats (Begg 1981b). Some of the variation in teat number has been attributed to geographical differences between populations, especially in *Antechinus*, and this may well account for the variation in the Eastern quoll (Lee & Cockburn 1985).

Godsell (1983) found that the period of denning was a critical event for females and one which initiated competition for den sites and resulted in a loss of young. Godsell made a direct correlation between the number of enlarged teats and the number of denned young but found that her estimates were higher than the figures based on the KTBA index (known to be alive index). Observations on captive animals in this present study confirmed that competition for nest sites and crowding in nest boxes certainly resulted in loss of young. However, the number of extended teats and lactating mammary glands was not an accurate guide to number of young in the den. Young are characteristically restless in the nest and actively migrate from one teat to another. In the wild most females appeared to retain the same number of lactating glands throughout lactation and it can only be assumed that a high proportion of young survive the period up to weaning.

Profiles for LH and progesterone obtained in this study cannot be represented accurately by monthly sampling. In most mammals LH is elevated only near the onset of oestrus or at ovulation as LH regulates ovarian cell development and induces ovulation in follicles suitably primed with FSH (Short 1975). In the Eastern quoll these events occurred in late May to early June when only two females were captured. Observations on captive females in Chapter 6 and a study by Hinds (1983) suggest that the Eastern quoll has a pattern of LH and progesterone secretion during the oestrous cycle and pregnancy, that is similar to other marsupials.

The Tammar wallaby, *Macropus eugenii,* shows a definite surge in LH of up to 60.0 ng per ml during the pre-ovulatory period of oestrus with ovulation occurring about 20 to 40 hours later (Sutherland *et al.* 1980; Tyndale-Biscoe *et al.* 1983). The female Kowari, *Dasyuroides byrnei,* shows an increase of between 7.0 to 12.0 ng in LH before entering oestrus which is

thought to be involved more with maturation of the ovary than with ovulation (Fletcher 1983). Juvenile females in this study did not show any early elevation in LH which might have been associated with maturation of the ovary. FSH is responsible for the processes of ovarian folliculogenesis and accessory gland development in the female mammal and generally no major peaks or troughs of LH occur during the prepubertal period (Ramaley 1979).

Most marsupials show significant increases in progesterone late in the oestrous cycle as the corpus luteum, which is the major site of progesterone secretion, is maximal by this time (Gemmell *et al.* 1980; Harder & Fleming 1981; Tyndale-Biscoe & Hearn 1981; Fletcher 1983; Walker & Gemmell 1983; Hinds 1983; Curlewis *et al.* 1985). Progesterone levels in the female quoll were 2.0 ng per ml or lower for most of the year and did not rise until near the breeding period. One female in the wild had a high progesterone level at mating (9.4 ng) but reasons and source of this elevation are unclear. Progesterone levels obtained in July from females with newborn young, were comparable with the levels found in captive females soon after birth (Chapter 6). These results confirm that a decrease in progesterone levels occur after birth and throughout the lactation period (Hinds 1983). All marsupials examined to date, except the bandicoot, show a rapid fall in progesterone just prior to birth and this fall was once considered an important stimulus triggering parturiton (Tyndale-Biscoe & Renfree 1987). The Tammar wallaby for example, shows dramatic surges in progesterone concentration over an eight hour period before and after birth (Tyndale-Biscoe *et al.* 1983). The Short nosed bandicoot, *Isoodon macrourus,* does not conform to this pattern but is unusual in that the corpus luteum of pregnancy persists until well into the lactation period (Gemmell *et al.* 1980). This may account for the variation in the progesterone profile.

The slightly higher progesterone levels in juvenile females at the time of weaning were probably related to maturation of the reproductive tract, more specifically the ovary and uterus. It is unlikely that the source of progesterone at this stage was luteal and more likely to be adrenal or from the granulosa cells of the ovarian follicle (Gower 1979). Godsell (1983) reported elevated androgen levels in juvenile females at weaning. However, as progesterone may be biosynthesised easily to form testosterone (Gower 1979), these elevated levels may have been a derivative of progesterone cross reacting with the antibody. Peripheral testosterone has been measured in the female

Brushtail possum (McDonald 1977; Allen & Bradshaw 1980) and Curlewis *et al.* (1985) found that the adrenal gland of the mature female possum had the ability to secrete large amounts of testosterone *in vivo* although they could not suggest a role for the steroid.

The duration of the lactation period was approximately five to six months for the quoll. This is slightly longer than other smaller dasyurids but comparable with the larger members such as the Northern quoll (Begg 1981b) and Tasmanian devil (Buchmann & Guiler 1977) and some other marsupial families (Tyndale-Biscoe & Renfree 1987). Prolactin levels were low during the non-breeding season but showed an increase during the mid- to late lactation period when the diameters of active mammary glands were large. The levels reported in this study for the Eastern quoll are within the ranges reported for the Brushtail possum (Hinds & Janssens 1986) and Tammar wallaby (Hinds & Tyndale-Biscoe 1985) throughout the lactation period. Although the prolactin profile in this study was lower than that found by Hinds and Merchant (1986), it is probable that the levels in wild females were reflecting similar trends.

One female quoll that did not produce young had a prolactin level similar to levels found in females after 150 days of lactation. This is interesting because in marsupials and eutherians, high levels of plasma prolactin have been attributed to the sucking stimulus of the young (Hinds & Tyndale-Biscoe 1985). Non-lactating possums, however, can show transient pulses in prolactin (Hinds & Janssens 1986), and the existence of transient pulses may well explain the levels in the quoll.

The profile of prolactin in marsupials contrasts with many eutherian mammals that generally show a rise in prolactin during early lactation (Cowie 1984). Considering that marsupial young are born at an 'embryonic' stage in development, this difference is perhaps to be expected.

Sharman (1970) hypothesised that the marsupial oestrous cycle is equivalent hormonally to gestation and that no endocrine maternal recognition of pregnancy occurs. This pattern has been verified for a number of marsupial families (Shorey & Hughes 1973; Harder & Fleming 1981; Fletcher 1983; Curlewis *et al.* 1985) and it is likely that the Eastern quoll may be included. In the Eastern quoll, the growth and development of the corpus luteum is similar in pregnant and non-pregnant animals (O'Donoghue 1912) and results from wild females in this study (and captive females in Chapter 6) have shown that the hormonal cycles of non-pregnant animals appear similar to those of

parous females. In contrast, however, some studies on Macropoidinae, have found subtle but significant differences in progesterone and prolactin levels and oestrous cycle lengths between pregnant and non-pregnant animals (Flynn 1930; Lemon 1972; Renfree & Tyndale-Biscoe 1973; Merchant 1979; Cake *et al.* 1980; Hinds & Tyndale-Biscoe 1982; Tyndale-Biscoe *et al.* 1983; reviewed by Tyndale-Biscoe 1984). This suggests that very detailed examination of hormonal changes are required if differences between cycles are to be detected.

4.4.5 Sex Specific Roles

Godsell (1983) proposed that the quoll exhibits sex specific spacing behaviour and intra-sexual competition and that these influence both the population size and structure. These behaviours arise due to the differences in requirements of each sex, namely "...mates for males and dens for females." This present study agrees with those observations and suggests the behaviour patterns also to extend to the specific role of each class within the population which may have arisen due of the longevity of the species.

First year females represent a effecient component of the breeding population because they retain the highest number of young. Females are characterised by a low dispersal rate and small home range. Males exhibit higher sex specific changes in body weight than females and have larger home ranges. First year males being lighter than older males are probably less likely to obtain a female at oestrous time and therefore socially the role of the first year male may be to increase competetion and intra- male aggression among resident males during the breeding season. Transient males sustain higher levels of LH and testosterone for slightly longer periods than other males and this could be advantageous when competing for food, permanent occupancy in an area or any females that have returned to oestrus. These factors inturn increase the pressure on the resident male and assist in periodically changing the composition of the population.

CHAPTER 5

PHOTOPERIOD

5.1 INTRODUCTION

Few studies have examined the effect of photoperiod on the reproductive cycle of marsupials even though most species breed seasonally. The most detailed investigations to date have been conducted on the female Tammar wallaby, *Macropus eugenii* (Sadlier & Tyndale-Biscoe 1977; Tyndale-Biscoe 1980; Tyndale-Biscoe & Hearn 1981; Hinds & den Ottolander 1983; McConnell & Tyndale-Biscoe 1985; reviewed by Tyndale-Biscoe & Renfree 1987). Evidence suggests that a sudden reduction in photoperiod (short days) near the summer solstice in December is the signal which reactivates the corpus luteum and subsequently the diapausing blastocyst in the female. During the period of increasing photoperiod (long days) an additional component may be responsible for causing anoestrus and seasonal quiescence (Sadlier & Tyndale-Biscoe 1977; Hinds & den Ottolander 1983). The male tammar produces sperm throughout the year and shows a seasonal change in prostate size. It has been suggested that the male probably responds more to cues from the female at breeding time than directly to a change in photoperiod (Catling & Sutherland 1980; Inns 1982; Tyndale-Biscoe *et al.* 1986).

The Short nosed bandicoot, *Isoodon macrourus,* breeds during a long day photoperiod and increasing daylength has been associated with an increase in plasma testosterone. While photoperiod is the most likely factor controlling seasonality in this species, breeding is not a synchronous event and additional factors such as the rate of change of minimum temperature, rainfall and nutrition may influence the reproductive activity of both sexes (Barnes & Gemmell 1984; Gemmell *et al.* 1985; Gemmell 1987).

The Brushtail possum, *Trichosurus vulpecula,* usually breeds from March to June (short days) throughout Australia but births may occur in other months depending on location. Photoperiod has been implicated as the primary cue influencing breeding in the female possum. However, as with the

bandicoot, locational variation in the onset of breeding suggests that additional local factors may be influential as a trigger to mating (Gemmell *et al.* 1986). In the northern hemisphere, female opossums, *Didelphis virginiana,* give birth earlier than expected when exposed to an increasing daylength (Farris in Tyndale-Biscoe & Renfree 1987). Male opossums show little response to photoperiod and produce high levels of testosterone prior to the female's oestrous cycle (Harder & Fleming 1986). There may exist some sexual dichotomy in the onset of reproductive activity in *Didelphis.*

The effect of photoperiod has been examined in several species of dasyurid: the Fat-tailed dunnart, *Sminthopsis crassicaudata,* the Kultarr, *Antechinomys laniger* and three species of *Antechinus.* The dunnart is a desert dwelling marsupial that has a long breeding season (6 to 8 months) extending from the winter to the summer solstice. In captivity the reproductive cycle of the female dunnart can be influenced by an artificial change in photoperiod (Godfrey 1969; Smith *et al.* 1978). Photoperiod is thought to have no discernible effect on the sexual development of the male dunnart and maturity is correlated more with age than with changing daylength (Godfrey 1969). There may, however, be indirect evidence to suggest that the male dunnart is influenced by photoperiod. The low breeding success of animals in an experiment by Smith *et al.* (1978) was attributed to incomplete fertilisation and a failure of the males to copulate with oestrus females. It may have been therefore that the physical or social environment, or the photoperiod regime were not conducive to male reproductive activity.

In a preliminary study by Valente (1984), the reproductive systems of male and female *Antechinomys laniger* were found to responded atypically to a change in photoperiod. Males maintained on a long photoperiod (14L:10D), normally conducive to the development of the scrotum and spermatorrhoea, displayed a slower pattern of scrotal development followed by a rapid rate of decline. Females responded to long photoperiods by entering oestrus significantly earlier than animals maintained on equinoctial or short photoperiods.

Male *Antechinus stuartii* housed in mixed colonies under a natural photoperiod produced a seasonal rise in androgen levels just after the winter solstice (Scott 1986). Artificial long day photoperiods inhibited reproduction in both male and female antechinus, while a short day photoperiod reduced and slightly delayed the seasonal rise in androgen concentration in males. Scott

suggested that the stimulus of short days was an important factor influencing breeding in this species although a combination of endogenous rhythm and photoperiod was responsible for the precise timing of breeding. The approximate timing of breeding in *Antechinus stuartii, A. swainsonii* and *A. flavipes* is thought to also be influenced by photoperiod controlling the endogenous circannual rhythm of reproduction (Dickman 1985; McAllan & Dickman 1986).

These latter studies suggest that the reproductive activity of male dasyurids may be responsive to photoperiod. The aim of this experiment was to determine whether the reproductive cycle of the male Eastern quoll was sensitive to a change in daylength. One experimental condition, long daylength versus naturally decreasing day length, was adopted to examine photosensitivity. The experimental hypothesis was whether Photoperiod males, maintained on a long daylength not conducive to breeding, would produce a pattern of hormonal and body changes that differed from Control males, experiencing a naturally decreasing day length conducive to breeding.

5.2 MATERIALS AND METHODS

5.2.1 Photoperiod and Control Animals

Photoperiod animals (n=7), were exposed to an altered daylength. The group consisted of four adult males (nos. 8, 16, 28 and LM) and three first year males (nos. 45, 82, 84). Control males (n=5) were animals housed under natural daylength and the group comprised three adult males (nos. 5, 26, 198) and two males maturing in their first year (nos. 42, 87). Males LM, 82, 84 and 87 had been caught recently from the wild whereas the remaining animals had been in captivity for some time.

5.2.2 Design of Experiment

In Tasmania, the quoll prepares for breeding and mates during the winter months (April to June, short days). This experiment was conducted from March until August and during this period males in the wild naturally develop to full reproductive status, they mate and then undergo a decline in reproductive activity. Control males were animals that experienced the

naturally decreasing daylength conducive to reproductive activity while photoperiod males were maintained on an artificially long day photoperiod. Long days were selected because in the wild they are not conducive to reproductive activity and therefore the regime was opposite to that experienced by control animals.

Some studies already mentioned have found that the reproductive cycle of the male marsupial may respond to a variety of cues including those from the female (Catling & Sutherland 1980; Fletcher 1983). Female quoll were therefore excluded from this experiment to remove any effect their presence may have on the male's hormonal cycle.

The experiment began in March 1985 when the natural light regime was approximately 12 hours light and 12 hours dark (12L : 12D) and before males commenced reproductive activity. Animals from both groups were exposed to a artificial equinoctial schedule of (12L: 12D) for 2 weeks before separation. This enabled some acclimation to the surroundings and experimental conditions. After 2 weeks Photoperiod males were commenced on a artificial long day photoperiod of 15 hours light and 9 hours dark (15L:9D). This schedule remained unchanged for 17 weeks and was similar to the hours of light occurring in late November and early December, just before the summer solstice. This regime was selected because it also coincides with males in the wild showing an increase in body weight (opposite to control condition).

5.2.3 Housing and Lighting

Photoperiod males were housed in a internal sound and light proof room constructed inside a galvanised shed. The room was illuminated by one daylight type 40 Watt fluorescent tube (Osram®) which, according to Sadlier and Tyndale-Biscoe (1977), most closely approximated the energy distribution curves of the sunlight at sea level. The light reading ranged from 120 to 160 lux around the room but was nearer to 140 lux in front of the cages. The duration of the light was regulated by a automatic timing device (Kambrook®) and the time for lights on was 7.00 a.m. and lights off 10.00 p.m. The control animals were housed immediately adjacent to the photoperiod room in the shed and were spaced evenly around a large glassed window that received natural light.

Animals were housed individually in wire cages (measuring 60cm

x 60cm x 60cm, plate 6b). The cages were raised above floor level to ensure that every animal was exposed to a strong field of light. No physical contact was possible between animals and it was assumed that all animals were exposed to the same changes in temperature, humidity and disturbance. Males were examined in their own sack and both groups were fed at the same time with a similar quantity of food. Blood samples and body measurements were taken from each group weekly then 2 to 3 times per week as the breeding season approached. Blood plasmas were stored at -20°C until assayed for LH and testosterone as described in Chapter 3.

5.3 RESULTS

Table 5.1 shows the hours of natural light experienced by control males. This was based on the hours of sunrise and sunset for Hobart (Eastern Standard Time) compiled by the Bureau of Meterology of Tasmania.

Table 5.1 Hours and minutes of light experienced by Control and Photoperiod males at each sampling point.

Date 1985	Sample	Control h. min	Photoperiod h. min
March 11	1	12.39	12.39
*	*	12.00	12.00
April 11	2	11.08	15.00
April 22	3	10.38	15.00
April 27	4	10.25	15.00
May 1	5	10.15	15.00
May 6	6	10.03	15.00
May 10	7	9.53	15.00
May 14	8	9.45	15.00
May 20	9	9.37	15.00
May 28	10	9.19	15.00
June 3	11	9.12	15.00
June 11	12	9.04	15.00
June 18	13	9.00	15.00
June 26	14	9.00	15.00
July 8	15	9.08	15.00
July 18	16	9.21	15.00
July 24	17	9.32	15.00
August 2	18	9.47	15.00

* 2 weeks exposure - no sample taken

The 2 weeks comprising 12 hours of artificial light (12L:12D) are situated between samples 1 and 2. Photoperiod males were exposed to 15 hours of artificial light after this period.

5.3.1 Between Groups

There was no significant difference in mean body weights between the two groups at the start of the experiment ($t=0.174$, d.f.10, $p>0.4$). To eliminate possible bias by the smaller body weights of the first year males, a percentage of weight loss for each individual was determined. This was calculated by dividing the body weight in April (after 2 weeks exposure to12L:12D) by the body weight at each sample point (x100).

From April until May there was little difference in either the mean weight loss or percentage weight loss between the two groups (Fig. 5.1). Photoperiod males began to show a increase in weight (represented by a smaller percentage weight loss) after May and this continued until the end of the experiment. There was a significant variation in the mean percentage weight loss between the two groups from June until August (ANOVA, $F_{[1,10]}=120.99$, $p<0.0001$). There was no significant variation in the percent weight loss between individuals in each group during this time (ANOVA, Photo., $F_{[5,35]}=0.053$, $p>0.25$; Cont., $F_{[5,16]}=0.449$, $p>0.25$). Control males lost body weight in a manner similar to that displayed by males in the wild.

The testes index was also converted to the percentage change in testes index to eliminate the bias of the smaller scrotal size of the first year males. As Fig. 5.2 shows, both groups of males showed a very similar profile of percentage change in testes index. Initially there was an increase in testes size (greater than 100% change), followed by a decrease. By the end of the experiment in August, both groups had reached an identical stage of decline. There was no statistical difference in the testes index between the two groups of males at the start of the experiment (Mann-Whitney, $U=10$; $p>0.05$) and it was unlikely that any difference occurred between the two groups throughout the experiment.

There was no significant difference in LH levels between the two

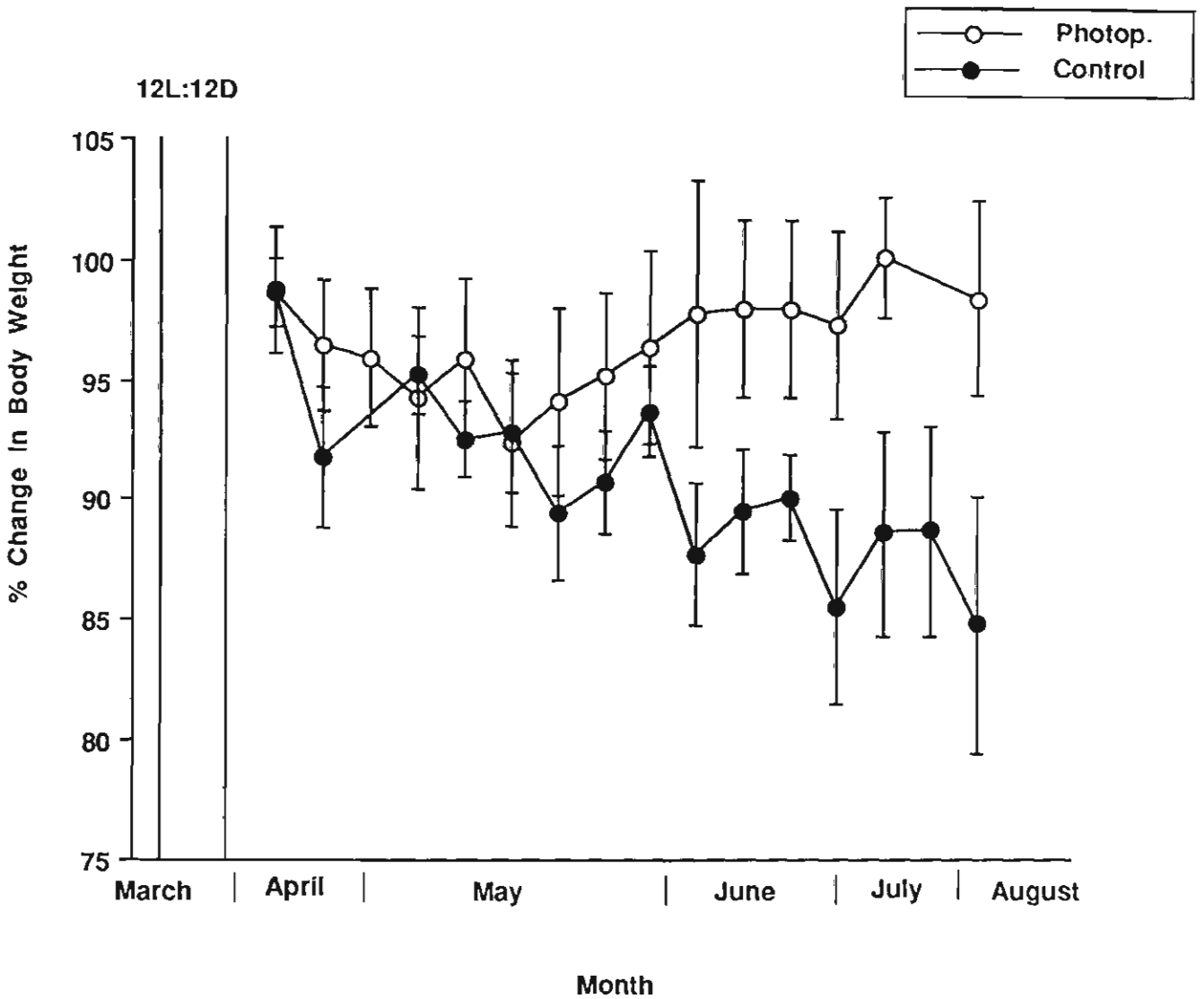

Fig. 5.1 Mean (± s.e.) % change in body weight for 7 Photoperiod males and 5 Control males. Photoperiod males were significantly heavier and increased in weight during May.

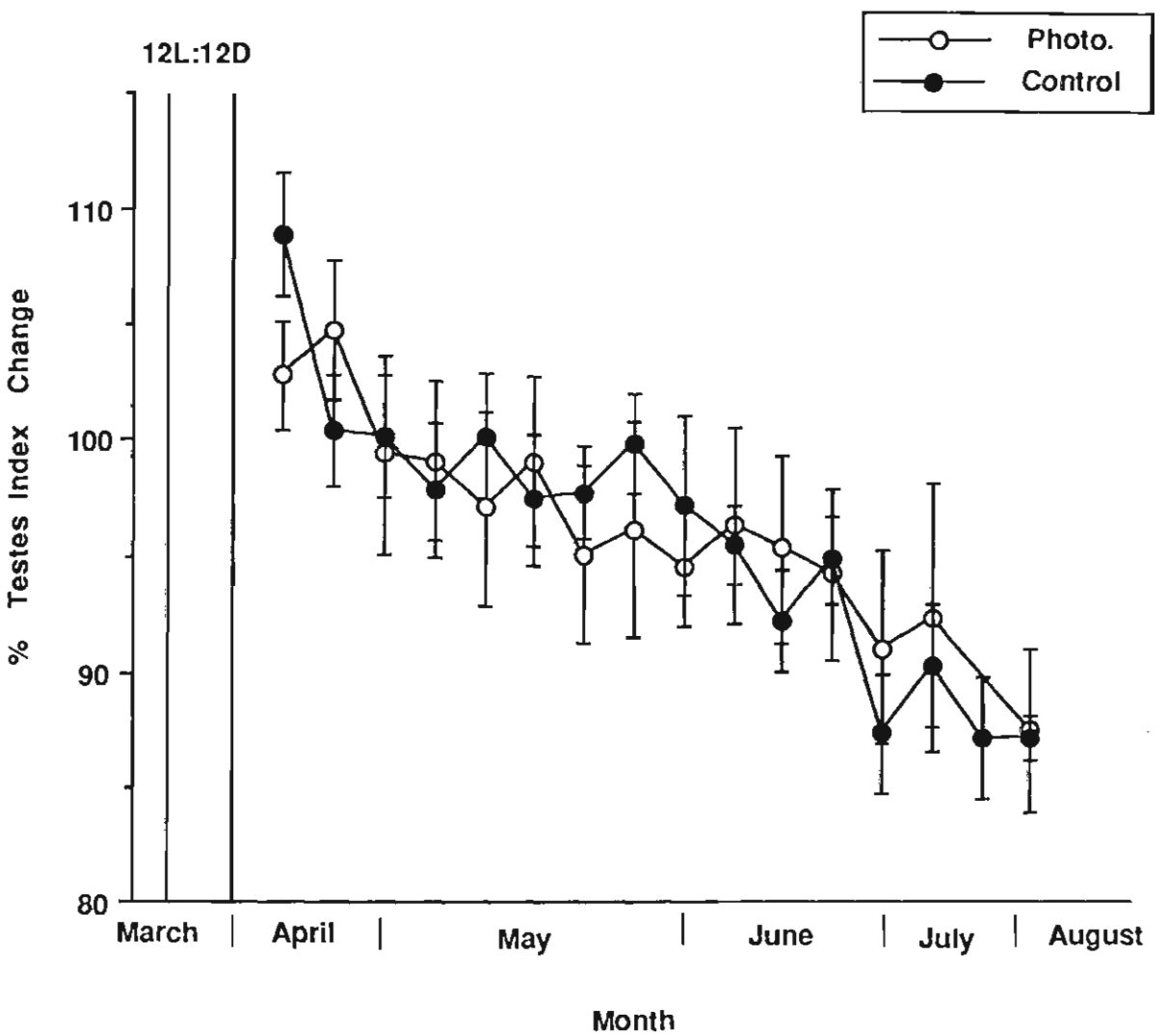

Fig. 5.2 Mean (± s.e.) % change in testes index for 7 Photoperiod males and 5 Control males. Both groups showed a similar decline in testes size.

groups of males at the start of the experiment ($t=1.54$, d.f.8, $0.05<p<0.10$). Figure 5.3 shows that LH levels in both groups generally declined over the experimental period. After May, however, control males had consistently higher levels of LH than photoperiod males. The variation in LH secretion between the two groups from June to August was significant (ANOVA, $F_{[1,10]}=14.51$, $0.0001<p<0.005$).

Control males had significantly higher testosterone levels in March than photoperiod males ($t=1.99$, d.f.10, $0.025<p<0.05$). After exposure to 2 weeks of 12L:12D the situation was reversed and photoperiod males had significantly higher levels ($t=3.08$, d.f.10, $0.005<p<0.01$). From April until the end of the experiment there was little variation in the testosterone levels between the two group of males. Both groups had testosterone levels that increased from basal levels in March to reach maximal levels during April (Fig. 5.4). After April, every male showed a steady decline in testosterone and had basal levels by May. No additional hormonal changes occurred during June. There was no significant variation in the mean testosterone levels between first year males in the photoperiod or control group over the experimental period (ANOVA, $F_{[4,73]}=0.43$, $p>0.25$).

There were no high correlations between the hormonal and body parameters and the hours of light (Table 5.2).

Table 5.2 Correlation coeffecient, r for hormonal and body parameters of male quoll and hours of light.

Parameter	Photoperiod artificial light	Control natural light
Body weight	0.630	0.247
Testes index	0.099	0.061
LH	0.388	0.136
Testosterone	0.197	0.266

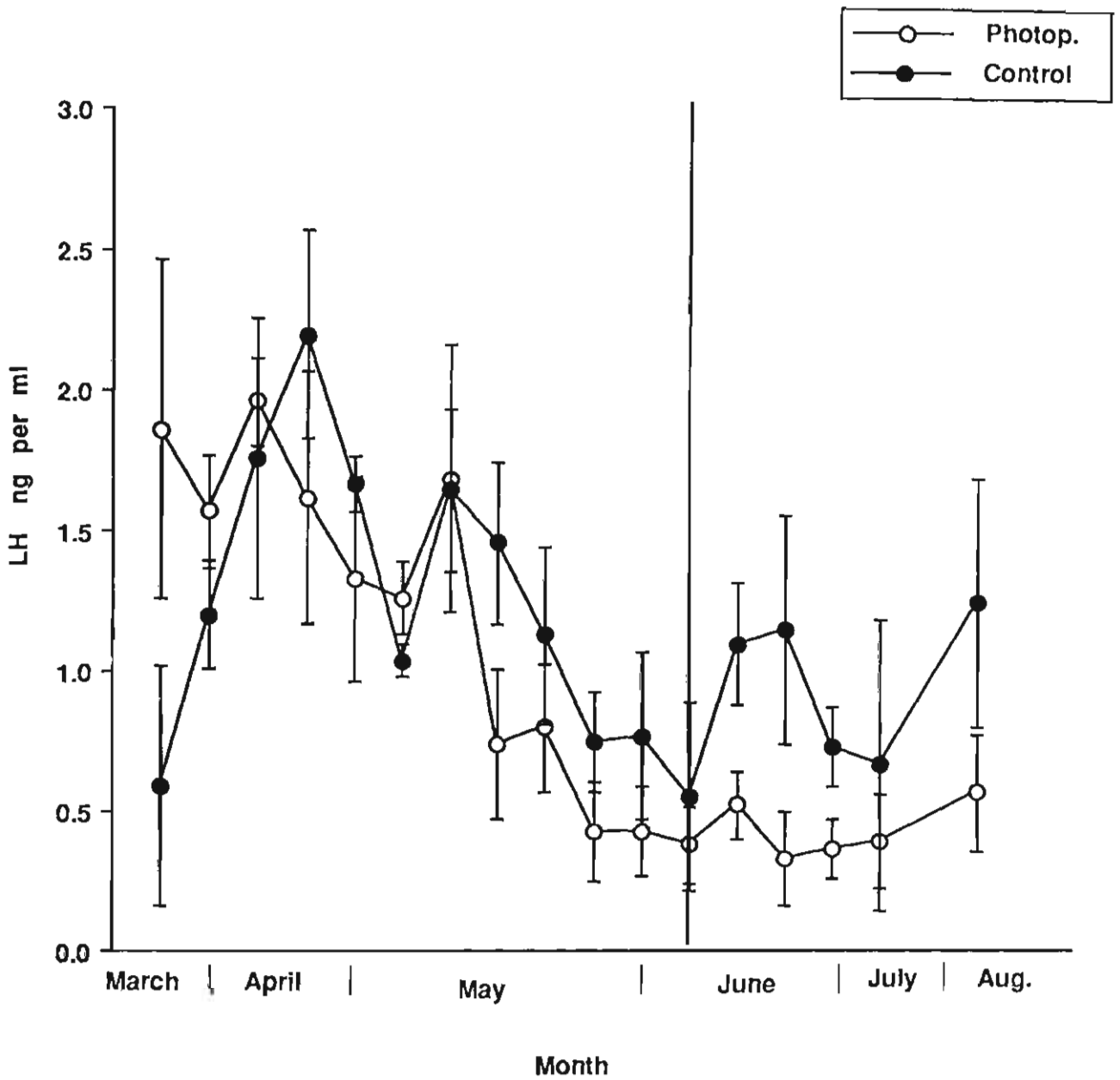

Fig. 5.3 Mean (± s.e.) LH for 7 Photoperiod males and 5 Control males. Control males had consistently higher LH which was significant after mid- June (indicated by line).

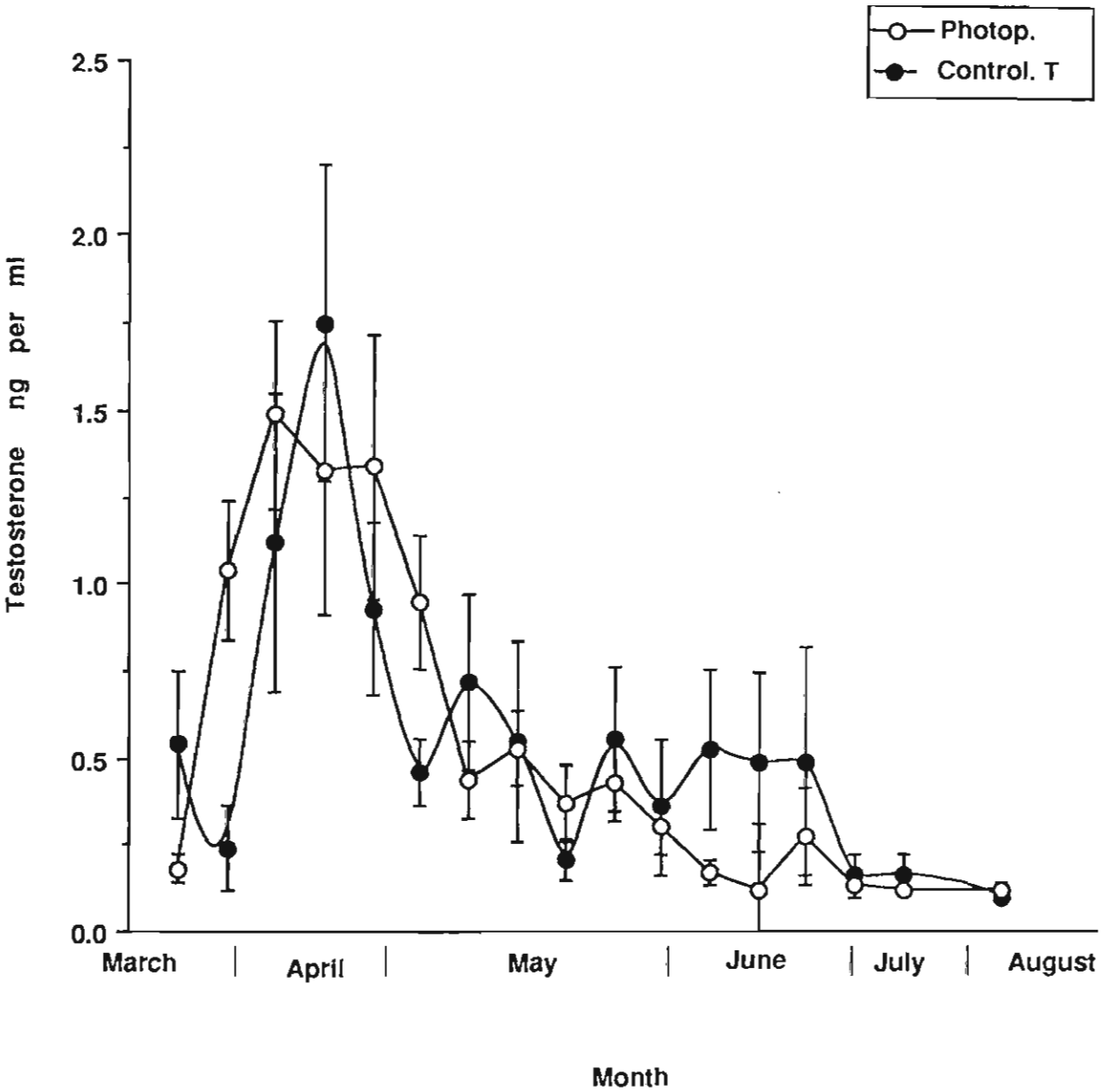

Fig. 5.4 Mean (± s.e.) testosterone for 7 Photoperiod males and 5 Control males. There was no significant difference between the two groups.

The hormonal profiles for both groups of males did not correspond with those of males in the wild at this time (Chapter 4). The LH and testosterone levels in the captive males reached a peak in April and then started to decline approximately 5 weeks before the expected breeding season.

5.3.2 Within Groups

Hormonal profiles are shown for each individual in the photoperiod and control group in Figure 5.5 and Figure 5.6. The profiles demonstrate the initial rise in hormones after the start of the experiment then the rapid decline to basal concentrations. They also illustrate the unison between LH and testosterone secretion in every animal.

There was a large degree of variation over the four parameters between individuals in each experimental group (Table 5.3).

Table 5.3 Analysis of variation (F) between individuals within each group (level of significance also shown).

Parameter	F	Photoperiod (n=7)	F	Control (n=5)
Body weight	42.75	$p<0.0001$	87.13	$p<0.0001$
Testes Index	15.96	$p<0.0001$	3.57	$0.01<p<0.025$
LH	2.28	$0.025<p<0.05$	3.86	$0.005<p<0.01$
Testosterone	2.76	$0.01<p<0.025$	4.13	$p<0.005$

This was probably due to the differing age structures of animals and variation in body sizes. However, as Table 5.4 shows, no individual dominated every category and the first year males were evenly dispersed between the older animals. In the control group, for example, male no. 198 had the highest LH and highest testosterone level whereas in the photoperiod group male no. 45 had the highest testosterone but one of the lowest LH levels (Fig. 5.5). Male no. 28 was significantly heavier than other photoperiod males but had the lowest

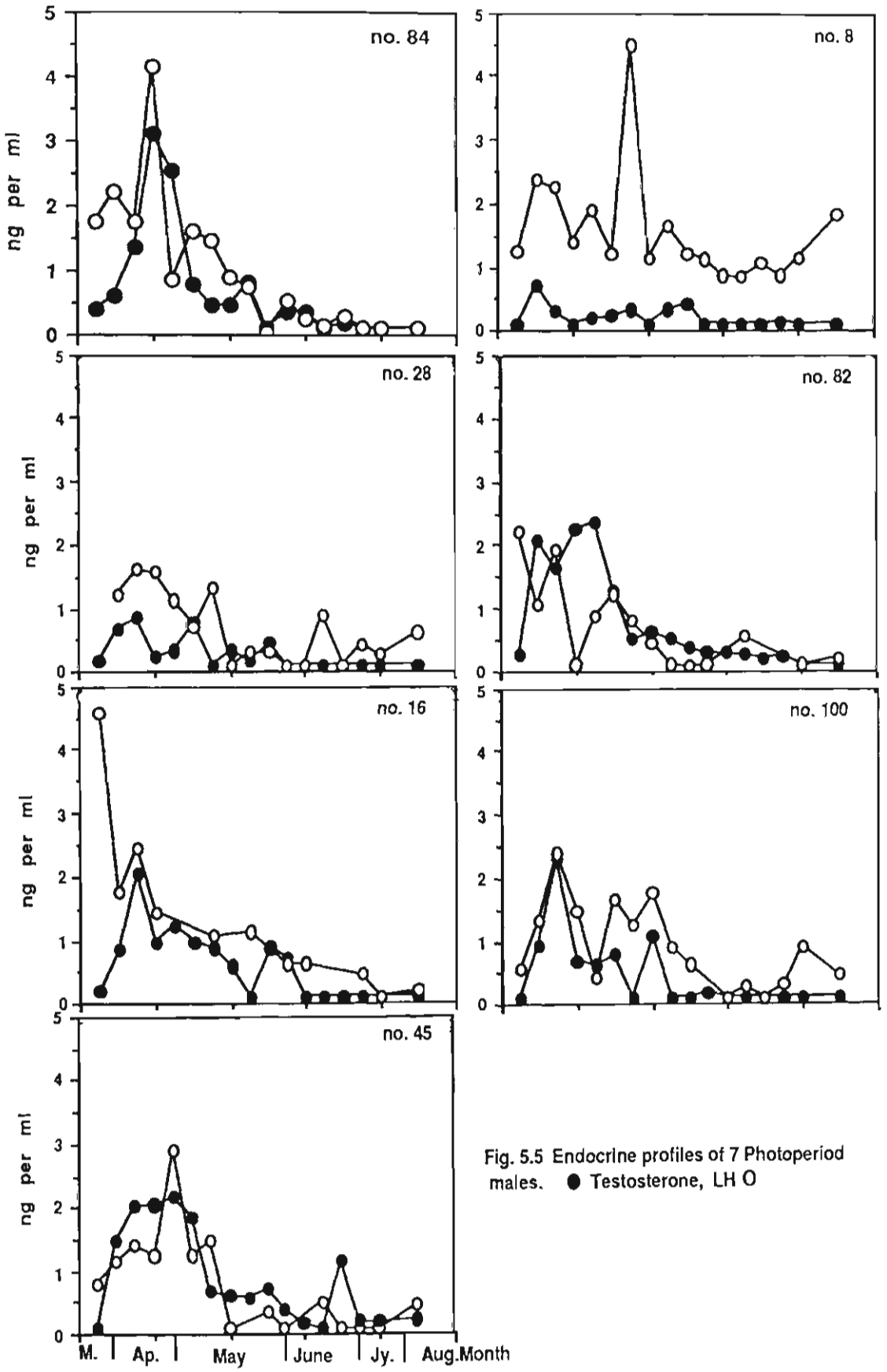

Fig. 5.5 Endocrine profiles of 7 Photoperiod males. ● Testosterone, LH O

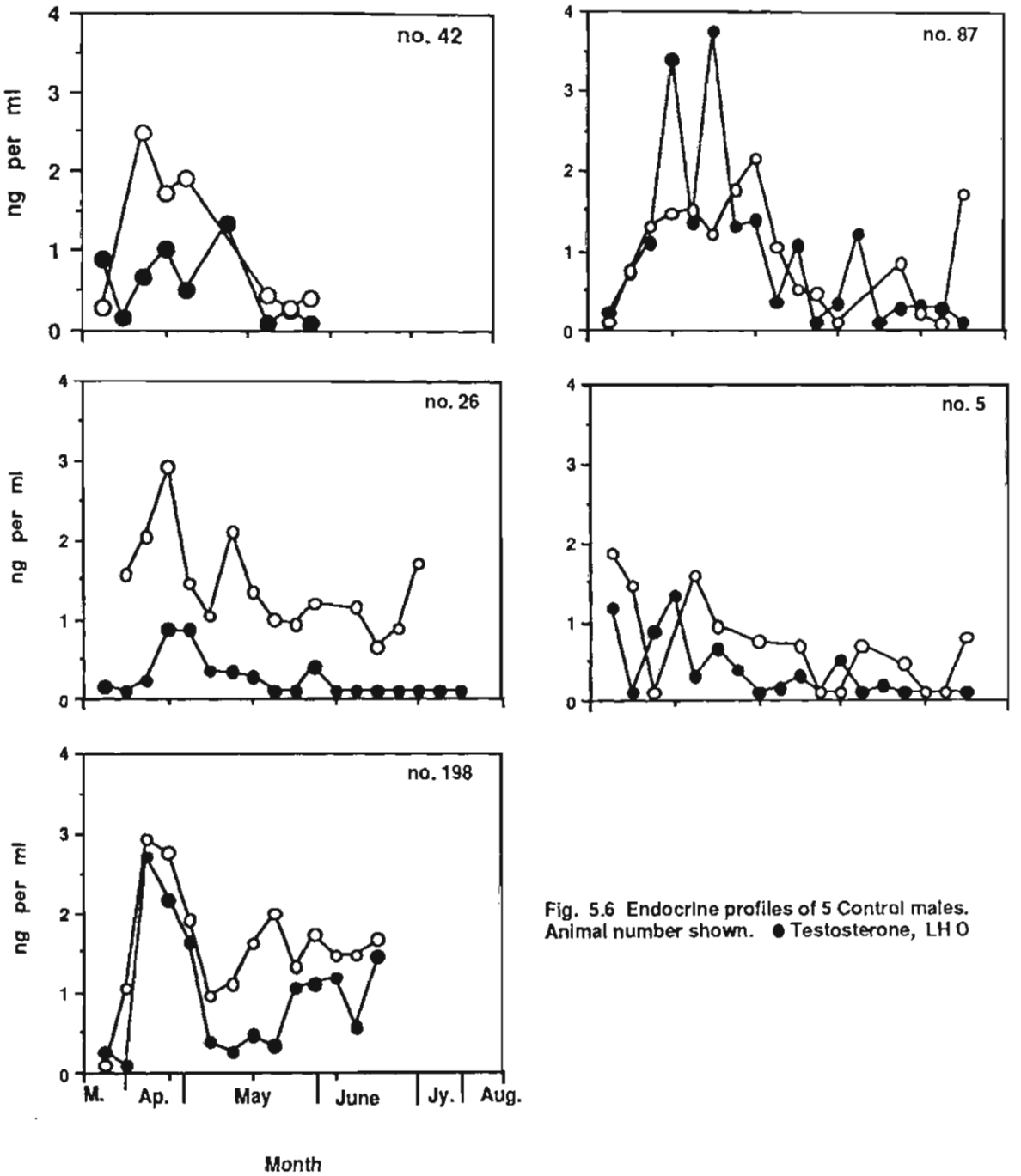

Fig. 5.6 Endocrine profiles of 5 Control males. Animal number shown. ● Testosterone, LH O

LH and second lowest testosterone levels. Males no. 16 and 28 scored consistently high on body weight, testes index and LH level but did not show a trend with testosterone concentrations.

Table 5.4 Males ranked from highest to lowest for the 4 parameters (male identity number shown)

Photoperiod	Highest			-------->		Lowest	
Weight	28	16	45	100	84	8	82
Testes Index	28	16	100	84	82	8	45
LH	8	16	84	100	45	82	28
Testosterone	45*	82*	84*	16	100	28	8
Control							
Weight	5	26	42	87	198		
Testes Index	87	26	198	5	42		
LH	198	26	42	87	5		
Testosterone	198	87*	42*	5	26		

* first year males

5.4 DISCUSSION

The results of this experiment suggest that some endocrine and morphological cycles of the male Eastern quoll can be influenced by changes in photoperiod. Within 4 to 6 weeks exposure to a long daylength, male quoll showed significant variation in body weight and LH secretion compared to control animals. As the artificial light regime approximated the non-breeding months of November and December, during which time males in the wild gain in body weight and have basal levels of LH (Chapter 4), it is possible photoperiod males were responding in the appropriate direction to an increase in daylength. The short duration of this experiment may have prevented more

obvious differences between the two groups from emerging.

The simplistic design of this experiment prevents any detailed analysis of the hypothalamic-pituitary mechanisms underlying the response to photoperiod change. In other species such as the Soay ram the effect of a long daylength is to inhibit hypothalmic and pituitary activity and suppress LH-RH (Lincoln & Short 1980). Feedback from the testes increases this inhibition causing further depression of gonadotrophic secretion. During short days, inhibition from the testes becomes less effective and the hypothalmus increases its secretory activity with an increase in the frequency of episodic LH-RH release. It is possible that the processes in the Eastern quoll (a southern hemisphere species) are similar to those in the ram. In this experiment the change in photoperiod from decreasing to long days, may have signalled the hypothalamus to alter its output of LH-RH. This may have been initiated independent of any final effect of gonadal hormones. As the quoll passes from the breeding to the non-breeding season (short days to long days), the pituitary-gonadal axis changes its sensitivity to the negative feedback effects of gonadal hormones. More detailed information on the mechanisms for this species are not available as yet.

Two interesting points have arisen from this experiment. Firstly, whether there was any response by testosterone and the testes cycle to a change in photoperiod and, secondly, the similarity and synchrony between some photoperiod and control cycles.

One reason why testosterone secretion and the cycle of testes growth in the quoll remained unchanged from the control condition may be that a change from short to long days 'switches on' the hypothalamic control of LH release but that it takes much longer to activate the pituitary-gonadal axis. For example, it may take many weeks for the Soay ram to achieve full reproductive development following a change in photoperiod even though the initial response may be rapid (Lincoln 1976; Lincoln & Short 1980). A similar time lag, coupled with the relatively short observation time of this experiment, may have obscured changes in these parameters.

Another reason for a lack of change could be that the long daylength inhibited the hypothalamic release of LH-RH necessary for LH secretion and this in turn failed to stimulate the Leydig cells to alter testosterone synthesis. Many mammals show a change in the sensitivity of the testes to changes in LH secretion (Lincoln & Short 1980; D'Occhio *et al.* 1984) and some species can

show a rise and fall in LH in response to photoperiod which is independent of testicular hormones (Clarke 1981). Likewise, the constancy of the photoperiod over 17 weeks may have had little effect after the previous experience of daylength or caused the male to become refractive or insensitive to the light regime. In this case there would be no additional change in testosterone secretion or testes regression. Exposure to a constant photoperiod fails to stimulate reactivation of the blastocyst in female tammars (Sadlier & Tyndale-Biscoe 1977) and also causes refractiveness in a number of other mammals (Almeida & Lincoln 1984; Boyd 1986).

The European rabbit, *Oryctolagus cuniculus,* responds to an altered light regime with an inhibition or reactivation of the testes but does not produce any alteration to testosterone or LH concentration (Boyd 1986). Boyd proposed that short-term stress caused by handling and anaethetising the rabbits when taking blood masked any endocrine changes. While it is possible that the quolls experienced similar stresses throughout this experiment, investigations reported in Chapter 7 suggest that male quoll are not unduly stressed by confinement and that restricting access to other animals appears to lower the levels of free cortisol.

There appears to be a difference in the sensitivity of male marsupials to photoperiod changes and the male quoll may be reflecting such species variation. Male *Antechinus stuartii* housed with females under different photoperiods show a significant change in androgen secretion after about 6 weeks (Scott 1986). Scott proposed that while the non-breeding photoperiod condition of long days suppressed reproduction, a short day photoperiod alone was not sufficient to activate the hypothalamo-gonadal axis. The reverse of this situation was found when *Antechinus swainsonii* held on a daylength similar to the natural mating season showed an earlier rise in androgens compared to experimental animals (reported by Lee & McDonald 1985).

Perhaps the most likely reason why male quoll failed to show any apparent change in testosterone secretion or testes size was that photoperiod alone was not sufficient to stimulate these changes. The male quoll may be like the Brown antechinus (Scott 1986), Short nosed bandicoot (Gemmell *et al.* 1985; Gemmell 1987), Brushtail possum (Gemmell *et al.* 1986) and Tammar wallaby (Catling & Sutherland 1980; McConnell *et al.* 1984), where factors in addition to photoperiod influence reproductive activity. Factors such as a

change in metabolic activity, diet, temperature or the presence of the female quoll, may be required to influence these cycles.

Alteration of the testicular cycle of the seasonally breeding Bush rat, *Rattus fuscipes,* requires changes in daylength, diet and environmental temperature (Irby *et al.* 1984). A combination of shortening daylength and decreasing temperature will induce partial regression of the testes in this species but a reduction in protein intake is required for complete regression. Similarly, photoperiod alone has no affect on the gonadal function of adult Deer mice, *Peromyscus maniculatus,* eventhough the body mass of young mice changes at altitudes with differing photoperiods (Weirner *et al.* 1984). Seasonal changes in body weight occur in Djungarian and Golden hamsters, ground squirrels, dairy cattle and lemmings with no concomitant change in food intake. This suggests that there is an alteration in metabolic processes either due to the alteration in daylength or inherent rhythms (Turek & Campbell 1979).

In three species of Antechinus, *Antechinus stuartii, A. swainsonii, A. flavipes*, there was little indication that photoperiod alone controlled the timing of reproduction (Dickman 1985; Dickman *et al.* 1987). The approximate timing of breeding appears to be controlled by an endogenous circannual rhythm, with the precise timing of reproductive activity synchronised by photoperiod and the influence of the female. In *A. stuartii* the onset of the mating period was correlated with the rate of change of photoperiod, rather than with critical photoperiod length (McAllan & Dickman 1986), however this can be overriden by endogenous circannual rhythm (Dickman *et al.* 1987). It is both the circannual rhythm and a response to this aspect of photoperiod that allows individuals to take advantage of the arrival of insect flushes and prepare for a single large reproductive effort. A similar circannual rhythm and response to photoperiod may be found in the Eastern quoll.

The second point of interest was that both the photoperiod and control males displayed highly synchronous hormonal profiles that peaked then declined in concentration much earlier than males in the wild. This supports the suggestion that additional factors apart from light were influencing the hypothalmic-gonadal axis of both groups of males. Preliminary observations have found that the onset of the breeding period may vary by up to 4 weeks at various locations around Tasmania (Appendix D). These are unlikely to be accounted for entirely by a change in photoperiod due

to the closeness of the locations.

Social factors have been well documented as influencing the breeding cycle of many animal species (Sadlier 1969; reviewed by Jewell 1973) and the absence of the female quoll may have caused an early decline in LH and testosterone. Male Kowaris and Tammar wallabies when segregated from females during the oestrous cycle fail to show a rise in plasma androgen levels near breeding (Catling & Sutherland 1980; Fletcher 1983). Male Antechinus housed with females but maintained on a daylength not conducive to breeding, completely suppress androgen production within 3 months of exposure (Scott 1986). Male Antechinus may be responding to the reproductive activity of the female which in turn is responding to a photoperiod cue. The effect of social factors on the reproductive activity of the male quoll are investigated in more detail in Chapter 6. The results of those investigations suggest that contact with the female quoll may be instrumental in initiating an androgen rise near breeding time in the male.

In conclusion, these results suggest that photoperiod may be one factor influencing seasonal breeding in the male Eastern quoll. Body weight and LH secretion were found to respond to a change in photoperiod within a relatively short time period. It is hypothesised that the precise timing of breeding, however, is unlikely to be controlled by photoperiod alone. Additional factors such as endogenous circannual rhythm, diet, temperature and social cues, such as the presence of the female, may initiate breeding.

There is sufficient evidence arising from this study to warrant a more detailed investigation of the effect of photoperiod on the Eastern quoll. Future work could focus on the presence of the female, the hierachy between photoperiod and social cues and also the effect of artificially increasing or decreasing the hours of light over a longer time period. Prolactin and melatonin may be additional hormones to investigate.

CHAPTER 6

FACTORS INFLUENCING BREEDING

6.1 INTRODUCTION

Godsell (1983) presented a comprehensive account of the types of communication and spacing behaviour of Eastern quoll in captivity. She found that although the quoll displayed a variety of social behaviours such as vocalisations, visual displays and olfactory cues, the animals generally avoided each other by using aggressive threats. Males tended to form a dominance hierarchy that was maintained by avoidance and submissive behaviour of subordinates. Females displayed aggressive behaviour only toward other females in relation to den sites or pouch young.

Godsell considered that although quolls appeared solitary and exhibited a low frequency of social behaviour, olfactory cues probably played an important role throughout the breeding season. Various forms of scent marking are common among dasyurids and other marsupials and range from behaviours such as cloacal dragging, urine dribbling, chin and sternal rubbing and a number of methods for distributing saliva (Buchmann & Guiler 1977; Eisenberg & Golani 1977; Croft 1982; Fanning 1982; Biggins 1984; Russell 1984; Fadem & Cole 1985; Conway 1988). In some Dasyuridae such as *Sminthopsis crassicaudata,* (Ewer 1968), *Sarcophilus harrisii*, (Buchmann & Guiler 1977), *Ningaui* spp., (Fanning 1982) *Antechinus* spp., (Croft 1982) and *Dasyurus maculatus,* (Conway 1988), scent is used to display sexual dominance and or receptiveness of the female. Godsell (1983) considered cloacal dragging and rubbing behaviour by oestrous female quoll, coupled with low activity levels and small home ranges in the wild, were behaviours that probably aided the male quoll in "homing-in" on females.

Detecting the presence and influence of social cues on reproductive activity can be difficult. Measuring endocrine changes in relation to changing social circumstances, however, can be employed as an indirect measure of response. For example, some male marsupials such as the Tammar wallaby,

Macropus eugenii, (Catling & Sutherland 1980; Inns 1982) and Kowari, *Dasyuroides byrnei*, (Fletcher 1983) show a significant rise in plasma testosterone concentration when in the presence of oestrous females. Males isolated from females during the breeding season do not produce this rise. In this way hormonal changes reflect a change in social experience and sexual status.

The aim of this chapter is to examine the effect of different social groupings on the pattern of endocrine secretion in male *Dasyurus viverrinus.* A number of treatments were designed to investigate whether proximity to the female quoll, competition and physical contact, influence the male's secretion of LH and testosterone during the breeding period. The aims of each treatment are listed below.

Captive Breeding: This experiment simulates the natural pattern of breeding by exposing captive males to new animals at breeding time. The aim of this treatment is to determine whether competition for females influences the male's pattern of hormone secretion.

Males with Females: The aim of this experiment is to determine whether males housed with females over the breeding season produce the same hormonal profiles as males experiencing competition at breeding time.

One Male with One Female: This study investigates whether a lack of competition for a female influences the male's secretion of LH and testosterone. In this treatment one pair of animals are housed together over the breeding season.

Males near Females: This experiment monitors the endocrine cycles in male quoll housed near but denied physical access to females. The quoll is solitary in the wild, therefore a cue from the female may be important in triggering mating activity.

Males isolated from Females: The aim of this treatment is to examine the importance of the female quoll by monitoring the hormonal cycles of males isolated from females over the breeding period.

6.2 MATERIALS AND METHODS

Details concerning methods of blood collection and hormone assays have been presented in Chapter 2 and Chapter 3. Blood samples were collected from each experimental group weekly and then more frequently (2, 3 and 4 times a week) as the breeding season approached. Smaller volumes of blood were collected during the mating period to minimise disturbance. Samples of blood plasma were stored at -20°C until assayed for LH and testosterone. Plasmas from females in contact with males were measured for LH, progesterone and prolactin.

6.2.1 Captive Breeding

Animals were housed in an external enclosure (measuring 16m length x 13m width x 2m height) containing artificial nest boxes and some natural vegetation (Plate 6a). Four adult males, 2 adult females and 2 juveniles (1 male and 1 female) were placed in the enclosure in January 1984. These animals formed a 'resident' population (5 males, 3 females).

Four males were added to the compound in March to replicate the natural influx after weaning. The males were removed in April to simulate dispersal. In May, 10 new animals (5 males and 5 females) were added to the enclosure. This addition was to simulate the influx of animals into the resident population near breeding time. Females were introduced to increase the opportunities of males to mate. For a short period the compound supported a high density of animals but this was alleviated quickly during and after mating. This sequence of events have been outlined in Table 6.1.

Disturbance was kept to a minimum over the mating period. Food was proportioned according to the number of animals in the compound and therefore was never a limiting resource.

The experiment was completed by October 1984 although females with young were not released until January 1985.

6.2.2 Males with Females

This experiment was conducted in a bushland enclosure (measuring 8m length x 5m width x 2m height) within the Zoology department grounds. Five males and 2 females were placed in the enclosure in April 1986 and remained together over one breeding season. No new males were introduced to the experiment but the 2 females had to be replaced with new

animals because of injuries suffered during mating.

Three of the males were adult (nos. 201, 205, 208) and 2 were first year males (nos. 202, 204). Both females (nos. 206, 207) were approaching their first breeding season. The experiment was completed in August 1986.

6.2.3 One Male with One Female

One juvenile male (no. 209) and one juvenile female quoll (no. 200) were placed in an outside pen (3m length x 1m width x 2m height) in April 1986. The pair remained housed together over one breeding season. The male was released in late July 1986 several weeks after the female had given birth.

6.2.4 Males near Females

The experiment was conducted in an external animal house adjacent to the zoology department, from April to August 1986. The animal house received natural light via glassed windows.

Three adult male quoll (nos. 210, 214, 216) were caged individually and the cages placed in a row facing two female quoll (AB, no. 215). The male cages were spaced several centimeters apart and approximately half a meter away from the female cages. The females were caged individually but had one adjoining mesh side to their cages. Visual and auditory communication was possible between animals but physical contact between males and between males and females was prevented. Animals were handled in their own sacks.

6.2.5. Males Isolated from Females

Males in this experiment were divided into two groups, both groups were totally isolated from females:

(1) Separated : Six male quoll were housed in a shed approximately one kilometer away from the zoology department. The shed received natural lighting and was not temperature regulated. This location was free from contact with other quoll and human disturbance. The animals were caged individually in wire mesh cages (measuring 1.0 m x 1.0 m x 1.0 m). Four of the males were first year animals (nos. 212, 217, 218, 219) and two were adult (nos. 220, 221). The males remained at this location from April until August 1986, over one breeding season.

(2) Combined: Four adult males (nos. 00, 01, 02, 5) were housed all

together in one brick and wire pen and isolated from females. The pen (measured 2.5m length x 3m width x 2.5m height) was enclosed inside the departmental holding facility, approximately 400 meters from other locations containing quolls. The experiment commenced in April 1984 and ended in January 1985. Due to the loss of some animals, an additional 4 males were added to the group in June 1984 (nos. 20, 21, 25, 1E).

Animals were examined in their own sacks and care was taken not to expose the group to any stimuli from females or outside males.

6.3 RESULTS

Body weight and scrotal dimensions will be discussed briefly or only when the cycles differ from the patterns already described in Chapter 4 and Chapter 5.

6.3.1 Captive Breeding

Table 6.1 outlines the sequence of events throughout the experiment, including the addition and removal of animals.

The mating period lasted approximately 14 days but comprised 2 periods of copulation. The first matings commenced between resident males and resident females on 1 June and extended approximately 5 days. Four of the 5 introduced females commenced mating 11 days later and matings continued between this group for approximately 5 days. The variation in the timing of mating is probably due to the derivation of animals from different populations (see Appendix D).

The mating period was characterised by aggressive displays and much daytime activity such as boundary running and calling. There was considerable crowding in selected nestboxes while previously used nest sites were no longer occupied. Several males received wounds about the head and neck around the time of mating and one animal suffered a broken tail. Females received bites to the neck and back during copulation and these in some cases became infected and required treatment.

Table 6.1 Sequence of events during the Captive Breeding experiment (removal or addition of animals is shown as - or +).

Jan.	Resident population of 5 males and 3 females
_ _	Juveniles weaned and retained in compound
Feb.	Maturation of juvenile male
_ _	
Mar.	+ 4 new males
_ _	
Apr.	- 4 males
__	
May	
__	+ 5 males + 5 females
June	Mating by 1 June
	- 2 males
	End of first mating
	Fem. nos. 10 & 14 gave birth, removed
	- 2 males -1 female
_ _	Female no. 18 gave birth
	Female nos. 27 & 15 gave birth
	Female no. 27 gave birth
July	Female no. 1 re-mating
_ _	Female nos. 15,17, 18, 19, 27 re-mating
Aug.	
_ _	Total 6 males, 5 females
Sept.	
_ _	
Oct.	End of experiment.

6.3.1.1 Males

Fig.6.1 shows the mean changes in body weight and testes index for the resident population of 5 males (4 adults & 1 first year). Adult males attained a maximum body weight of 1.61 ± 0.06 kg ($\bar{x}$ ± s.e., n=4) by mid- May. By the onset of mating, every resident male had lost between 0.10 and 0.40 kg in weight and all continued to lose weight until late in the year. Three resident males (nos. 8, 28, 29) and one introduced male (no. 260) were identified copulating with females. The resident males were approximately 350 grams heavier than other males but male no. 260 was 100 to 200 grams lighter than resident males at the time of mating.

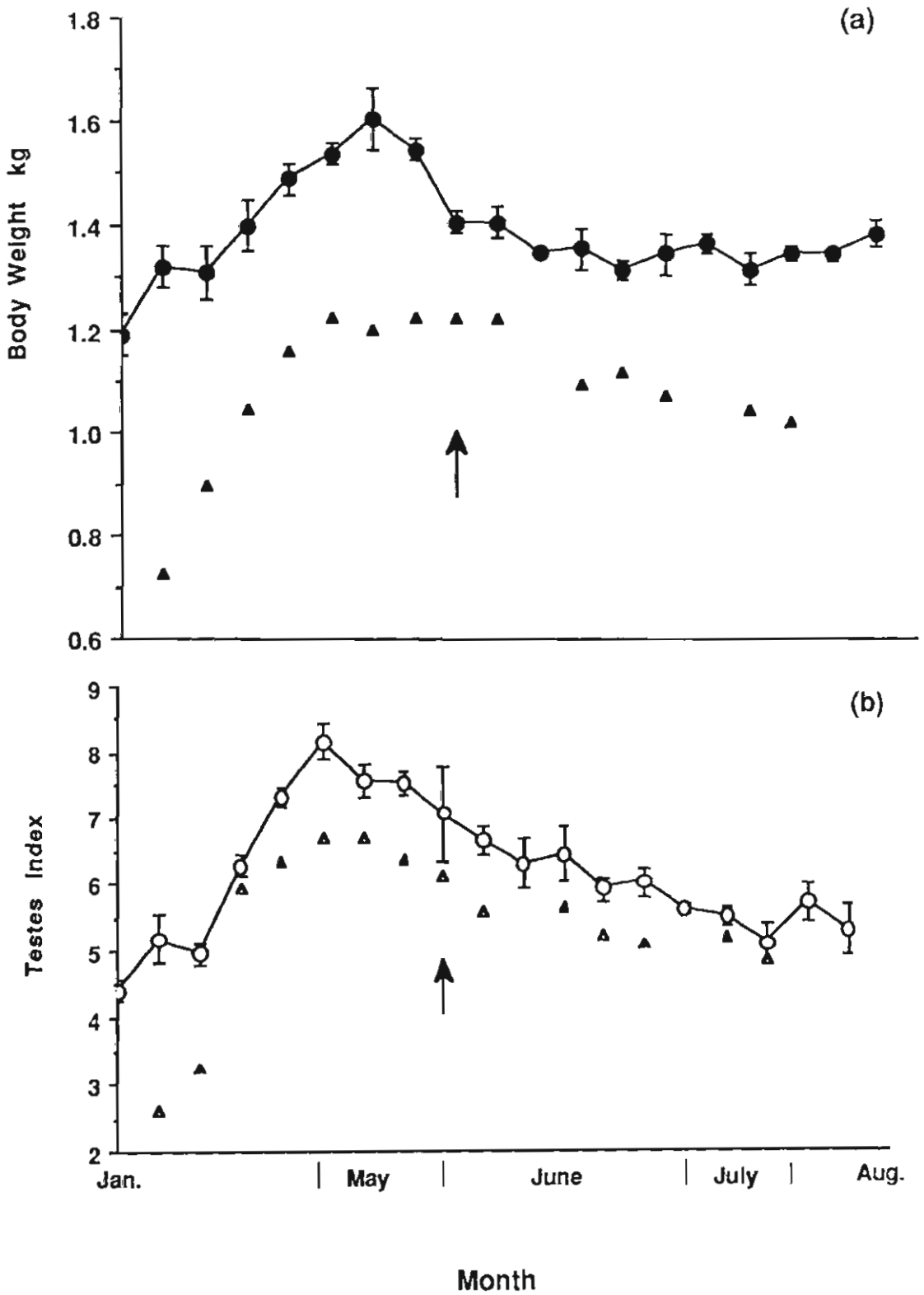

Fig. 6.1 Mean (± s.e.) (a) body weight (b) testes index for 4 adult resident males. Both parameters had declined by the onset of mating (arrow). Measures for the juvenile no. 198 are shown below.

Adult males reached a maximum testes index of 8.2 by early May, one week before reaching maximum body weight. The testes steadily declined in size during and after May. Body weight and scrotal profiles of the first year resident male are shown in Fig. 6.1 and are similar to the pattern shown by first year males in the wild.

LH levels fluctuated between 0.50 and 1.60 ng before and after breeding. This range was comparable to the range recorded in wild males at similar times (Chapter 4). LH increased to a mean level of 4.96 ng per ml at mating time (Fig. 6.2a). Although this was the highest mean LH level recorded in captive animals, the level was approximately 3 times lower than that observed in wild males during mating (13.90 ng LH per ml). LH decreased to a basal level of 0.56 ng per ml 9 days after the start of copulation. A small rise of 1.17 ng LH in mid- June coincided with the onset of mating in the introduced females (see Table 6.2) but the rise to 2.60 ng per ml in August did not appear to coincide with any event such as mating, the female's oestrous cycle or removal of animals.

Testosterone concentrations in resident males fluctuated at basal levels between January and May. The rise and large standard error in the February sample was biased by one high level from the first year male (see Fig. 6.4). Testosterone gradually increased as the breeding season approached and at the start of mating (1 June) had reached a mean level of 1.66 ng per ml (Fig. 6.2b). This pattern was similar, although considerably lower, to that observed in adult resident males in the wild. Males in the wild showed a mean testosterone level of 5.00 ng during mating with some individuals reaching between 6.00 and 7.00 ng per ml. One week after the start of mating, captive males had basal levels of testosterone. The small rise in testosterone observed some days later was coincident with the rise in LH and consistent with the onset of the second set of matings. No additional changes in testosterone levels occurred during the introduction or removal of animals.

Figure 6.3(a,b) shows the mean LH and testosterone levels in resident and new males combined. The levels for resident males alone are also inserted on the graph to show the variation between the two groups over the mating period. The effect of introducing new males near breeding time was to increase the mean LH and testosterone levels of the population during mating. Resident males alone showed a sharp decrease in hormonal levels throughout mating. After mating there was little variation in the hormonal

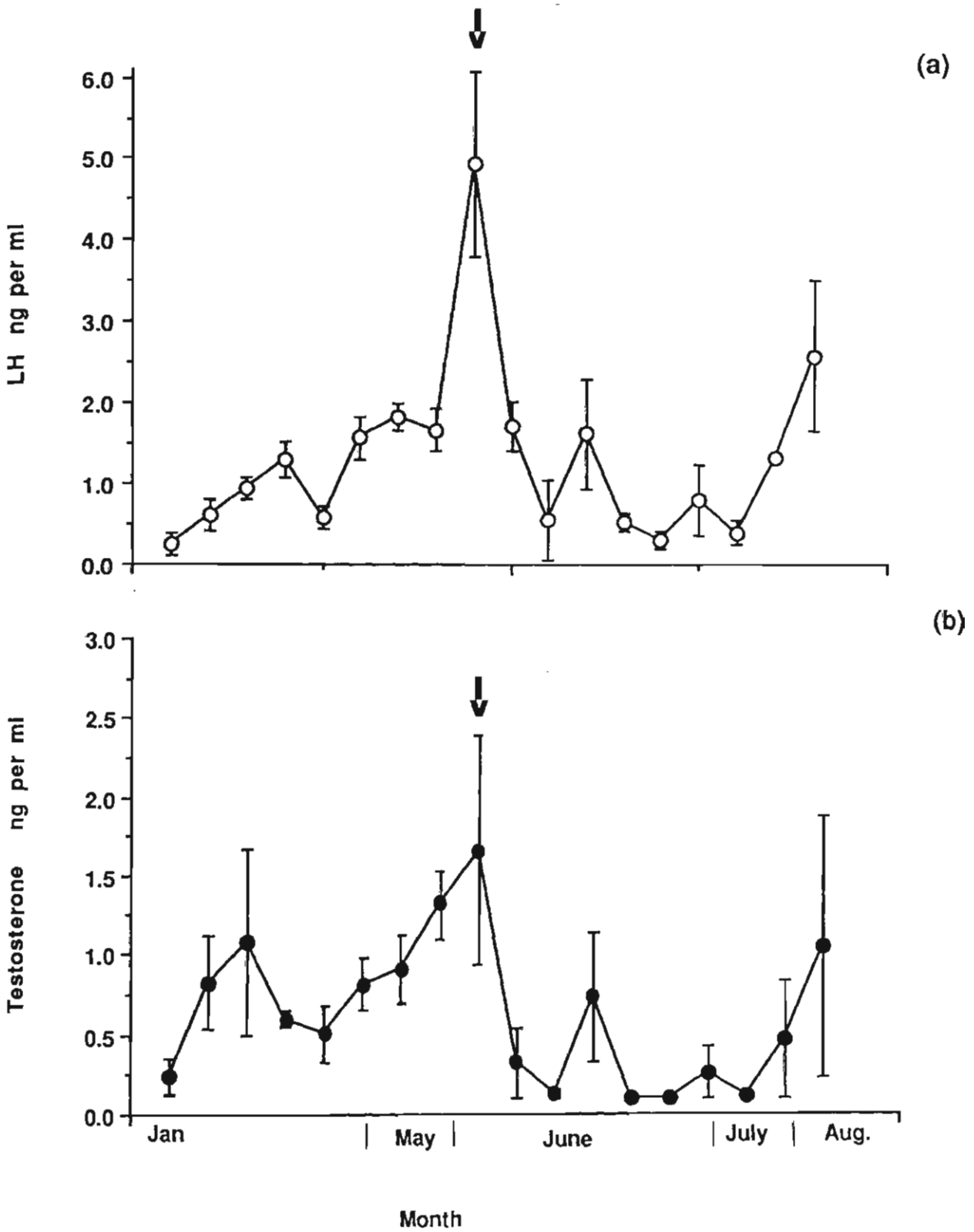

Fig. 6.2 Mean (± s.e.) (a) LH and (b) testosterone levels in 5 resident males. The onset of mating is indicated.

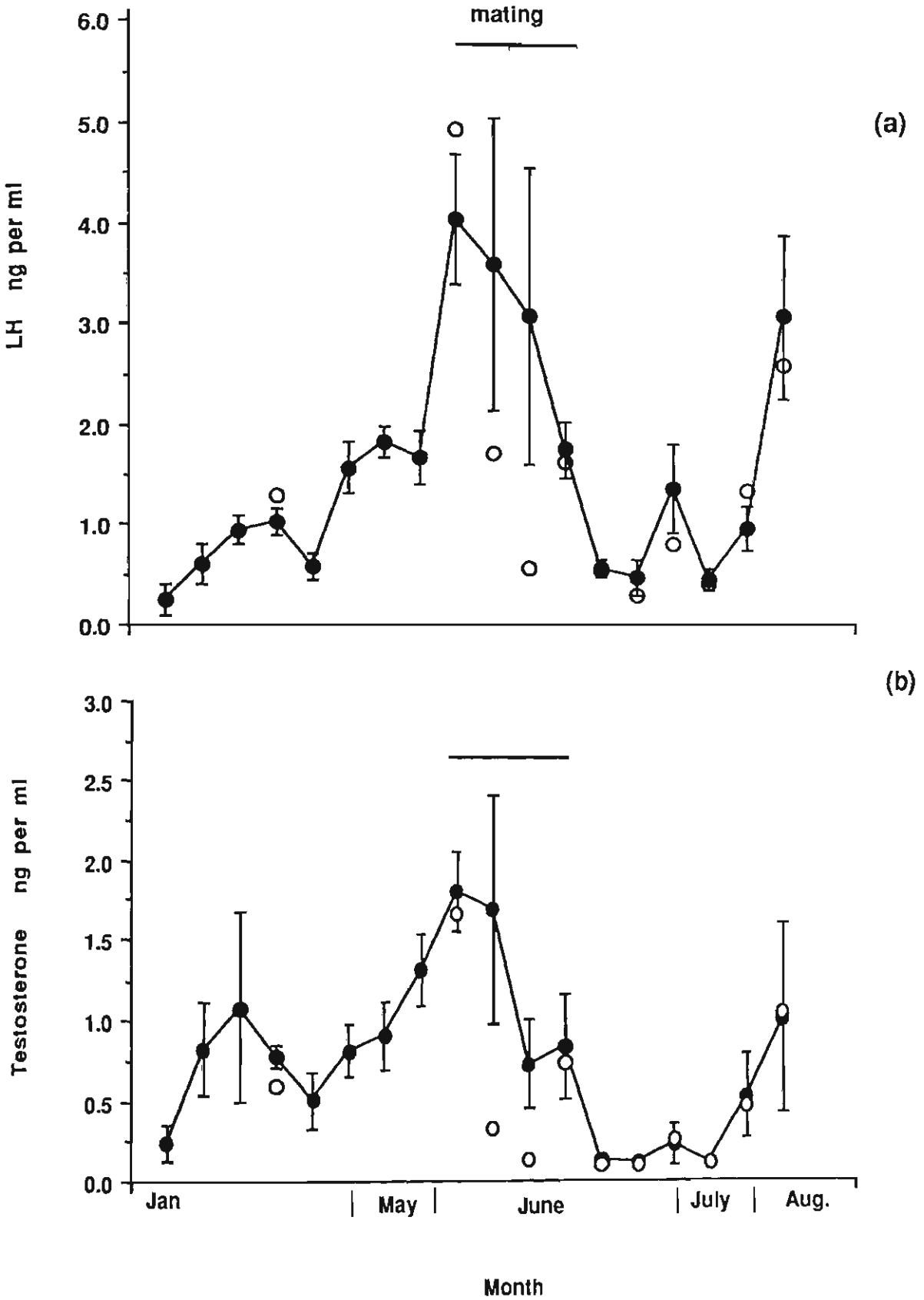

Fig. 6.3 Mean (± s.e.) (a) LH and (b) testosterone levels in resident and introduced males. O represent the mean levels for resident males as shown in Fig. 6.2. Mating is indicated.

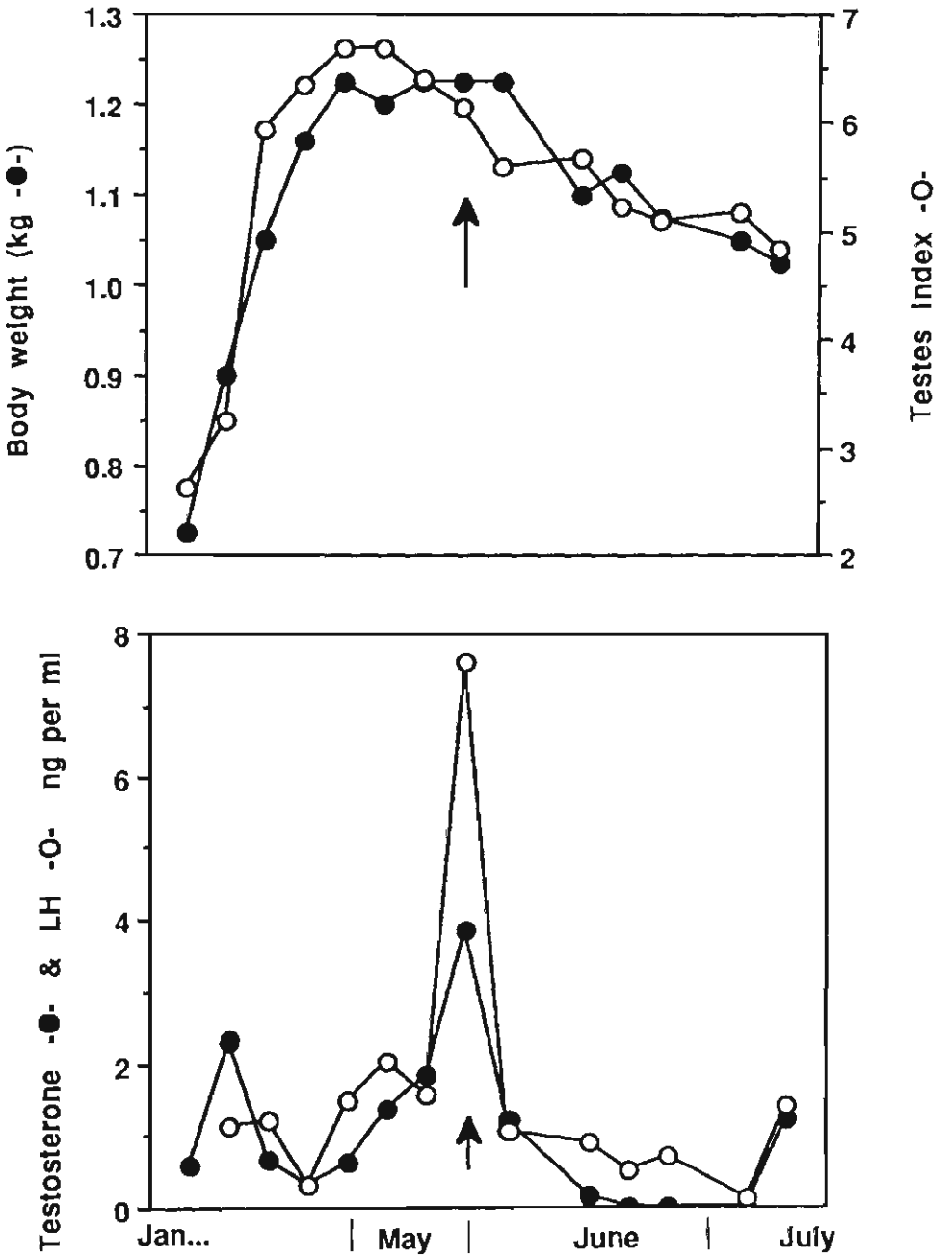

Fig. 6.4 Profiles for first year male no. 198. Breeding is shown by arrow. Maturity is represented by a weight between 0.9 to 1.0 kg and testes index of 5.0 to 6.0.

levels between resident and new males.

The weight, testes index and hormonal cycles of the first year resident male (no. 198) were similar to those of first year males in the wild (Fig. 6.4). Body weight and testes index showed a large increase between February and May. The testes declined in size by late May although body weight remained stable until early June. A rise in testosterone of 2.3 ng per ml in February coincided with the increase in body weight and testes size. LH and testosterone increased steadily from April and culminated in a peak of 7.6 ng LH and 3.9 ng testosterone in early June. Four days later hormone levels had decreased to basal concentrations which suggests that the rise was of relatively short duration. The synchrony between LH and testosterone secretion can be seen clearly in Fig. 6.4. This animal was not observed copulating with any females.

6.3.1.2 Females

The timing of mating and parturition for 3 resident and 5 introduced females is presented in Table 6.2. Every resident females was observed mating. Two (nos. 10 & 14) were observed with 6 newborn young each, 19 days after mating. Both females raised their young until weaning. The third resident female (no. 1) had a pouch 20 days after mating that was typically post-partum, but was not carrying young. She re-mated but was not observed with young

Every introduced female showed evidence of having mated (Chapter 2, sect. 2.2.3) but every female that gave birth lost her first litter of young. Two animals returned to oestrus and re-mated within 5 and 6 days respectively of losing young. In the other females this period ranged between 11 and 20 days.

Females without young had reduced, tight pouches by October. When released in January 1985 they showed little evidence of mammary or teat development and had regressed pouches typical of wild non-lactating females at this time (Appendix C).

Resident females reached maximum body weight by the end of May. During the mating period, females lost between 50 and 150 grams in weight or 12.5% to 15.0% of total body weight. Females did not continue to lose weight after mating or throughout the lactation period.

LH levels were low or below the sensitivity of the assay for much of the experiment (Fig. 6.5). Some individuals showed an increase in LH after

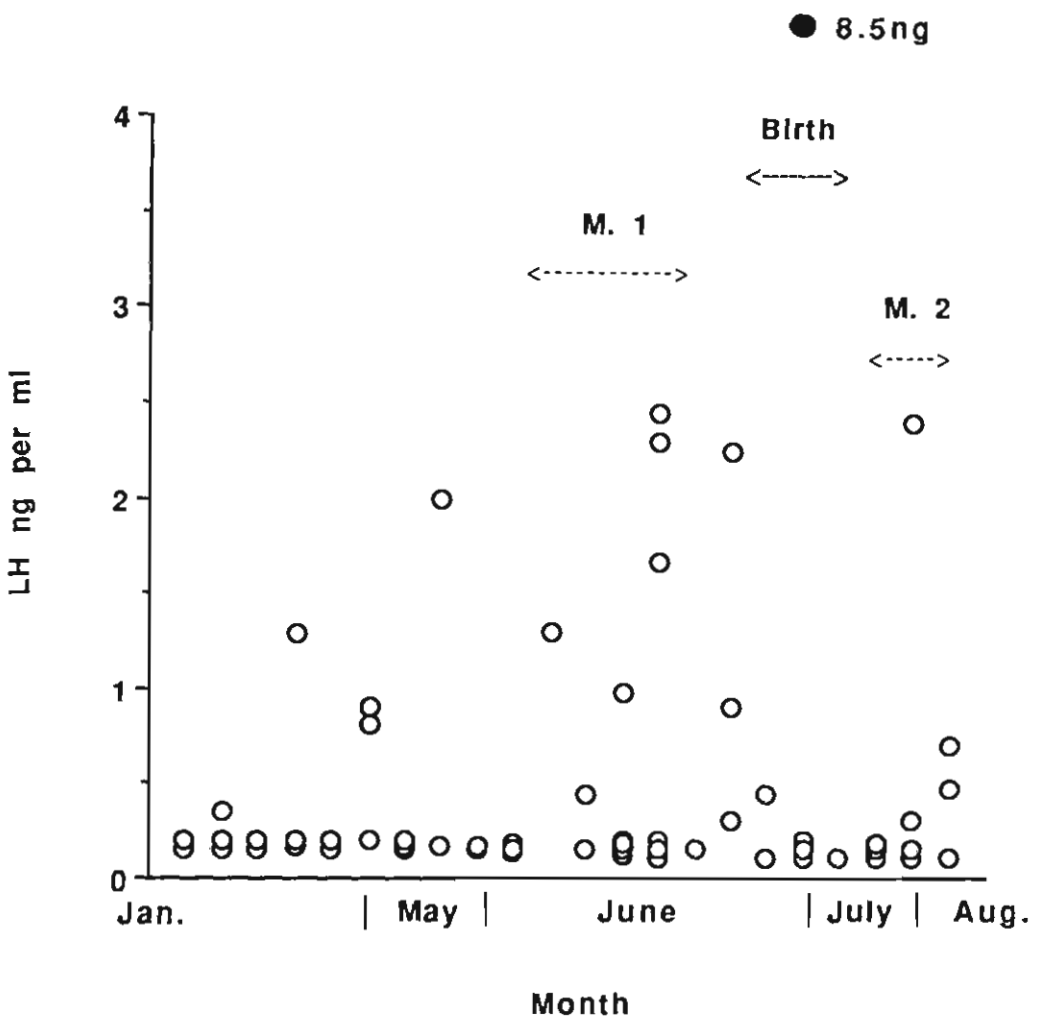

Fig. 6.5 LH levels in captive females (n=3 to 8). Every female mated at M.1. Those that failed to give birth or lost their young remated at M.2. One high LH level of 8.5ng was obtained from a female on the day she gave birth. Females retaining young had LH below the sensitivity of the assay.

mating and before parturition but no consistent pattern emerged. The highest level of LH recorded in this experiment was 8.5 ng per ml. This was produced by one female 18 to 24 days after mating but as this animal did not appear with young, its timing in relation to birth could not be determined.

Table 6.2 Reproductive traits of females in the Captive Breeding experiment.

Fem.	1st mated (June)	Birth	Gestat'n (days)	No. of young recorded	All young lost by	2nd mating (July)
1	2	? 21 June	19 - 20	-	-	12
10	2	20 June	19	6	retained	-
14	2	20 June	19	6	retained	-
18	6	25 June	19	3	13 July	by 18
15	13 - 18	6 July	18 - 23	2	12 July	by 18
27	13 - 18	6 July	18 - 23	6	12 July	by 18
19	14	-	-	-	-	on 18
17	13 - 18	? 7 July	?18 - 24	-	-	by 18

Progesterone levels were approximately 2.0 ng per ml or lower for most of the experiment. A rise in progesterone was detected late in pregnancy and immediately prior to birth (Fig. 6.6a). Levels increased gradually after mating and in two females were between 9.2 and 17.2 ng per ml one day before parturition. Progesterone levels decreased soon after the birth period and fluctuated near or below 4.0 ng for the remainder of the experiment. There was no apparent difference in progesterone levels between resident or introduced females.

Prolactin levels increased in concentration after mating and during the lactation period (Fig. 6.6b). Some individuals showed a rise in prolactin during pregnancy and one female recorded a level of 87.0 ng per ml one day prior to birth. Females with pouch young showed an increase in prolactin concentration during the lactation period. One female (no. 27) recorded a prolactin level of 79.0 ng while carrying 2 pouch young 5 days old. The highest prolactin levels were recorded in two resident females carrying pouch young

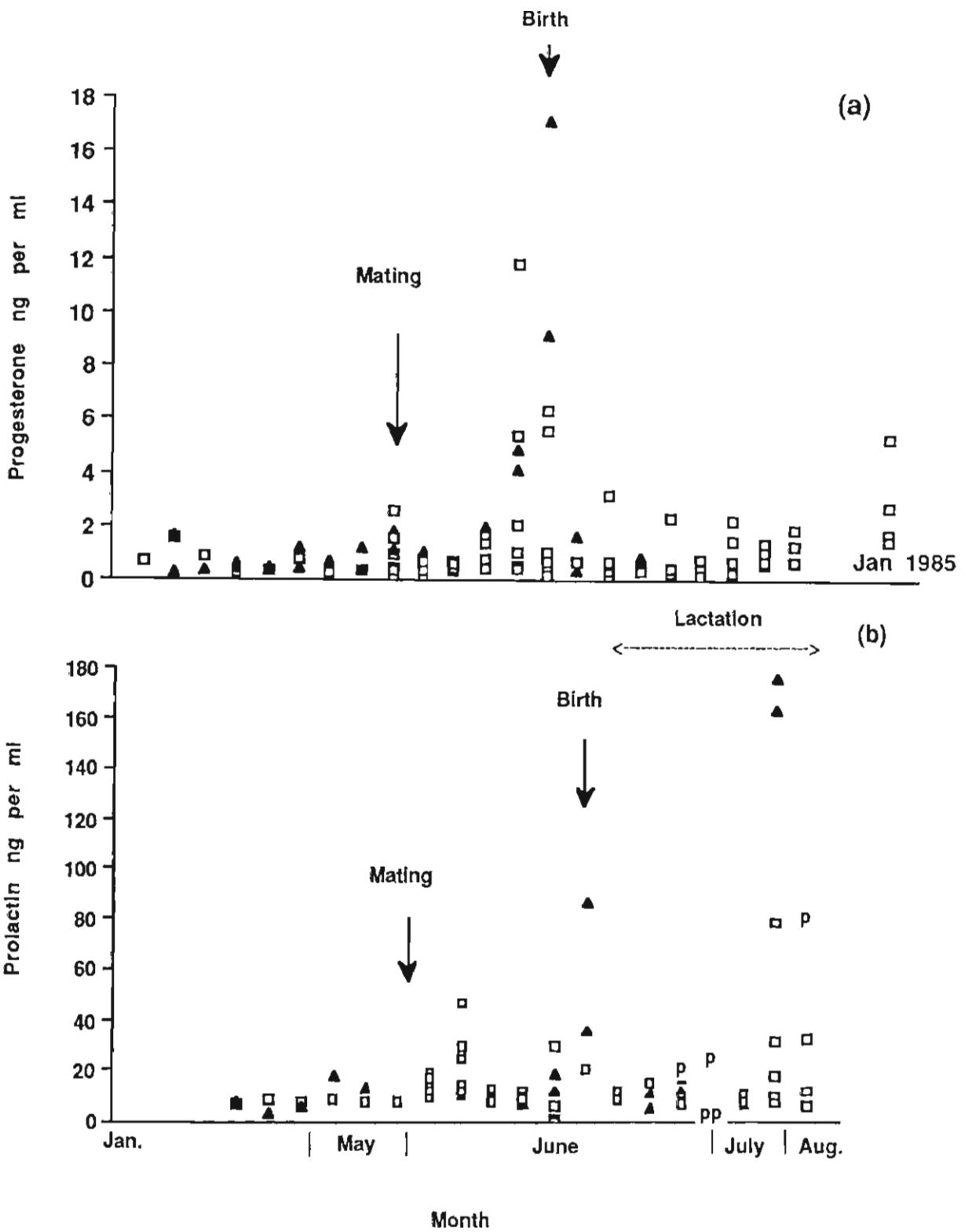

Fig. 6.6 Progesterone (a) and prolactin (b) levels in captive females (n=3 to 8). The onset of mating and birth are indicated. The ▲ indicates 2 females that retained their young and 'p' indicates females lactating at the time of sampling.

62 days of age (164.0 ng and 176.0 ng per ml). No prolactin measures were obtained after this stage of lactation. There was no apparent difference in the prolactin levels between resident or introduced females.

6.3.2 Males with Females

From April until May there was no obvious pattern of nest sharing between males and females. By the end of May one male would share with one or both of the females while the remainder of the males nested alone. The three adult males dominated the nest sharing. The start of nest sharing also coincided with the onset of pouch development in the females.

Resident female no. 207 mated on the 14 June. Due to cuts and a large gash on her underside this animal had to be removed from the compound and was replaced by female no. 215. Female no. 215 was observed mating one day after being placed in the enclosure and copulation proceeded throughout the day until 8 p.m. that evening. Female no. 215 also had to be removed from the compound due to injuries obtained during mating. The replacement female (AB) nested with male no. 208 one day prior to her mating on the 6 July. The second resident female (no. 206) mated on the 25 June. A summary of these events is contained in Table 6.3 .

Table 6.3 Reproductive traits of females housed with males.

Female No.	Entered enclosure	Removed	Date mated	Male no.	Gestat'n. days	No. of young
207	5 April	16 June	14 June	?	19	1
215	16 June	18 June	17 June	201	?20	-
AB	18 June	-	6 July *	?208	?20	-
206	5 April	-	25 June	?	?19	-

* re-mated 28 July

Females did not continue nesting with males after copulation and paired nest sharing ceased altogether by the end of the mating period. No

evidence was detected of female's being pursued by other males after their first copulation.

Male no. 201 was noted to be very aggressive and difficult to sample throughout the experiment. Every male lost weight during June but male no.201 remained 350 to 725 grams heavier than the other males (20% to 60%). Males incurred small losses of fur, bites to the back and scarring about the nose during mating.

LH concentration increased toward the breeding period but levels were relatively low compared to wild males (Fig. 6.7a). Testosterone levels ranged between 2.0 and 3.3 ng per ml prior to mating (Fig. 6.7b) and were similar to the levels recorded in wild males at this time. Three males (nos. 202, 204, 205) showed an increase in testosterone during the mating period, whereas the 2 males most likely to have copulated (nos. 201, 208), had basal levels of testosterone at this time. The large degree of individual variation is reflected in the large standard error for the June samples and in Fig. 6.8. Every male had a basal level of LH and testosterone by the time females were expected to give birth.

There was a significant variation in LH levels between the 5 males throughout the experiment (ANOVA, $F_{[4,83]}$ =3.19, $0.01<p<0.025$) with first year male no. 204 showing significantly lower levels. There was no significant variation in testosterone concentration between the males (ANOVA, $F_{[4,86]}$=1.19, $p>0.025$) although first year male no. 202 had consistently higher levels.

6.3.3 One Male with One Female

The male quoll in this experiment reached a maximum testes index of 8.15 by early May and maximum body weight of 1.175 kg by mid- May. Both parameters declined after May.

The pair were observed mating on the 4 June. This was 11 days earlier than reported in other groups. The female was observed with 6 pouch young on the 23 June, 19 days after mating. The male became increasingly agitated throughout the experiment and was separated from the female on the 18 July. The female raised the six young until their release in December 1986.

LH measurements were not available on every sample day due to insufficient plasma. Nevertheless, LH increased from 2.8 ng per ml at the start of the experiment to 9.0 ng per ml 7 days before mating (Fig. 6.9). It is

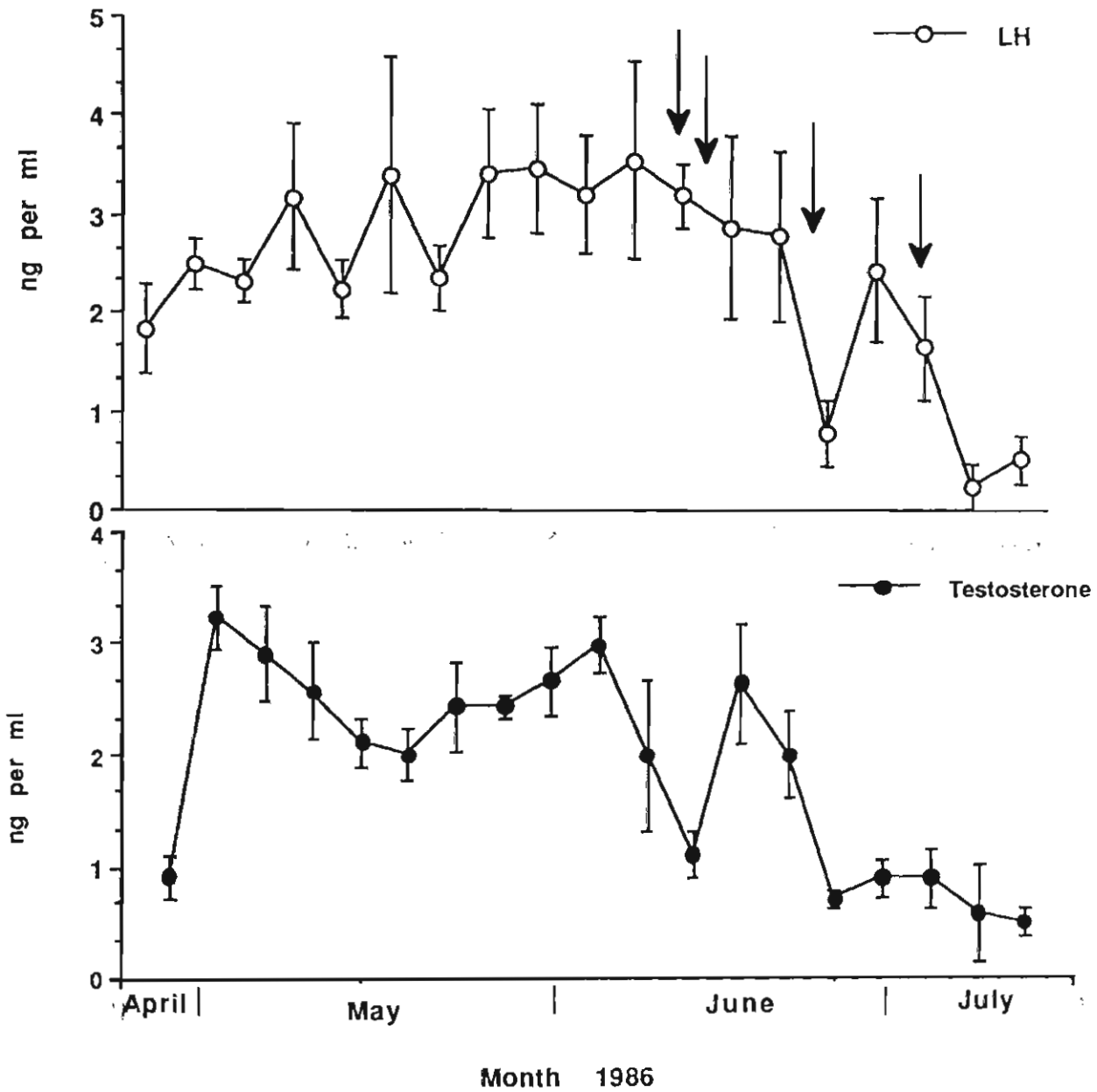

Fig. 6.7 Mean (± s.e.) LH and testosterone levels for 5 males housed with females over the breeding season. Arrows indicate time of matings. No new animals were introduced.

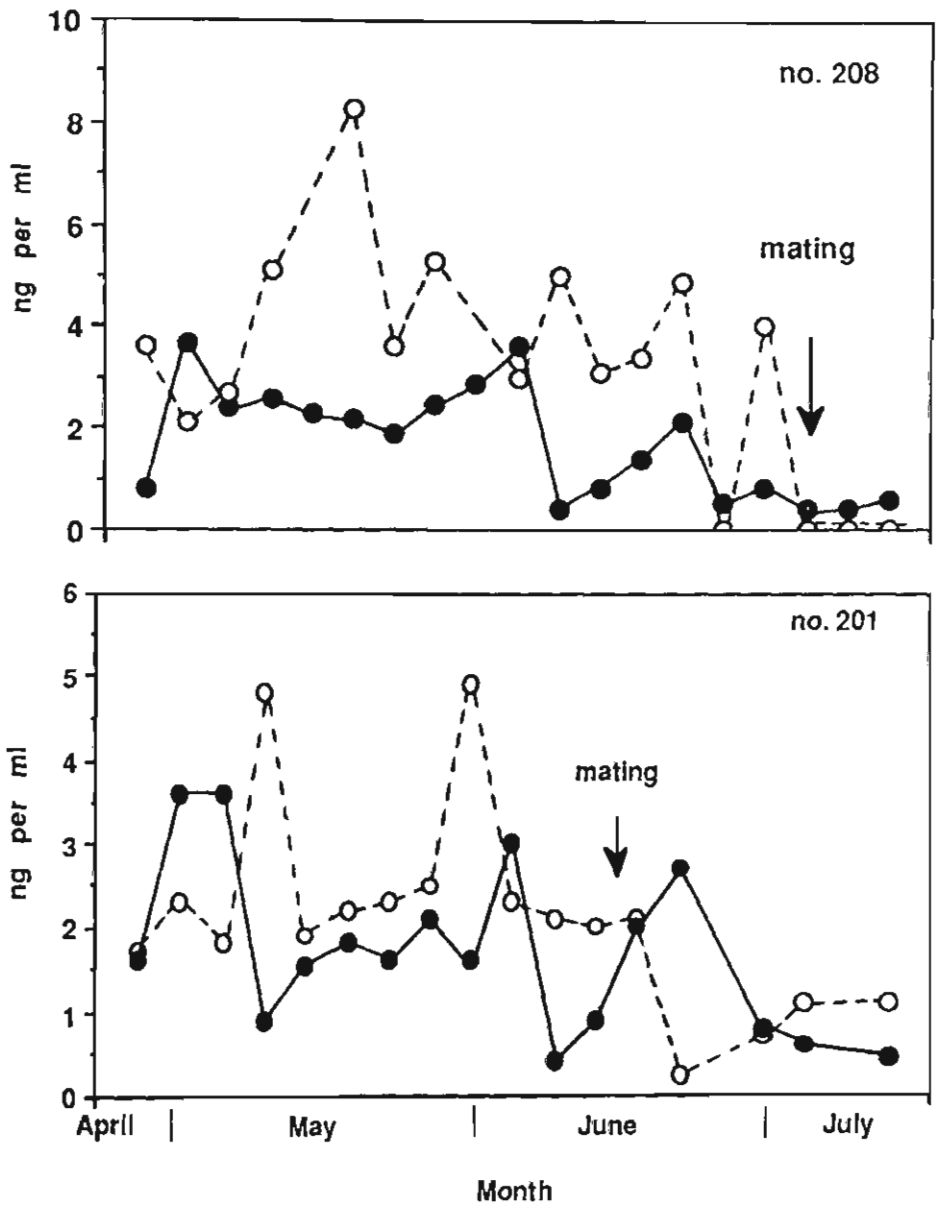

Fig. 6.8 LH and testosterone profiles for 2 males that were observed copulating with females. These males were heavier than the others and dominated the nesting with females. (LH O, testosterone ●).

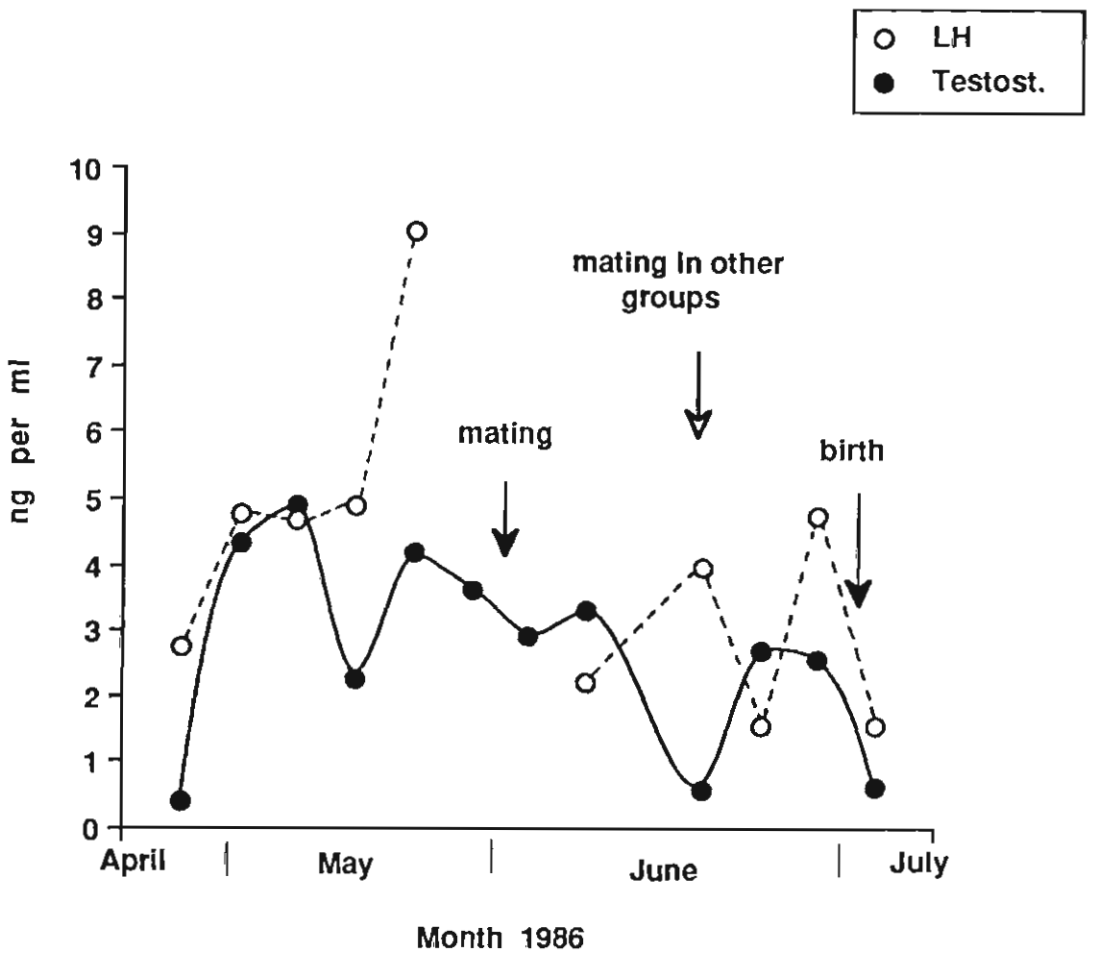

Fig. 6.9 LH and testosterone profile for 1st year male no. 209 paired with one female over the breeding season. Mating and birth are indicated. Not all the testosterone samples have a corresponding LH value.

possible that this level may have increased even further before mating. Five days after mating, LH had decreased to 2.2 ng per ml and showed sporadic fluctuations for the remainder of the experiment.

The male maintained testosterone levels of between 3.0 and 5.0 ng per ml throughout the experiment (Fig. 6.9). These were high compared to males in other captive groups but comparable to males in the wild. The highest testosterone levels were produced 2 to 3 weeks before mating. The reason for the depression in testosterone in mid- May is unknown, however, the depression in mid- June coincided with the end of the female's oestrous cycle and also the onset of mating in other groups.

6.3.4 Males Near Females

Males reached a maximum scrotal size by mid- May and maximum body weight by late May. A similar sequence of decline occurred in both these parameters after May.

The two females in this experiment displayed signs of behavioural oestrus between the 12 and 18 of June. The males also showed noticeable behavioural changes at this time such as guttural vocalisations, pacing and bouts of daily activity. In contrast to previous experimental groups, the males did not become aggressive and were relatively easy to handle during this time.

The profile for LH secretion in the males has been presented in Fig. 6.10. The highest mean levels of LH were recorded in May, prior to oestrus, however, individual variation was apparent. One male (no. 214) showed a large peak of 27.5 ng per ml at the end of May but no similar increases were detected in the other two animals.

Testosterone secretion declined over the study period. Teststerone was at a basal level throughout the female's oestrous cycle and until the end of the experiment.

There was no significant variation in LH or testosterone secretion between individual males (ANOVA, LH, $F_{[2,47]}=1.82$, $0.10<p<0.25$; testosterone, $F_{[2,48]}=1.05$, $p>.25$), although male no. 214 maintained consistently higher levels of both these hormones.

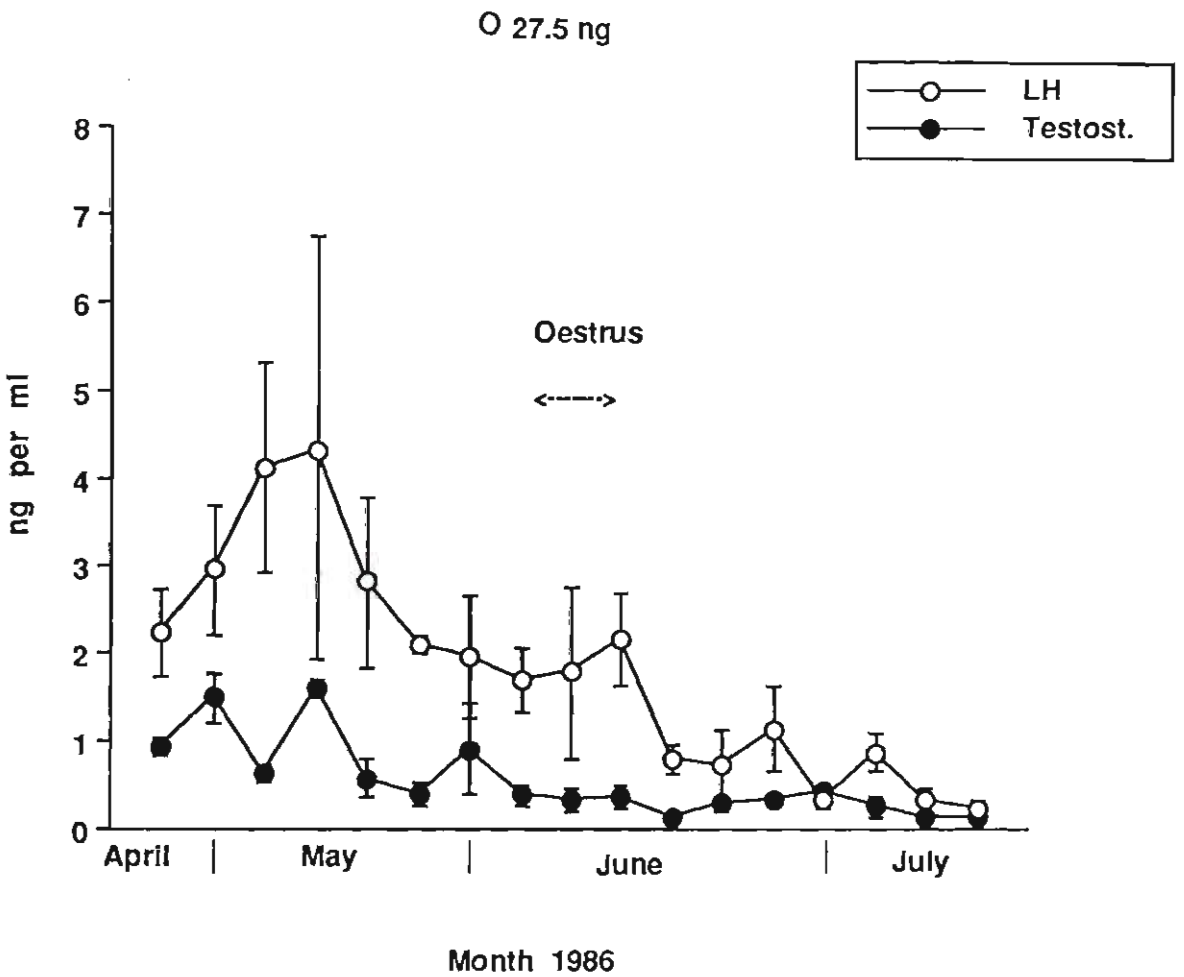

Fig. 6.10 Mean (± s.e.) LH and testosterone in 3 males housed near but without access to females. One male had LH of 27.5 and testosterone of 2.0 ng in early June.

6.3.5 Males Isolated from Females

6.3.5.1 Separated

LH and testosterone levels fluctuated in the 6 males isolated from females (Fig. 6.11). The large peak in LH at the end of May was due to two individuals with levels of 19.0 and 10.2 ng per ml, compared to ae mean of 3.1 ng per ml for 4 males. Testosterone levels increased during May but declined after May. This decline was well before the onset of oestrus in females from other groups. There was no significant variation in LH secretion between the 6 males (ANOVA, $F_{[5,60]}=0.52$, $p>0.25$) and no significant variation in testosterone levels between 5 males (ANOVA, $F_{[4,56]}=1.41$, $0.1<p<0.25$). First year male no. 212 had significantly higher levels of testosterone compared to other males and this was displayed behaviourally by a high level of agitation throughout the experiment.

6.3.5.2 Combined

The results of this experiment were difficult to assess but are included because they identify a discrepancy between testosterone levels and levels of aggression. Only two males were present over the entire study period (nos 5, 02). Two males escaped early in April (nos. 00, 01) and of the four new animals introduced in June, one had died within a week. A further two suffered injuries about the face necessitating one to be humanely killed. The remaining animals became so aggresive they were separated individually in late July to avoid further injuries.

Male no. 5 had attained a maximum body weight and scrotal size by the end of April. Male no. 02 reached a maximum scrotal size by mid- May and a maximum body weight of 1.95 kg by the end of June. This animal was between 0.55 and 0.80 kg heavier than other males.

Male no. 5 showed a decline in LH over the study period although there was a sporadic rise in LH late in April and August (Fig. 6.12). Male no. 02 showed a similar profile but with a sporadic increase in LH late in May. This increase appeared to coincide with the addition of new males into the experiment and with the onset of mating in other groups.

The interesting feature of the hormonal profiles of males no. 5 and no. 02 were the relatively low levels of testosterone. Both males had

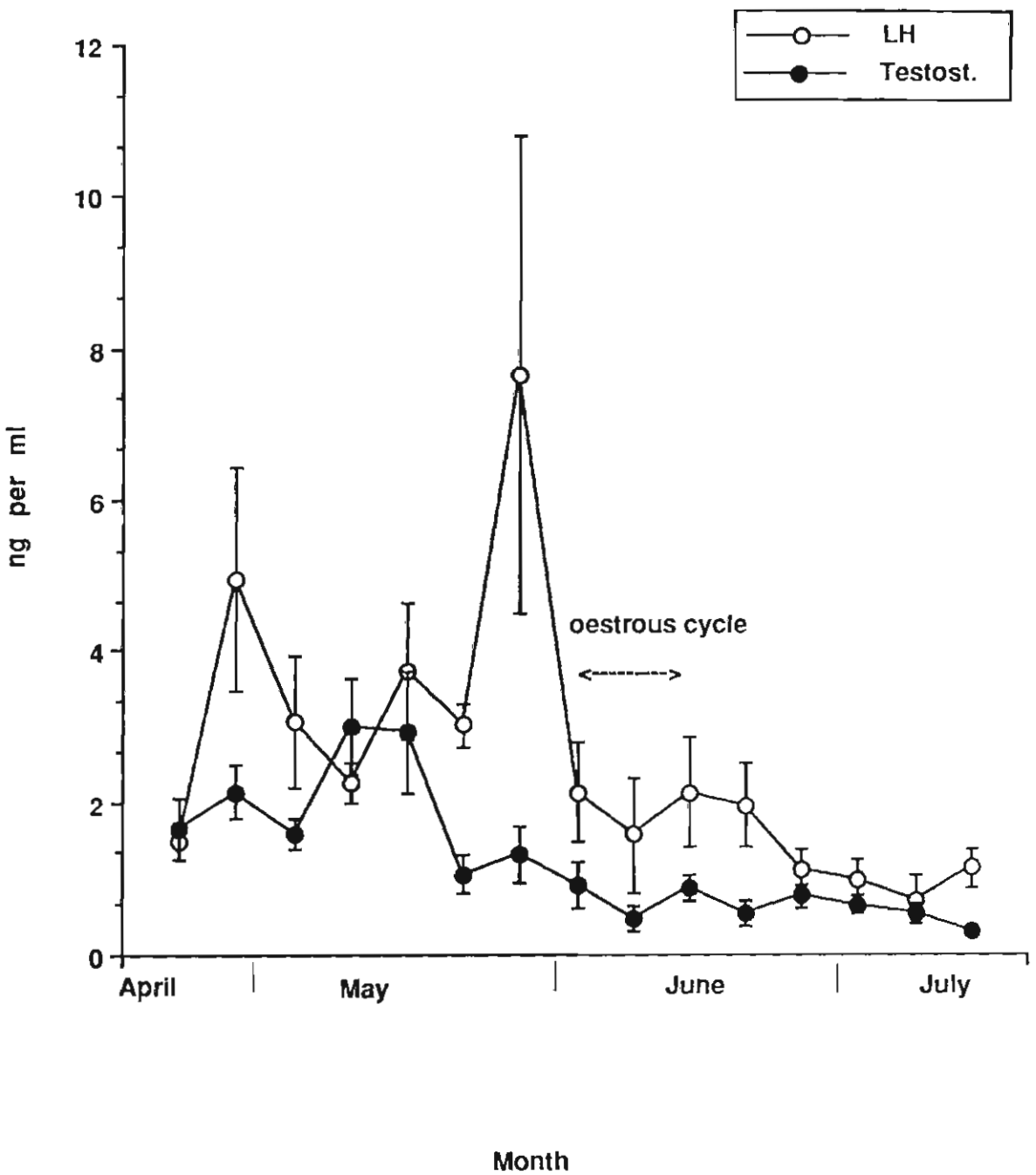

Fig. 6.11 Mean (± s.e.) LH and testosterone levels in 6 male quoll isolated from females. The time of oestrus in females from other groups is shown.

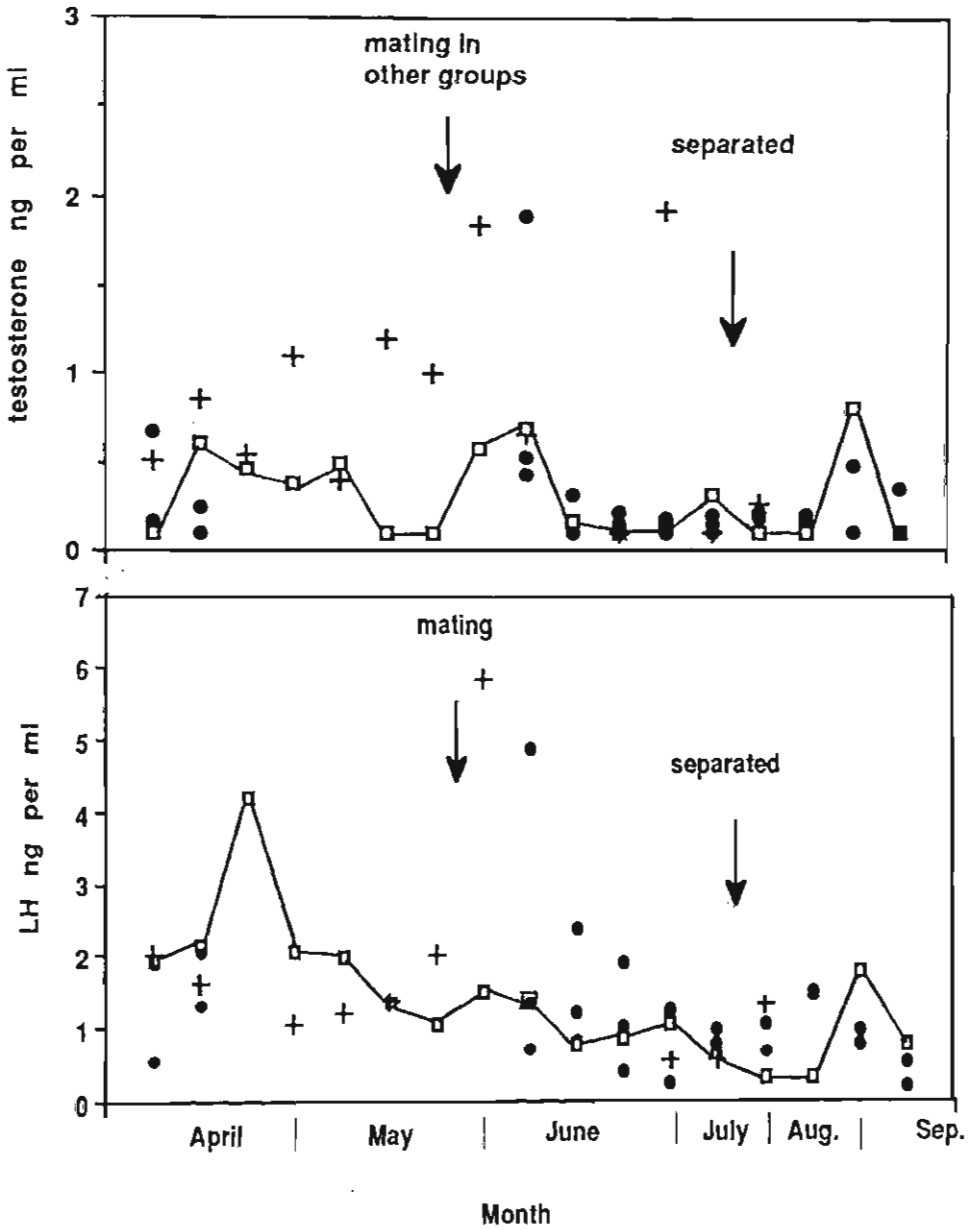

Fig. 6.12 Scattergram of testosterone and LH levels in males that were housed together but isolated from females. Two individuals are shown (no. 5♂, 02♀) and 2 to 4 introduced males ●. Animals were separated to avoid further injuries.

testosterone levels which mostly fell below 1.0 ng per ml over the experimental period. Testosterone levels were highest near the time of mating (in other groups) but were well below those of males in the wild. Male no. 02 had significantly higher levels of testosterone than male no. 5 (paired t-test, t=2.86, d.f.12, $0.005<p<0.01$) and was noted to be a very aggitated and dominant male. LH was not significantly different between the two males (paired t-test,t=0.77, d.f.9, $0.1<p<0.375$).

6.3.6 Comparative Results Between Groups

There was some degree of variation in the pattern of LH secretion between groups and individual variation was apparent. There appeared to be an increase in LH either before and/or during the mating period. This increase was not as high as recorded in wild males and was of short duration. In every experimental group, however, LH declined to basal levels by the time females had given birth (late June to July).

Patterns of testosterone secretion were divided into 3 categories depending on the levels recorded during the female's oestrous cycle and the mating period. The first category showed the highest and most prolonged period of testosterone secretion. This category included males with physical access to females. The second category of males produced an elevation in testosterone prior to the female's oestrous cycle but thereafter levels steadily declined. This group comprised males isolated from females during the breeding season. The third category had low levels of testosterone throughout the breeding season with no changes during oestrus or the expected time of mating. This category comprised males housed near but without physical access to females.

Figure 6.13 presents a diagramatic relationship between the experimental groups and their testosterone profiles.

An analysis of variance was conducted on the LH and testosterone levels for the 3 categories of males. The means were calculated from 11 sample days at similar stages throughout the breeding season. The three categories of males included: (a) males with females (n=6), (b) males near females (n=3) and (c) males isolated from females (n=6).

There was no significant variation in the LH levels between the three groups ($F_{[2,30]}=0.05$, $p>0.25$). Testosterone levels showed a significant

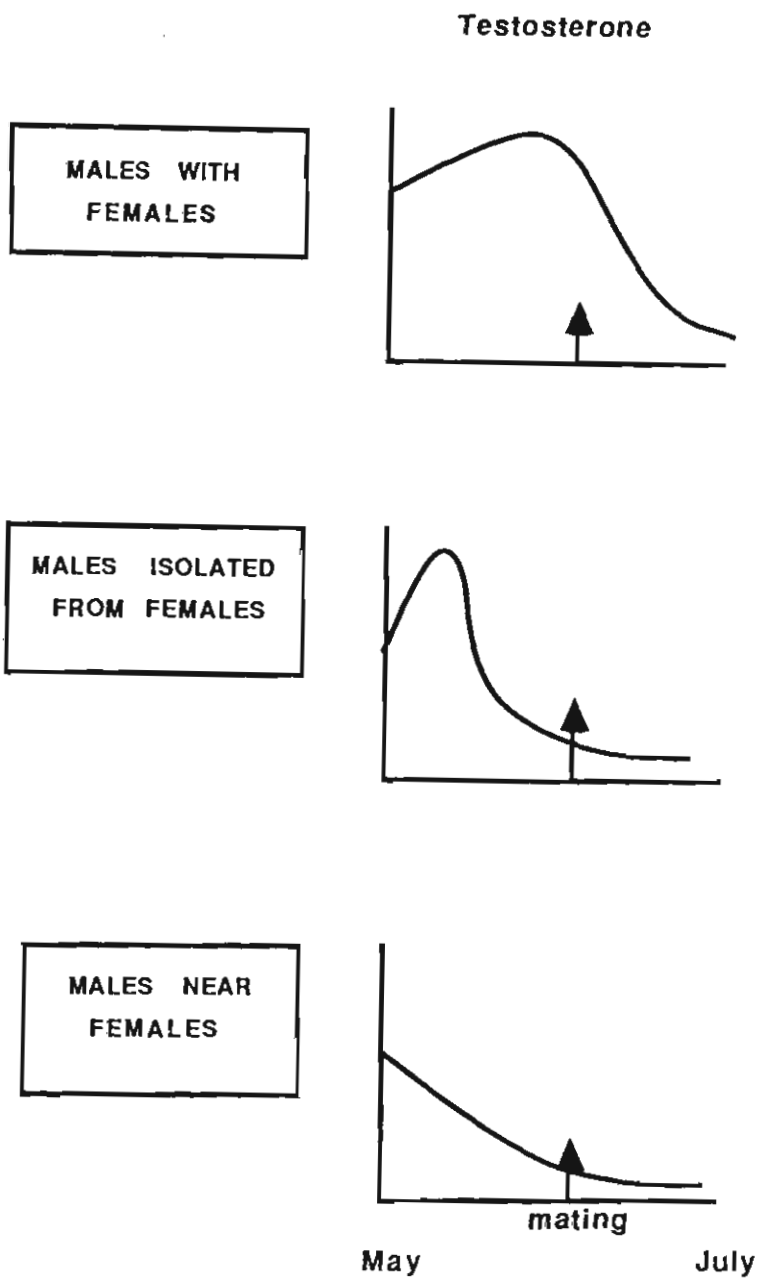

Fig. 6.13 Schematic testosterone profiles for male quoll housed under different social conditions at the expected. time of mating

degree of variation between groups ($F_{[2,30]}$=10.14, 0.0001<p<0.005) with each category varying significantly from the other (a,b, $F_{[1,20]}$=19.00, 0.0001<p<0.005; a,c, $F_{[1,20]}$=4.62, 0.025<p<0.05; b,c, $F_{[1,20]}$=6.84, 0.01<p<0.025).

6.4. DISCUSSION

6.4.1 Males

Frequent sampling during captive experiments has enabled a better understanding of the reproductive cycles of the male Eastern quoll than was possible from the wild. The cycles for body weight and testes size of quoll in captivity were similar to those of males in the wild and appeared to be unaffected by a change in social groupings. The cyclic nature of both these parameters suggests that they are seasonal rhythms which centre around the breeding period, and are largely independent of food resources. This supports a previous suggestion in Chapter 4 that while changes in body weight and scrotal size follow the cycle of insect abundance, a time differential implicates other factors, probably associated with reproduction, as regulating these cycles. In captive quoll, testes reached a maximum size before body weight and both parameters declined prior to the mating period. This pattern is true for many other *Dasyuridae* (Woolley 1966; Taylor & Horner 1970; Inns 1976; Begg 1981a,b; Calaby & Taylor 1981; Cuttle 1982; Fletcher 1983; Kerr & Hedger 1983; Woolley & Ahern 1983; Read 1984; Wilson & Bourne 1984; Kitchener *et al.* 1986).

One noticeable feature of every experiment was that LH and testosterone levels were lower in captive animals compared to males in the wild. This suggests that maintaining animals in captivity has some moderating effect on the endocrine cycle of this species and is an important consideration when interpreting any effect caused by social groupings. There may be many reasons for the differences in hormone concentration between captive and wild animals. In the wild, an increase in testosterone was related to an increase in activity levels and mobility of the male. Changes to the

normal pattern of behaviour imposed through confinement could be responsible for the moderating effect on hormone secretion in captive males. Factors relating to the stress of confinement, handling and captivity are also likely to influence hormone secretion and are considered in more detail in Chapter 7.

The secretion of LH and testosterone has been shown to contain a diurnal component that results in cyclic variations during the day and night (Appendix E). Cyclic fluctuations and variations should be considered as a natural characteristic of hormone secretion and may account for some of the differences between single samples collected from the wild and the more frequent and longer periods of sampling from animals in captivity. These observations support other workers who caution against the use of single or monthly samples to represent the 'true' hormone status of an animal (Lincoln 1978; Curlewis & Stone 1985a; McDonald 1986; Gemmell *et al.* 1987).

This series of experiments has found that the pattern of endocrine secretion in the male Eastern quoll may be influenced by a change in social groupings. Treatments such as proximity to the female quoll, competition and physical contact significantly influence the male's secretion of LH and testosterone surrounding the breeding period. The endocrine profiles of males experiencing a change in social circumstances have been divided into three categories for discussion.

Males with Females

The hormone profiles of males with physical access to females are similar to many other male mammals that show a increase in androgen concentration in response to the breeding season. Most profiles in the three groups of males with access to females showed a steady increase in LH and testosterone before breeding with the highest levels occurring just prior to mating. During mating and towards the end of the breeding period LH and testosterone steadily declined.

The finding that testosterone levels are declining or at basal levels during copulation is both an interesting and important one. It would seem that the high levels of androgens normally associated with the breeding period occur before mating and that during copulation testosterone levels are

reduced. In addition, males displaying a high level of aggressive behaviour during breeding (usually the heaviest and largest animals), have testosterone profiles that are similar to other males. As Godsell (1983) points out "...the relationship between body size, dominance and reproductive success is yet to be established" and it would seem that the role of androgens in the population structure and mate selection of this species is a complex one.

The Tasmanian devil, *Sarcophilus harrisii*, like the Eastern quoll, displays aggressive behaviour during mating and males frequently receive severe wounds during competitive bouts for females (Buchmann & Guiler 1977). Preliminary results of this study suggest that devils in the wild do not show a major rise in testosterone during copulation (Appendix B). Blood samples collected from male devils denned with females and known to be copulating, contained low levels of testosterone. It may be that the low levels of testosterone during copulation provides a common element in the reproductive strategy of both these large species and is a marked contrast to the high androgen levels found during mating in many of the smaller *Antechinus*. The social structure of the devil, determined in part by its greater longevity, is different to the quoll (Buchmann & Guiler 1977; Hughes 1982; D. Pemberton, pers. comms) although the reproductive cycle and developmental stages are similar.

Results suggest that introducing new males near breeding time (i.e. increasing male competition) increases LH and testosterone secretion in the population generally. Godsell (1983) found that the addition of an unknown quoll to a community was socially disruptive and resulted in increased activity levels, distorted social patterns and increased aggression between individuals. The pattern of space utilisation, territory and dominance exhibited by the quoll may have been affected by the high number of males, more so than in the wild because of the confines of the enclosure. The endocrine results are therefore reflecting such social conflict. Social conflict is known to also affect plasma testosterone levels of Rhesus monkeys living in social groups (Rose *et al.* 1975). Increased testosterone levels in monkeys reflected an increase in social stimuli and was accompanied by a higher probability of aggression.

Overcrowding that produces a change in social structure, is thought to regulate the density of many small mammal populations (Christian 1950; Sadlier 1969; McDonald & Taitt 1982; Dunbar 1985; Lee & McDonald 1985; Moberg 1985b). Christian (reviewed by Lee & McDonald 1985) hypothesised that

a number of endocrine changes occur in mammals as a result of stress induced by a population increase. These are: increased ACTH secretion, increased corticosteroid secretion, decreased sex steroid secretion, decreased fertility and increased mortality. In the present study the low reproductive success rate of introduced females suggests that some factor is influencing reproductive activity. The stress incurred by the social dominance of resident females over introduced females could be expressed by infertility and reflected by *in utero* loss and or resorption of the embryo, delayed maturation or impaired lactation.

When no new males were introduced to the population, resident males maintained comparatively high levels of LH and testosterone during the breeding season. The highest testosterone levels were recorded about three days before the start of mating. The three older quolls responsible for most of the mating, had hormonal profiles that were similar to those in other males. They did, however, show a decrease in testosterone at the time of copulation, a pattern that was reported in the previous experiment (sect. 6.4). In some possums and bandicoots, individual variation in testosterone secretion during breeding is thought to arise from differences in nutrition, social dominance and exposure to receptive females (Gemmell *et al.* 1985; *et al.* 1986). These types of factors may also be important in this experiment especially as three animals assumed dominance and one in particular maintained an aggressive nature and increased body size.

Although only a single pair of animals were used to examine a lack of male competition, the hormonal profiles of male no. 209 were similar to those outlined in previous experiments. The male showed a rise in LH and testosterone soon after being housed with the female and comparatively high levels of testosterone occurred over the breeding period despite there being no other males present. The results of this pairing also confirm that male and female Eastern quoll can breed successfully in their first year.

One major difference in this experiment was that mating commenced 11 days earlier than found in other groups. This result will require closer investigation to determine its significance. One possible explanation for its occurrence is that a lack of competition from males, or isolation of the female quoll from conspecifics may have promoted spontaneous ovulation in the female and hence result in an earlier mating. Female *Antechinus stuartii* isolated from other animals also exhibit asynchronous

ovulation (Scott 1986) so it is possible such a reaction may occur in the female quoll.

Males near Females

When male quoll were denied physical access to females during oestrus, a steady decline in LH and testosterone occurred. Males did not respond to females with a rise in testosterone levels although one animal did show an increase in LH a few days before the onset of oestrus. Subsequently, the hormonal levels recorded for males in this experiment were among the lowest of any captive group examined to date.

These results contrast to the growing evidence that suggests many male marsupials respond to females at oestrus with a rise in androgen levels. Male *Macropus eugenii,* penned with oestrus females show a significant elevation in LH and testosterone compared to isolated males (Catling & Sutherland 1980). Male *Dasyuroides byrnei,* housed alone but given access to females, produce a marked increase in testosterone concentration when females enter oestrus (Fletcher 1983). Male *Antechinus stuartii* housed together but given bedding from oestrus females, produce significantly higher androgen levels than control animals (Scott 1986).

There is, however, no single pattern of hormonal response by male or female marsupials and it is probable that a variety of social and environmental factors are operating to influence hormonal secretion at breeding time. For example, the testosterone profile of the male Short nosed bandicoot, *Isoodon macrourus,* is not influenced by the female and a rise in testosterone occurs in males housed well apart from females (Dr. R. Gemmell, pers. comm.) Male opossums, *Didelphis virginiana,* housed near but denied physical access to females, show a decrease in testosterone secretion when the females become anoestrus. Males produce subsequent rises in testosterone that may precede activity in the female by several months (Harder & Fleming 1986). Gray short-tailed opossums, *Monodelphis domestica,* become 'reproductively inactive' if isolated for extended periods and the female opossum shows synchronous oestrus periods if housed with males (Fadem 1985; Fadem & Rayve 1985). It is known that scent marking and odour cues play an important role in the social interaction of the opossum and it has been suggested that gonadotropin secretion in both species is probably triggered by a

pheromone produced by the male acting through the olfactory system of the female (Fadem & Cole 1985).

Some mammals, particularly small rodents, synchronise their breeding or oestrous cycles and become receptive when in the presence of fertile animals (Whitten 1956; Epple 1973; Bronson 1985; Scott 1986). If the male quoll likewise requires some factor to stimulate a rise in androgens at breeding time, the lack of direct contact between male / male and male / female quoll in this study, may have restricted some interchange between the sexes. In the wild, the increased activity of males during the breeding period most likely leads to an increase in physical interaction. The restricted access between males may have therefore limited behavioural cues leading to a state of arousal.

Any one or a number of these factors may have influenced the endocrine cycle of the male quoll in this experiment. Due to the importance of these results it would be of value if additional experiments were conducted in the future using a larger number of animals.

Males isolated from Females

When male quoll are denied contact with females during the oestrous cycle, testosterone levels decline near breeding time. The decline occurs regardless of the degree of physical interaction between males. This finding suggests that the female quoll may have some primary influence on the endocrine cycle of the male.

Social factors are thought to influence the breeding cycle of many marsupial species. Dickman (1985) suggests that the general timing of reproduction in *Antechinus* is determined by the female although males may help to synchronise the precise onset of oestrus. Male *Antechinus stuartii*, housed together but isolated from females or supplied with bedding from oestrus females, show higher testosterone secretion than males housed with females (Scott 1986). Gemmell *et al.* (1986) has found that testosterone secretion in the Brushtail possum is influenced by social dominance. Physically smaller possums show depressed testosterone profiles compared to the larger and more dominant animals.

When male quoll were housed together but isolated from females, severe aggressive bouts developed, some resulting in death. These displays,

however, were not apparently accompanied by any major increase in testosterone level and the reasons for this remain unclear. In other treatments an increase in testosterone in the male was clearly noted to coincide with an increase in aggression. Perhaps stress factors, due to overcrowding or physical domination, may have had a suppressive effect on androgen secretion in the quoll as they do in some larger mammal species (Dunbar 1985).

In a study by Scott (1986) Antechinus males produced low androgen levels when totally isolated from males and females. Scott concluded that olfactory cues from males increased androgen concentration whereas cues from females had a moderating effect. The synchrony of ovulation was controlled by the female and probably mediated by pheromones. Scott suggested that social cues did not influence male androgen levels in any consistent way but that the isolation of male Antechinus caused a depression of plasma androgens due to a lack of social stimulation. A lack of social stimulation may well have caused the depressed testosterone levels in male quoll in this study but at no time was it apparent that the female quoll had a moderating effect on androgen secretion in the male.

Male Tammar wallaby having undergone olfactory bulbectomy do not show a rise in plasma testosterone when housed with females. This finding suggests the hormonal response of the male is olfactory mediated (McConnell *et al.* 1984). Maximum androgen levels occur in tammars approximately three weeks before females come into oestrus (Catling & Sutherland 1980) and as this period coincides with the reactivation of the female blastocyst, the male is thought to be responding to a pheromone cue mediated by progesterone or estradiol during this time (McConnell *et al.* 1984; Tyndale-Biscoe *et al.* 1986). The isolation of males from female quoll prevented physical contact which could be one prerequisite for the androgen rise prior to mating. Other prerequisites may be in the form of directly perceiving odour signals from either the urine or after distributing over the body using behaviours such as face washing and grooming (Einsenberg & Golani 1977).

6.4.2 Females

While this study did not examine the effect of social groupings on the reproductive activity of the female quoll, it did obtain new information which is of interest. This study confirmed the female Eastern quoll to be

polyoestrus and able to return to oestrus between 5 and 20 days after losing her first litter of young. This time period is comparable with other female marsupials including the bandicoot, possum, wallaby and some smaller dasyurids (Tyndale-Biscoe 1984). The gestation length of the quoll was found to be 19 ± 2 days (n=12). This is slightly shorter than 21 ± 2 days (n= 4) reported by Fletcher (1985), although Fletcher did find that three of the four animals had gestation lengths of 20 days. The restrictions imposed on animals through confinement and disturbances over the breeding period, may account for some variation between the two estimates.

Observations suggest that female quoll mate with only one male but that it was possible one male may have mated with several females. This contrasts to Godsell's (1983) proposal that both male and female quoll are promiscuous breeders. Godsell reported that "...both males and females mated several times during the breeding season though, after mating once, females became increasingly agonistic to the advance of males, particularly ones with which they had already mated." It may be that any differences in observations between the two studies are an artefact of captive maintenance and may differ from the situation as it is in the wild.

The endocrine cycles of captive female quoll, in conjunction with the female cycles reported in Chapter 4, suggest that the relationship between LH, progesterone and prolactin at oestrus, parturition and during lactation, are similar in this species as found in other female marsupials (Tyndale-Biscoe & Renfree 1987). The female quoll showed little change in LH or progesterone concentration until the onset of oestrus and parturition. Female mammals in general only show changes in LH near the time of ovulation and then only when follicles have been primed adequately with FSH (Baird 1984). In the quoll transient pulses in LH occurred during the oestrous cycle and prior to parturition. These were similar to those reported in the female dasyurid Kowari (Fletcher 1983) and also to the Tammar wallaby that demonstrates a well defined LH surge 20 to 40 hours prior to ovulation (Sutherland *et al.* 1980).

The monophasic profile of progesterone produced by the quoll is similar to that of the Brushtail possum (Hinds 1983), Virginia opossum, (Harder & Fleming 1981) and Kowari, (Fletcher 1983). The profile is also similar to that of the Quokka (Cake *et al.* 1980) and Tammar wallaby (Tyndale-Biscoe *et al.* 1983) throughout the latter stages of pregnancy (second half of the biphasic profile). The pattern of a short, sharp rise followed by a

decrease in progesterone immediately prior to giving birth, is now reported to occur in many other marsupials (reviewed by Tyndale-Biscoe & Renfree 1987).

Prolactin levels were generally 20.0 ng per ml or lower throughout the non-lactation period. A rise in prolactin occurred near parturition and levels were raised throughout the lactation period. Maximum changes in prolactin occurred after the young were able to relinquish the teat (approx. 60 days). This coincides with Hinds and Merchant (1986) and Green *et al.* (1987) who have found a good correlation between prolactin levels, changes in milk composition and tooth eruption in the quoll. The profile of prolactin in this study was comparable to that found by Hinds and Merchant (1986) for the quoll but contrasts to the much lower levels observed in females from the wild at similar stages of lactation (Chapter 4). Factors such as stress or diet may be responsible for these differences. Recently, some studies have shown differences in growth rate occur between wild and captive born young and that these may be due to the dietary differences of the mothers (*Bettongia gaimardi*, Taylor & Rose 1987). Variation in prolactin levels may also be a means of detecting these differences in the future.

6.4.3 GENERAL CONCLUSIONS

This series of experiments have demonstrated that the female quoll has an important influence on the reproductive activity of the male. When male quoll were isolated from, or denied access to females, testosterone levels declined earlier than in males housed with females. This suggests that the female may be important in initiating a testosterone response in the male near breeding time. It is interesting to speculate that the cue may be in the form of an olfactory cue such as a pheromone transmitted in the urine or cloacal rubbings of the female. The male might detect this directly by sniffing the female's body or indirectly from her general odours. The social behaviours displayed by the quoll, such as paired nesting, an upright stance and cloacal sniffing, enhance the receiving of chemical signals and have been identified as having this role in other marsupials families (Croft 1982; Fletcher 1983; Biggins 1984; McConnell *et al.* 1984; Russell 1984; Fadem 1985; Fadem & Cole 1985; Gemmell *et al.* 1986; Scott 1986; Conway 1988).

CHAPTER 7

ADRENAL FUNCTION AND BLOOD PARAMETERS

7.1 INTRODUCTION

Many small male Dasyuridae display a syndrome, or sequence of physiological and endocrinological events, during mating that has been attributed to rapid aging (Woolley 1966) and stress (Wood 1970; Lee & Cockburn 1985; Lee & McDonald 1985). The males produce an increase in testosterone and adrenocortical activity prior to and during mating (Barnett 1973; Braithwaite 1974; Kerr & Hedger 1983; Bradley 1987). In some species, the rise in plasma androgens and total corticosteroids are coupled with a decrease in the plasma corticosteroid binding globulin (CBG), referred to as the maximum corticosteroid binding capacity (MCBC). This results in a dramatic increase in free, biologically active cortisol concentration. One consequence of this imbalance is the suppression of the animal's immune and inflammatory responses. This leads to an increased sensitivity to pathologic and parasitic organisms and increased incidence of haemorrhaging from gastrointestinal ulceration. Males become anaemic due to infections and haematological profiles reveal marked lymphopenias and neutrophilias (Cheal *et al.* 1976; Barker *et al.* 1978). Male *Antechinus* show an increase in liver glycogen, glucogenesis of protein, decrease in body weight, decrease in plasma sodium concentration and enter a state of negative nitrogen balance shortly before death (Barnett 1973; Inns 1976; Bradley *et al.* 1976, *et al.* 1980; McDonald *et al.* 1981; Lee & Cockburn 1985).

Few studies have examined the role of stress factors in the life cycle of other marsupial families or the larger members of the Dasyuridae. In some eutherian and marsupial mammals, the haematology and blood biochemistry are known to change in response to season, breeding activity, age and condition (Sealander 1964; Presidente & Correa 1981; Humphreys *et al.* 1984). Brushtail possums,*Trichosurus vulpecula,* in poor condition or under stress show significant changes in their serum glucose, cholesterol and triglyceride

concentration (Parsons *et al.* 1971a; Presidente & Correa 1981). Little work has been conducted on the haematology of the Eastern quoll since Parsons *et al.* (1971b) first provided haematological parameters for two male quolls of unspecified age and status. A number of comparative studies have been conducted on the physiological aspects of the blood of other Dasyurid species (*Antechinus stuartii*, Cheal *et al.* 1976; *Sarcophilus harrisii,* Nicol 1982; and *Dasyurus hallucatus,* Schmitt *et al.* 1988) and these provide information on a change in blood constituents throughout the year.

This chapter contains the details of investigations into a number of aspects of the life cycle of the Eastern quoll. Collectively the studies aim to determine what role, if any, stress plays in the breeding biology of this species. This project has already found that the pattern of hormonal secretion in the male quoll may be influenced significantly by social circumstances (Chapter 6). For example, LH and testosterone levels decline when males are denied access to females during the oestrous cycle. Being a relatively long lived animal, the quoll is unlikely to show a stress response during the mating period which leads to total male mortality. The quoll does, however, display a number of features during its reproductive cycle that are similar to some smaller, short lived species. These include a androgen rise during breeding, inter-male aggression and decreased survivorship of males after mating (Godsell 1983). These similarities warrant a closer investigation.

Plasma cortisol, partitioned into high affinity bound (CBG-bound), albumin bound and free compartments, was used as an indicator of adrenocortical activity in adult male quoll. Moberg (1985a) points out that while a focus on a pituitary hormone will not provide a complete measure of an animal's well-being, at least this approach offers the greatest potential for understanding the impact of stress. The first experiment in this chapter stimulates cortisol secretion in the Eastern quoll using synthetic ACTH. This was necessary to obtain a guide to the potential range of cortisol levels and responsiveness of the adrenal cortex in this species, and also to provide some basis for assessing and interpreting the cortisol levels found in wild and captive quolls. This chapter also examines whether any significant changes in plasma biochemical parameters occur during the breeding cycle. Triglycerides (neutral fats) and cholesterol (sterol) are monitored because of their role in fat metabolism, and protein and glucose concentration are

measured because they are indices of energy balance and nutrition (Ganong 1965). Finally, the range of haematological parameters found in apparently healthy Eastern quoll are defined in order to determine whether anomalies occur in the blood profile of adult animals that suggest a deterioration in health due to 'stress' or associated disease. Collectively these experiments should indicate whether stress plays any part in the breeding biology of this species.

7.2 MATERIALS AND METHODS

The following abbreviations have been adopted throughout this chapter: ACTH, adrenocorticotrophic hormone; CBG, corticosteroid binding globulin bound; MCBC, maximum cortisol binding concentration and Alb, albumin-bound cortisol. Cortisol is measured as either total cortisol or represented by the summation of the CBG, Alb and free cortisol fractions. Testosterone levels have been measured using the techniques outlined in Chapter 3 and values have been converted to nM for easy comparison with cortisol.

7.2.1 ACTH Stimulated Cortisol Levels

Corticotrophic secretion was induced by an injection of synthetic ACTH (Synacthen Depot ®, Ciba-Geigy, Australia Ltd.). This preparation is reported by Ciba-Geigy to have a similar chemical structure and display the same pharmacological properties as that of endogenous ACTH. The Synacthen Depot preparation contains zinc phosphate which provides a slow release of tetracosactrin. In humans, this causes plasma cortisol levels to remain elevated for at least 24 to 36 hours after an injection of 1 mg.

Six males were injected (intra-muscular rump) with 1 mg ACTH per ml per kg (100 I.U.). This dosage was proportional to doses administered to *Antechinus.* (Bradley *et al.* 1980; I. McDonald pers. comm.). Three males were injected in December during the non-breeding period. Blood samples were collected every 2 hours for 8 hours after injection and again the following morning, 24 hours later. Three males were injected during the breeding period in July and were sampled a total of 8 times during 12 hours. A blood sample was collected from every animal prior to injection and injections were

given between 9.00 a.m. and 9.10 a.m.

The blood samples were centrifuged immediately after collection and the plasma stored at -20°C. Total cortisol was measured by RIA using a tritiated cortisol tracer. The assay was sensitive to between 1.93 and 2.43 nM cortisol. The materials, methods and validations of this assay have been detailed in Chapter 3. Partitioning of cortisol into CBG bound, Alb bound and free fractions were determined by Dr. Adrian Bradley, Zoology Department, University of Western Australia. The samples were analysed using equilibrium dialysis and a gel filtration technique with a specific high affinity association constant applied for the Eastern quoll (Bradley *et al.* 1976, Bradley *et al.* 1980; McDonald *et al.* 1981).

7.2.2 Bound and Free Levels of Total Cortisol

Blood samples were collected throughout the year from males in the wild and from males housed in captivity under various social conditions. The social conditions have been described in detail in Chapter 6 and are grouped into three categories: (a) males with females, (b) males near females and (c) males isolated from females. Plasmas were stored at -20°C until assayed for total cortisol and cortisol partitioning (as described in sect. 7.2.1).

7.2.3 Plasma Biochemistry

Glucose, total protein, cholesterol and triglycerides were measured from 100µl aliquots of blood plasma that had been stored previously at -20°C. The samples were analysed using a spectrophotometer (<Cobas> Mira, Diagnostica, Roche). The spectrophotometer incorporated 8 interference filters with a range of 340 to 750nm wavelength and accuracy to ± 3nm. Specifications include a pathlength of 6mm, linear absorbance range of 0 to 2.4 Å (deviation <1%) and a photometric reproducibility of 0.0009 Å.

The spectrophotometer was calibrated using human standards obtained from diagnostic kits. Samples were diluted and reacted in a single cuvette at an incubation temperature of between 20°C and 25°C. The reaction time for each test was approximately 45 seconds making a total measurement time of 4 minutes per sample.

The measurement for glucose was based on a enzymatic reaction with hexokinase (HK) and determined by recording the absorbance at 340, 334

and 365nm wavelengths. Total body protein was determined using a biuret reaction with perchloric acid. Values were recorded photometrically at a wavelength of between 530 and 560nm. Triglycerides were measured using a enzymatic colorimetric method with iodonitrotetrazolium chloride (INT). The concentration of the formazan in the reaction was directly proportional to the concentration of triglyceride at an absorbance range of 490 to 550nm. Cholesterol was measured using an enzymatic colorimetric method similar to that for triglycerides but by converting cholesterol esterase to cholesterol oxidase. The range of wavelengths for measuring cholesterol were 470 to 550 nm.

7.2.4 Haematology

Blood samples were obtained from animals trapped at Cradoc or other areas around Tasmania (1984 to 1986), and from animals detained in captivity for less than one month. This work was conducted using equipment made available by the Haematology Department, Royal Hobart Hospital and was partly funded by the David Collins Leukaemia Foundation.

Blood samples were collected into EDTA anticoagulant vials and blood films were prepared immediately onto glass slides (see sect. 2.3.1). Haemoglobin (Hb), Packed cell volume (PCV), mean red cell volume (MCV), mean red cell haemoglobin concentration (MCH), red and white cell count (RCC, WCC) were measured on a Coulter model S haematology analyser. Platelets were counted with a Coulter 'Thrombocounter'. Reticulocytes and Westergren erythrocyte sedimentation rates were estimated by standard methods (Dacie & Lewis 1984). Red cell inclusions were stained by incubating 1 part blood with 2 parts methyl violet stain (1% methyl violet in 0.9% sodium chloride). The mixture was left at room temperature for 10 minutes prior to preparing the films.

Haemoglobin electrophoresis was conducted on cellulose acetate using a tri-edta-borate buffer (0.025 M, pH 8.6) and on agar gel at pH 6.2. Alkali resistance of haemoglobin was determined by the method of Betke *et al.* (1959) in accordance with the recommendations of Molden *et al.* (1982). Blood films for differential counts and red cell morphology were stained with 50:50 Leisham-Giemsa stain using an Ames 'Hematek' automated staining machine. A x10 eyepiece and x100 oil immersion objective were used to classify 100 leucocytes and study red cell morphology on each slide.

7.3 RESULTS

7.3.1 ACTH stimulated Cortisol Levels

Prior to injection with ACTH, the mean total and free cortisol levels in December males were not significantly different from July males (total cortisol, $t=1.45$, d.f.4, $0.1<p<0.375$; free cortisol, $t=1.64$, d.f.4, $0.05<p<0.1$). Every male recorded the highest level of total cortisol 2 to 4 hours after injection (Fig. 7.1a). These ranged from 89.2 to 143.9 nM total cortisol (115.1 ± 8.8 nM,$\bar{x}$ ± s.e., n=6) during this time. Every male showed a decline in total cortisol within six hours of injection. The highest free cortisol concentrations also occurred 2 to 4 hours after ACTH injection (Fig. 7.1b) and levels ranged from 7.0 to 15.7 nM (11.5 ± 1.3 nM,$\bar{x}$ ± s.e., n=6) during this time.

Table 7.1 Comparison between the mean (+s.e.) cortisol levels in male quoll after ACTH injection (n=6)

Parameter (nM)	Non-Breeding males	Breeding males	Significance ANOVA
Total cortisol	92.9 ± 6.3	71.4 ± 5.9	$0.10<p<0.25$
CBG bound	75.1 ± 6.8	61.6 ± 4.9	$p>0.25$
MCBC	216 ± 2	257 ± 5	$p<0.0001$ *
Free cortisol	9.7 ± 0.6	5.8 ± 0.6	$0.005<p<0.01$ *
Free as % of total	10.0 ± 0.3	7.8 ± 0.2	$p<0.0001$ *
Alb bound	8.3 ± 0.7	3.9 ± 0.4	$p<0.0001$ *

only samples comparable on day of injection used in calculations
* significant variation

Males showed an immediate increase in total cortisol after ACTH injection. In Figure 7.2(a,b) total cortisol has been partitioned as CBG bound, Alb bound and free circulating cortisol. Total cortisol comprised 75 - 90% CBG bound, 5 -15% Alb bound and 6 - 10% free cortisol. There was no significant difference in the mean cortisol secretion or CBG between or within the two

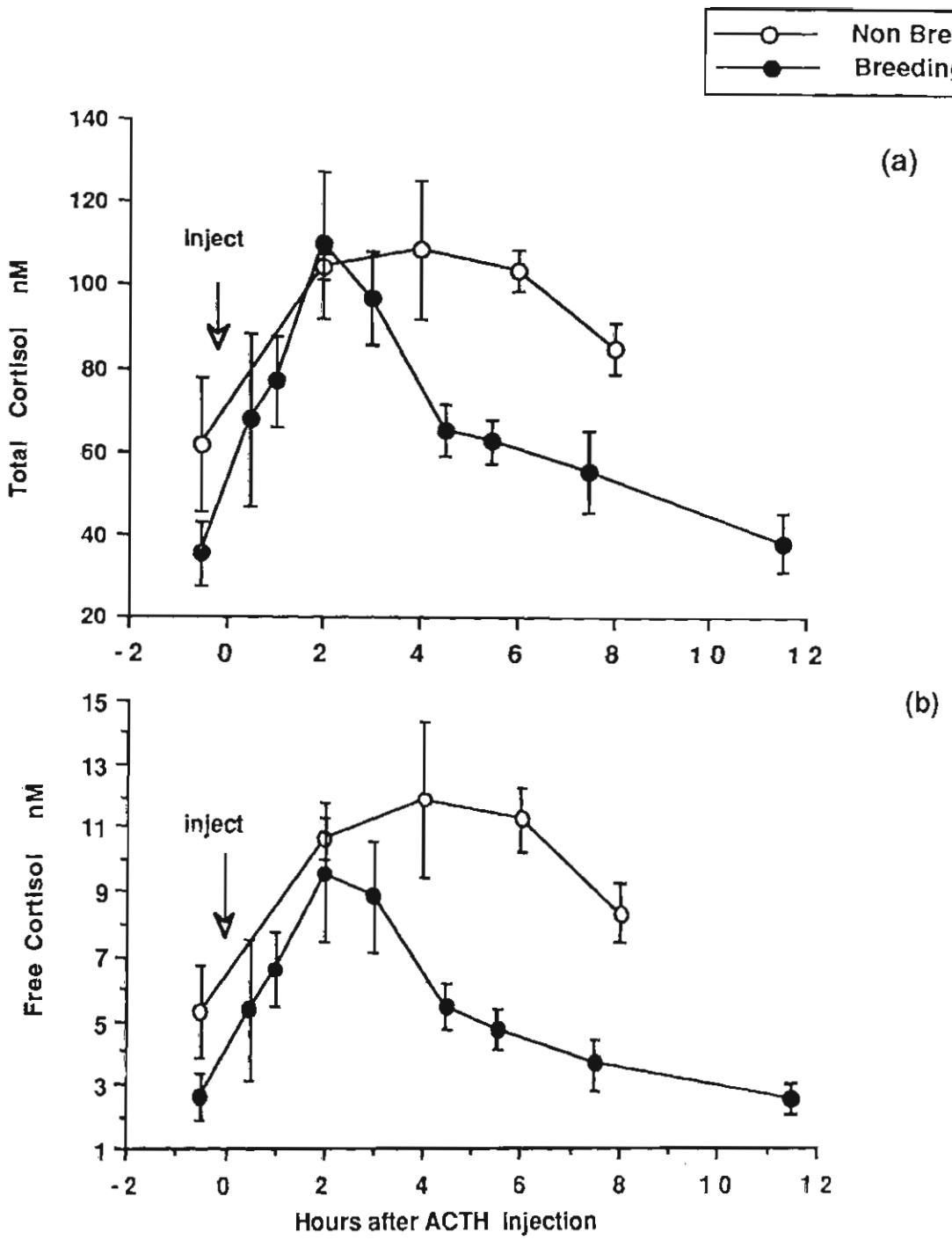

Fig. 7.1 Mean (± s.e.) total cortisol (a) and free cortisol (b) in males injected with ACTH in the breeding (n=3) and non breeding (n=3) season. The highest cortisol levels occurred 2 to 4 hours after injection.

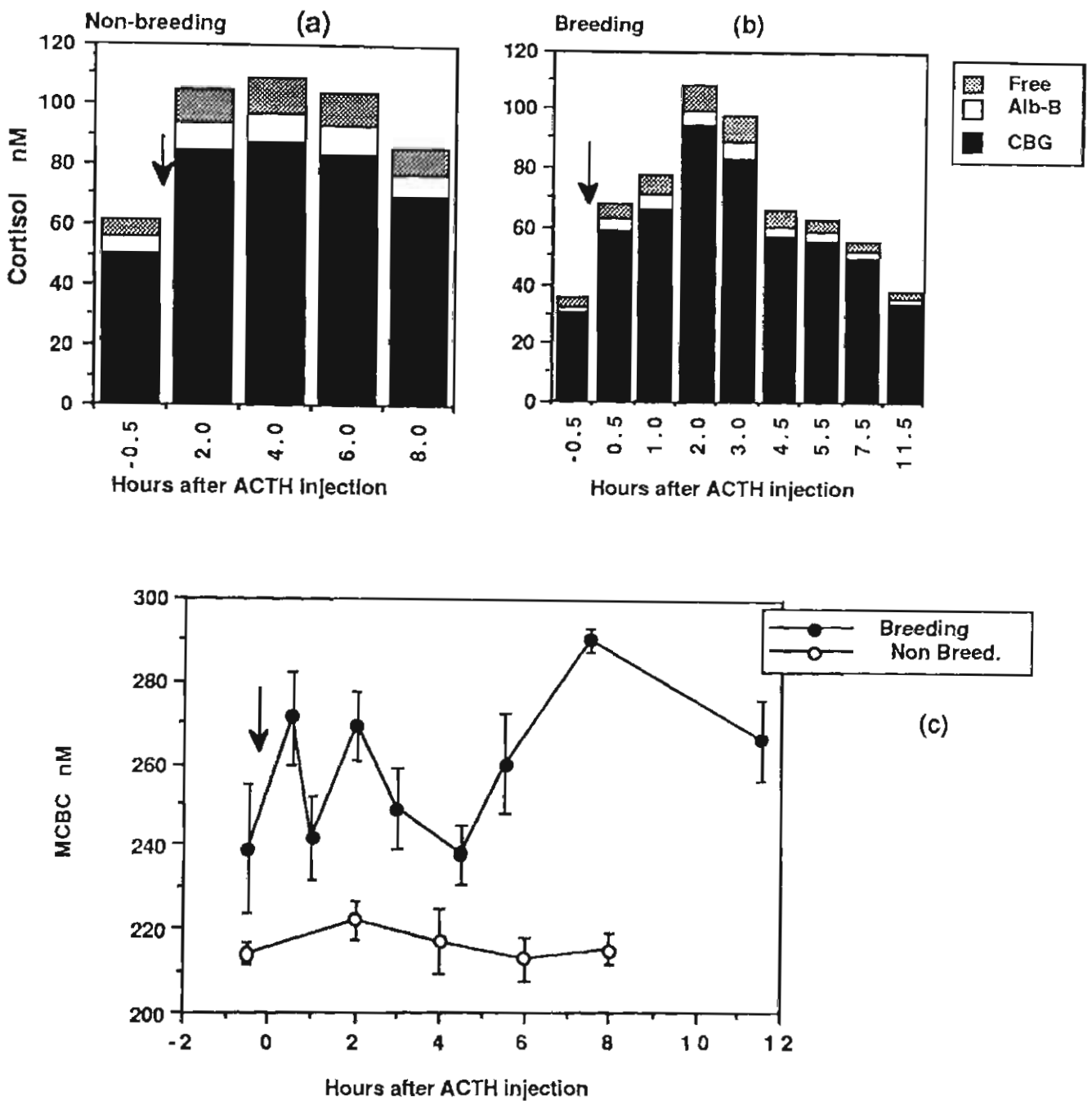

Fig. 7.2 Total cortisol expressed as free, albumin bound and CBG bound in the (a) non-breeding and (b) breeding season. (c) Mean (± s.e.) MCBC is also shown for these periods (n=3 males). Arrow = time of injection.

groups of males (Table 7.1). Every male had a MCBC greater than their total cortisol concentration. December males had lower MCBC but more steroid bound to Alb than July males (Fig. 7.2c). Four hours after ACTH injection, December males had significantly higher total and free cortisol levels than July males (total, $t=2.40$, d.f.4, $0.025<p<0.05$; free, $t=2.56$, d.f.4, $0.025<p<0.05$). Free steroid ranged from 2.7 to 15.7 nM in December males and 1.5 to 13.5 nM in July males throughout the experiment. Free cortisol as a percentage of total cortisol ranged from 7.9 to 11.7% in December males and from 6.0 to 10.3% in July males throughout the experiment.

The levels of total and free cortisol found in the six males (shown in Fig. 7.1a,b) were used as the basis for determining a 'degree of stress'. Although the categories selected were arbitrary, they were considered broad enough to allow for a variety of situations and yet still useful as an aid to the biological interpretation of cortisol levels. 'Low stress' cortisol levels included those recorded prior to ACTH injection and also encompassed the levels found in long term captive animals. 'Moderately stressed' levels comprised the majority of cortisol levels that fell in the mid- range of the graph either before 2 hours or 4 hours after injection with ACTH. The total and free cortisol levels recorded between 2 and 4 hours of injection with ACTH were suggestive of 'high stress' and (in terms of this experiment) were considered the only levels to be biologically important. These categories have been outlined in Table 7.2.

Table 7.2 Degree of stress suggested by the level of total and free cortisol in the Eastern quoll (n=6).

Total Cortisol nM	Free Cortisol nM	Level of Stress
0 to 60	0.0 to 5.0	low stress
60 to 80	5.0 to 7.0	moderately stressed
80 to 100 or >	7.0 to 10.0 or >	highly stressed*

* biologically important

Prior to ACTH injection LH levels were significantly higher in July males than in December males ($t=2.38$, d.f.4, $0.025<p<0.05$). There was no difference in testosterone levels between the two groups ($t=1.24$, d.f.4, $0.1<p<0.375$). Every July male showed a decrease in LH and an increase in testosterone concentration in the first sample collected after ACTH injection. Subsequent samples fluctuated in concentration over the 24 hour period showing peaks and troughs in a manner similar to that described for the diurnal cycles in Appendix E.

7.3.2 Bound and Free Levels of Total Cortisol

7.3.2.1 Males in the Wild

The yearly variations in MCBC and total cortisol partitioning in male quoll from the wild are presented in Fig. 7.3. There was a significant degree of fluctuation in MCBC throughout the year (ANOVA, $F_{[10,35]}=3.15$, $0.005<p<0.01$) with the MCBC in September to January being higher than for other months (Fig. 7.3a). There was only a small degree of variation in total cortisol throughout the year with no significant difference between months (ANOVA, $F_{[10,35]}= 1.65$, $0.10<p<0.25$). The highest mean total cortisol levels occurred in November, February and June but sample sizes were small (Fig. 7.3b).

The highest mean levels of free cortisol were recorded in November, February and June but there was no significant difference between months (ANOVA, $F_{[10,35]}=1.64$, $0.10<p<0.25$). Free cortisol levels were at a 'unstressed' to 'moderatly stressed' level for most months of the year. During November, February and June the levels were approaching or had reached a 'high' stressed condition. There was no obvious increase in free cortisol during June when testosterone levels were maximal (Fig. 7.3c).

7.3.2.2 Captive Males with Females

Figure 7.4 shows the change in MCBC, total cortisol and testosterone concentration in five males housed with females over the breeding season. MCBC showed no significant variation over the breeding season

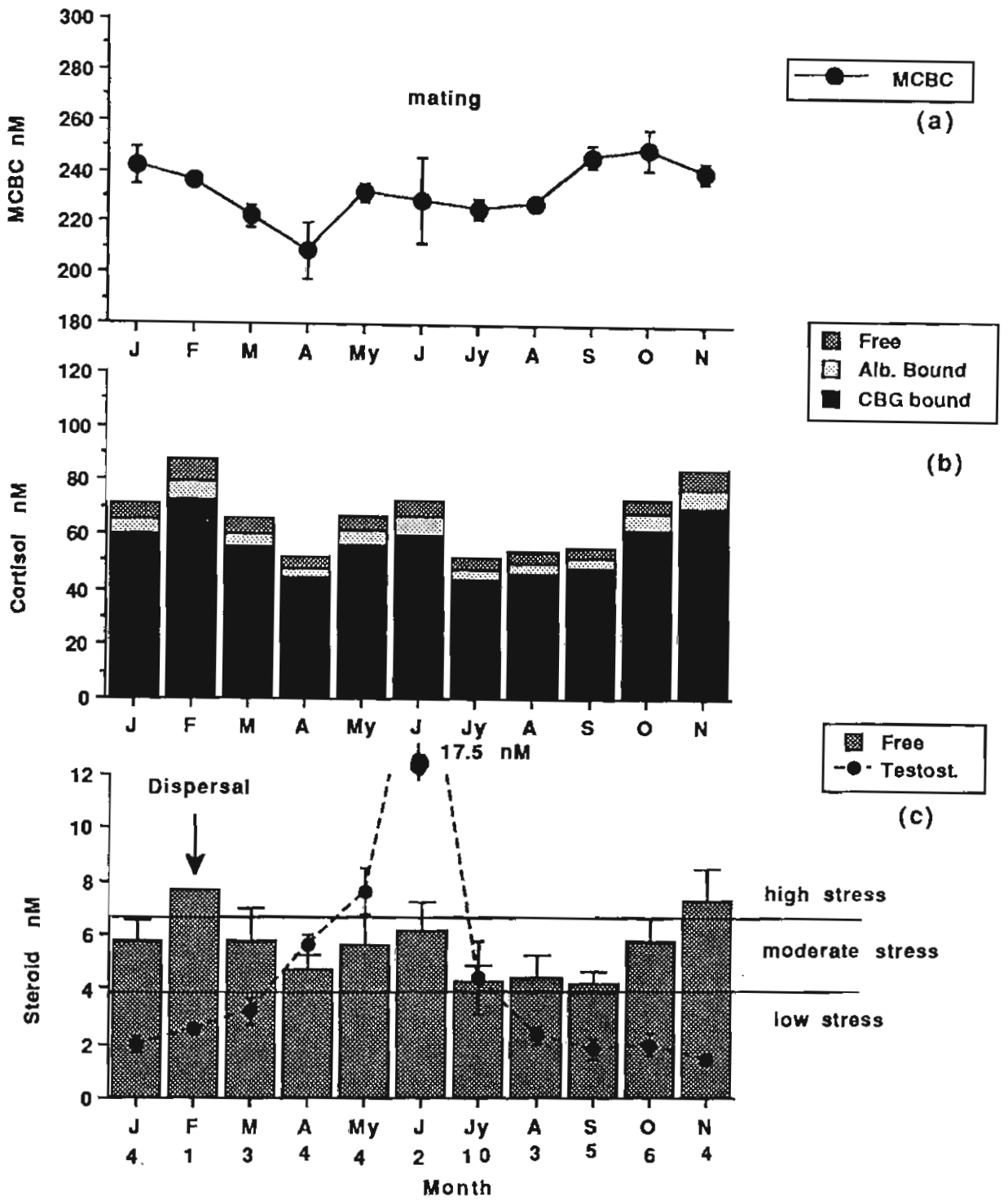

Fig. 7.3 (a) Mean (+s.e.) MCBC (b) CBG + alb + free cortisol,(c) Enlargement of free cortisol and testosterone levels in wild Eastern quoll. Mating was in June , dispersal after November. Sample size below month. Free cortisol stress category shown.

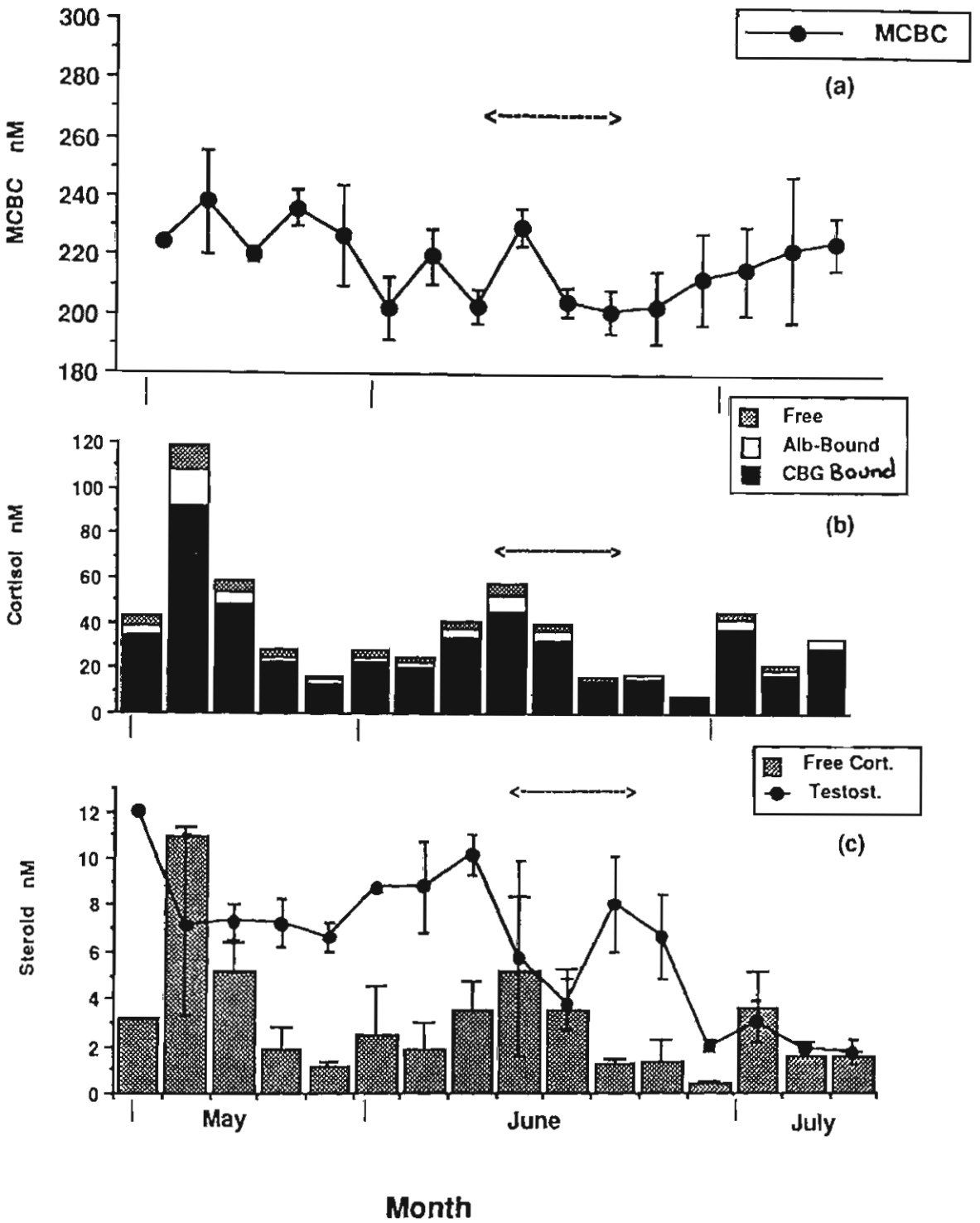

Fig. 7.4 Males with females (n=5). (a) Mean (± s.e.) MCBC (b) CBG + albumin-bound + free cortisol (c) Enlargement of free cortisol and testosterone levels. The duration of the breeding season is shown.

(ANOVA, $F_{[14,40]}=1.44$, $0.10<p<0.25$). A large increase in total and free cortisol production occurred shortly after the males were housed together (Fig. 7.4b,c). These levels quickly subsided and remained at low or moderate stress for the remainder of the experiment. There was a significant degree of variation in free cortisol levels over the breeding season (ANOVA, $F_{[4,52]}=2.58$, $0.025<p<0.05$) with the highest mean level occurring in early May (10.95nM). Free cortisol secretion showed no obvious correlation with the pattern of testosterone secretion (Fig. 7.4c).

7.3.2.3 Captive Males Near Females

Males housed near females produced comparatively low levels of total and free cortisol levels throughout the experiment. The scale in Fig. 7.5 has been reduced in order to show cortisol partitioning. MCBC levels ranged between 228 to 261nM and were comparable to the ranges measured in other groups. Total cortisol levels fluctuated widely with the highest concentrations coinciding with oestrus in the females. The highest total cortisol concentration of 20nM was low compared to the levels found in other groups at similar times (ranging from 50nM to 80nM). There was no significant variation in free cortisol over the experiment with most levels being 2.0nM or lower (ANOVA, $F_{[11,19]}=1.34$, $p>0.25$). The testosterone levels found in this treatment were among the lowest of any experimental group.

7.3.2.4 Captive Males Isolated from Females

Males isolated from females had the highest range of MCBC levels for this series of experiments. At mating time levels reached a maximum of 290nM compared to 240nM in other groups (Fig. 7.6a). Total and free cortisol levels also showed a dramatic increase at the expected time of breeding (Fig. 7.6 b,c). The increase in free cortisol is reflected by the significant degree of variation between samples (ANOVA, $F_{[13,60]}=4.11$, $p<0.0001$). The increased levels of free steroid, however, were still low compared to other groups and were unlikely to have produced a biological stress effect. Testosterone levels gradually declined over the experimental period with no significant changes occurring at breeding time.

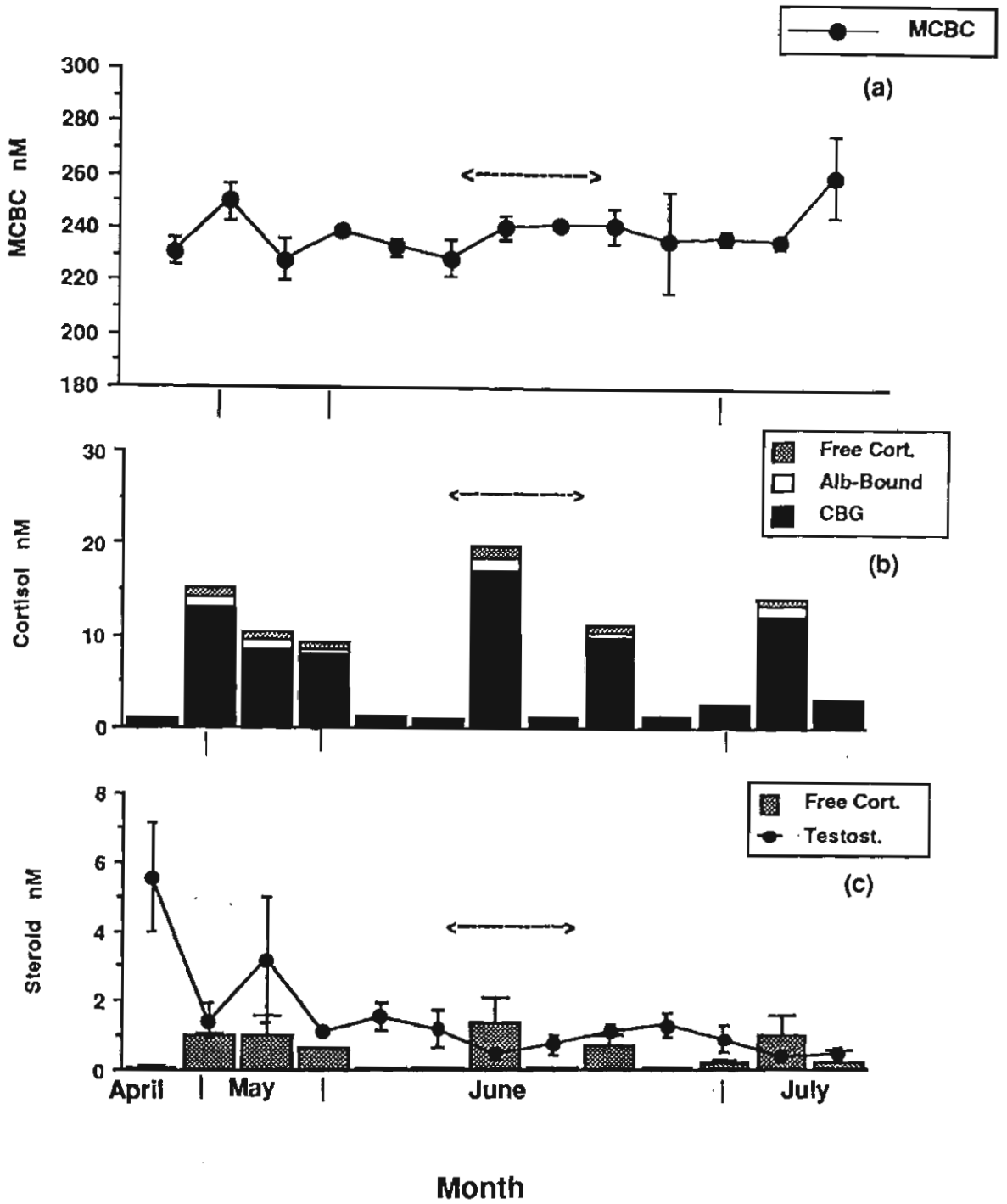

Fig. 7.5 Males housed near females (n=3). (a) Mean (± s.e.) MCBC (b) CBG + albumin-bound + free cortisol (c) Enlargement of free cortisol and testosterone levels. The timing of the female's oestrus cycle is shown. Note the change in scale.

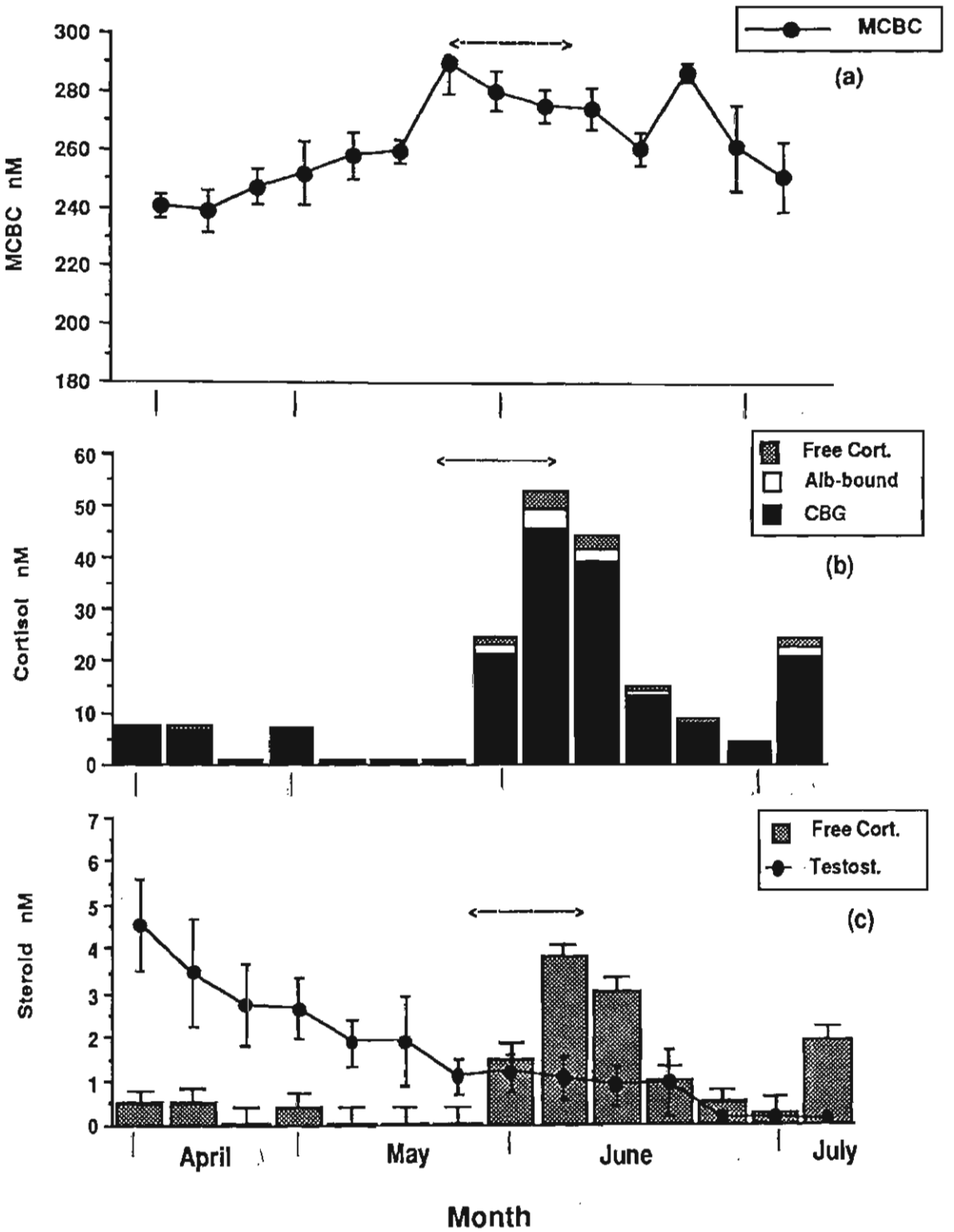

Fig. 7.6 Males isolated from females (n=3). (a) Mean (± s.e.) MCBC (b) CBG + albumin-bound + free cortisol (c) Enlargement of mean free cortisol and testosterone levels. The timing of the breeding season is shown. Note a change in scale.

7.3.2.5 Comparative Results

Table 7.3 compares the ranges of testosterone and cortisol partitioning between the four groups of males (wild plus 3 captive groups) over the months surrounding the breeding period (May, June and July). An obvious feature of the results are the lower range of total cortisol partitioning in males housed near or isolated from females and the low range of CBG bound cortisol in captive animals compared to wild males.

Table 7.3 Range of testosterone and total cortisol partitioning levels between groups in the months surrounding the breeding season (May, June, July).

		Captive		
Parameter nM	Wild Males (n=16)	Males with Females (n=5)	Males near Females (n=3)	Isolated Males (n=6)
MCBC	225 - 232	202 - 238	228 - 261	247 - 290
CBG-bound	43.8 - 60.2	5.3 - 92.1	0.9 - 17.1	0.9 - 46.0
Alb-bound	3.7 - 6.8	1.0 - 15.5	0.1 - 1.3	0.1 - 3.5
Free	4.2 - 6.1	0.4 - 11.0	0.1 - 1.4	0.1 - 3.8
Testosterone	5.0 - 17.5	1.7 - 12.0	0.3 - 5.6	0.1 - 4.6

A correlation coeffecient (r) was calculated to determine whether there were any high correlations between the secretion of testosterone and cortisol and whether this correlation changed in relation to the social situation of the animal. Table 7.4 shows the correlation between testosterone and cortisol components. High correlations were found between total cortisol and CBG bound, Alb bound and free cortisol and these correlations appeared similar for every social grouping including males in the wild. No high correlations were found between testosterone levels and any cortisol parameter.

Table 7.4 Correlation coeffecient (r) between testosterone and cortisol components for male Eastern quoll.

a : males in the wild (n=46 samples)
b : males with females (n=57 samples)
c : males near females (n=33)
d : males isolated from females (n=78 samples).

Parameter		Tot. Cortisol	CBG	MCBC	Alb	Free
Testost.	a	-0.11	-0.13	-0.35	0.04	-0.07
	b	0.07	0.06	-0.17	0.11	0.11
	c	0.09	0.08	-0.27	0.19	0.11
	d	-0.10	-0.10	-0.31	-0.09	-0.09
Tot. Cortisol	a	-	1.00*	0.53	0.94*	0.98*
	b	-	0.99*	0.44	0.97*	0.99*
	c	-	1.00*	0.16	0.98*	0.97*
	d	-	1.00*	0.33	0.98*	0.99*
CBG bound	a	-	-	0.56	0.92*	0.98*
	b	-	-	0.46	0.96*	0.99*
	c	-	-	0.17	0.98*	0.97*
	d	-	-	0.34	0.98*	0.99*
MCBC	a	-	-	-	0.33	0.38
	b	-	-	-	0.37	0.36
	c	-	-	-	0.10	0.14
	d	-	-	-	0.28	0.28
Alb bound	a	-	-	-	-	0.95*
	b	-	-	-	-	0.97*
	c	-	-	-	-	0.96*
	d	-	-	-	-	0.99*

* high correlation

7.3.3 Plasma Biochemistry

Table 7.5 provides the mean levels for the biochemical parameters measured in wild quoll during four periods in the year. The four periods were selected because: from March to April the male quoll is increasing in body

weight and scrotal size in preparation for breeding; June to August surrounds the breeding season; September to November are non-breeding months; and from December to January juvenile quoll disperse into the population.

Table 7.5 Plasma Biochemistry (mean ± s.d.) of male Eastern quoll in the wild.

Month	n	Glucose mM	Protein gL	Triglycerides mM	Cholesterol mM
Mar. - Apr.	5	6.00 ± 2.1	68.92 ± 15.5	0.69 ± 3.1	2.78 ± 1.2
June - Aug.	7	7.31 ± 2.1	65.99 ± 8.3	1.28 ± 0.6	3.63 ±1.1
Sept. - Nov.	11	6.04 ± 3.2	69.88 ± 10.3	1.09 ± 0.4	3.84 ± 1.2
Dec. - Jan.	5	7.38 ± 1.3	76.87 ± 14.7	1.22 ± 0.5	5.76± 2.1

There was no significant variation in glucose, triglyceride or protein concentration during the four periods examined (ANOVA, glucose, $F_{[3,24]}=0.62$, $p>0.25$; triglyceride, $F_{[3,24]}=1.63$, $0.10<p<0.25$; protein, $F_{[3,24]}=0.76$, $p>0.25$). Cholesterol levels varied significantly throughout the year (ANOVA, $F_{[3,24]}=4.29$, $0.01<p<0.025$) with the highest levels occurring in the December to January period.

Table 7.6 shows comparative biochemical levels in captive male quoll sampled more frequently during the breeding season. In contrast to males in the wild, captive animals did not experience a seasonal change in diet and were all considered to maintain good physical condition.

The range of the parameters are comparable between wild and captive males although total proteins and glucose generally were lower and triglycerides and cholesterol generally higher in captive animals. Glucose and cholesterol levels varied significantly over the months (ANOVA, glucose, $F_{[11,117]}=1.89$, $0.025<p<0.05$; cholesterol, $F_{[11,133]}=5.29$, $p<0.0001$) but neither showed any significant variation during the mating period in June. There

were no significant differences between the remaining parameters during June (ANOVA, $p>0.05$).

Table 7.6 Plasma Biochemistry (mean + s.d.) of captive male Eastern quoll surrounding the mating period.

Month	n	Glucose mM	Protein gL	Triglycerides mM	Cholesterol mM
Mar.	4	4.48 ± 2.2	66.68 ± 10.5	1.23 ± 0.4	7.98 ± 1.7
Apr.- early	5	5.24 ± 0.7	58.50 ± 2.3	0.87 ± 0.7	4.68 ± 1.4
Apr.- late	12	4.79 ± 0.7	52.49 ± 11.3	1.10 ± 0.4	5.49 ± 2.6
May - early	16	4.71 ± 1.3	53.32 ± 8.5	1.73 ± 0.8	3.48 ± 1.1
May - mid	19	5.61 ± 2.2	58.95 ± 13.6	2.11 ± 1.2	4.17 ± 0.8
May - late	17	5.78 ± 1.8	52.53 ± 16.4	1.38 ± 0.7	4.09 ± 1.9
June - early*	13	6.15 ± 1.6	59.19 ± 12.2	1.27 ± 0.7	5.51 ± 1.8
June - mid*	24	5.91 ± 2.8	56.05 ± 9.7	1.64 ± 1.3	4.21 ± 1.1
June - late	10	5.38 ± 1.3	61.64 ± 9.9	1.71 ± 1.1	5.48 ± 1.5
July - early	12	5.77 ± 2.3	60.42 ± 15.3	1.32 ± 0.7	5.46 ± 1.6
July - late	14	6.38 ± 1.1	59.54 ± 9.5	1.95 ± 0.9	6.69 ± 1.5
Aug.	8	8.53 ± 5.4	56.93 ± 12.8	1.06 ± 0.5	5.37 ± 2.5

* mating

7.3.4 Haematology

Table 7.7 shows the range of haematological parameters for 40 adult male and 40 non-lactating adult female quoll, all apparently in a healthy condition. There was no significant difference in the levels between wild or captive animals or between male and female adults (series of t-test, $0.1<p<0.375$). The large standard deviation recorded in some parameters possibly reflects seasonal and age differences which could not be accounted for because of the discontinuous collection of samples over sites.

Table 7.7 Haematological parameters for adult male and female Eastern quoll (mean + s.d).

Parameter (units)	Male (n=40)	Female (n=40)
Erythrocytes		
Hb (g / dl)	17.6 ± 2.3	15.9 ± 2.8
RCC (x 10^{12} L^{-1})	10.1 ± 3.0	9.8 ± 1.6
PCV (%)	42.9 ± 12.8	40.1 ± 6.2
MCV (fl)	40.6 ± 5.9	40.4 ± 2.8
MCHC (pg)	15.6 ± 0.5	15.2 ± 3.1
Platelets (x 10^9 L^{-1})	220 ± 30	220 ± 30
Leukocytes		
WCC (x 10^9 L^{-1})	5.7 ± 3.8	5.4 ± 4.4
Basophils	Absent	Absent
Neutrophils (x 10^9 L^{-1})	3.1 ± 1.3	2.4 ± 1.3
Neutrophils (%)	54.4 ± 22.0	44.5 ± 24.0
Lymphocytes (x 10^9 L^{-1})	3.2 ± 0.7	2.2 ± 0.9
Lymphocytes (%)	56.2 ± 12.2	38.6 ± 15.8
Monocytes (x 10^9 L^{-1})	0.3 ± 0.01	0.3 ± 0.05
Eosinophils (x 10^9 L^{-1})	0.2 ± 0.05	0.3 ± 0.04
Ring cells (x 10^9 L^{-1})	0.1 ± 0.02	0.1 ± 0.04
Reticulocytes (x10^9 L^{-1})	130 ± 80	144 ± 96
Sedimentation rate (mm / hr)	3.0 ± 3.0	5.0 ± 4.0
Alkali res. haemoglob. (%)	4.7 ± 3.2	4.9 ± 3.9

Examination of the blood films revealed an absence of basophils although a small number of basophilic granules were identified among the eosinophilic granules. This condition is not uncommon in animals (Parsons

et al. 1971a,b; Wintrobe *et al.* 1981) and usually suggests that eosinophil performs a dual role.

In general, three types of red blood cell profiles were identified. All three profiles contained small numbers of Howell Jolly bodies, 'Ring cells', nucleated red cells and red cell inclusions resembling 'Heinz' bodies. Approximately 50% of the samples had blood pictures that contained mainly discocytes and a small number of spherocytes and spicule cells (Type 1). Approximately 30% of animals had nearly all spherocytes and spicule cells (Type 2). In the third group of animals (20%), Pappenheimer bodies (siderotic granules) and Heinz bodies were prominent and the blood picture was typical of the 'hyposplenic' blood picture observed in humans suffering from an absent or non-functioning spleen (Type 3).

7.4 DISCUSSION

7.4.1 Adreno-cortico activity

Cortisol is the major C-21Δ^4, 3-oxo steroid in most marsupials (Chester Jones *et al.* 1964; Weiss & McDonald 1966, 1967; Weiss & Richards 1971; Oddie *et al.* 1976; reviewed by McDonald 1977) and in the Eastern quoll represents 85 to 87% of the total peripheral plasma corticosteroid concentration (McDonald in Godsell 1983). Nine Δ^4-3-oxosteroids have been identified in the adrenal venous drainage of the quoll but only cortisol and corticosterone have been identified in the peripheral circulation (Weiss & Richards 1971). This implies that a measure of total cortisol in the quoll is a reasonably good indication of the secretory activity of the adrenal cortex.

ACTH is secreted by the anterior pituitary gland and is the prime regulator of glucocorticoid production. Most eutherian mammals show a rise in corticosteroid secretion following injection of synthetic ACTH (Ganong 1965; Turner & Bagnara 1976; McDonald & Waring 1979; Kelley 1985; Roth 1985). In some marsupials such as the Brushtail possum, the secretion rate of cortisol and corticosterone increases after ACTH injection due to an increase in cortisol production (Than & McDonald 1973). In some kangaroo species such as *Macropus eugenii*, *M. giganteus* and *M. rufus*, injection of ACTH produces varying results although most animals show a definite stimulation of cortisol

after administration (Weiss & McDonald 1967; Cooley & Janssens 1977). In a previous study by Weiss and Richards (1971) only one of three Eastern quoll infused with ACTH showed any minimal increase in cortisol secretion, the other two animals showed no effect. The results presented in this chapter confirm that an injection of ACTH does produce a rise in total cortisol in the quoll and that the rise is comparable to other marsupial species examined to date (Oddie *et al.* 1976; reviewed by McDonald 1977).

The quoll showed a rise in cortisol within 30 minutes of injection and within approximately two hours the concentration had increased from 48.6 to 115.0nM cortisol. This rise was comparable to that occurring in the Brushtail possum, Dingo and Koala and similar, but lower, to that found in castrate *Antechinus stuartii* (Weiss & McDonald 1966; Than & McDonald 1973; Oddie *et al.* 1976; Bradley *et al.* 1980; McDonald 1986).

This study found no difference in total or free cortisol levels between male quoll prior to injection but observed differences after injection. December males (non-breeding) produced and maintained higher levels of total cortisol with a higher percentage of biologically active free cortisol after injection than July males (breeding). Godsell (1983) also found the highest total corticosteroid levels occurred in male quolls in the wild during December. She associated this with the period of maximum stress as a result of increased population density. The stress was due mainly to juvenile emergence and dispersal which in turn created pressures for food resources and den sites. It is difficult to associate the levels recorded in captive animals in this experiment with social changes occurring in the wild, especially as there was no difference between animals prior to injection. It may be, however, that an inherent or seasonal component related to hypothalamic or adrenal sensitivity may account for the differences between groups after ACTH injection. This could also be related to a different metabolic clearance rate of cortisol in breeding males compared to non-breeding animals and to the variation in body weight between these times. Adrenal steroids are known to reduce the rate of protein synthesis by antagonising the effects of insulin, thus reducing the rate of energy production through the breakdown of glucose (Turner & Bagnara 1976). This reduction in the rate of energy production may be important in July males that experienced a substantial loss in weight during the mating period.

The aim of injecting animals with ACTH was to determine the potential range of cortisol levels in this species. An application of these results

has been the interpretation of cortisol levels, and in particular free cortisol, into a stress category. Although there is some conjecture, many workers believe that only the free unbound fraction of cortisol is biologically active (Ballard 1979; Bradley 1986) and therefore the most useful component of total cortisol to adopt as a guide to levels of stress. This project adopts the free fraction of total cortisol (and provides total cortisol levels for comparison with other species) as an indice to represent 'stress' but acknowledges that a variety of other systems may be contributing to stress related behaviour without registering a change in free cortisol levels. For example, catecholamines such as norepinephrine and epinephrine are also directly related to levels of stress and may vary widely between and within species (Turner & Bagnara 1976; Levine 1985). Many animals, including man, may show a significant rise in catecholamines as a reaction to stress while the cortisol levels remain unchanged (Ganong 1969; Kelley 1985).

The range of total cortisol levels measured in the Eastern quoll ranged between 30nM and 144nM; slightly lower but similar to those found in other marsupials. The criteria chosen to determine the level of biological important 'high stress' was 80nM to 100 nM or higher of total cortisol, or 7nM to 10 nM or higher of free cortisol. In other marsupials, the ranges vary between 55 and 165nM total cortisol in some macropodids, 160nM to 200nM in some possums, bandicoots and the Koala, and 80nM to 200nM in three species of Dasyuridae (McDonald 1977; Bradley 1987). Plasma cortisol levels suggestive of stress range from 160nM and 500nM in some sheep and dogs, approximately 800nM in rodents, and approximately 1,400nM to 2,700nM, in some primates and rabbits (McDonald 1977).

The second series of experiments investigating cortisol levels in wild and captive quoll have demonstrated that male Eastern quoll are sensitive to a change in social circumstances and can reflect this sensitivity by a change in cortisol levels. It is clear, however, that male Eastern quoll do not experience high total or free cortisol levels during the breeding period suggestive of 'high stress' nor do they show the same pattern of endocrine responses characteristically associated with the post-mating mortality seen in some smaller dasyurids (Barnett 1973; Bradley *et al.* 1976, Bradley *et al.* 1980; McDonald *et al.* 1981; Lee & McDonald 1985; Bradley 1987). This finding is

consistent with the quoll being a long lived member of the Dasyuridae.

An increase in free cortisol in the male quoll is not associated with a decrease in MCBC or CBG bound cortisol and, as Table 7.4 shows, there is poor correlation between testosterone secretion and cortisol levels. The situation where an increase in free cortisol is coincident with high steroid levels, is less likely to arise in this species. The cortisol binding profile of the Eastern quoll is similar therefore to male *Sminthopsis crassicaudata,* and to the Northern quoll, *Dasyurus hallucatus* (McDonald *et al.* 1981; Schmitt *et al.* 1988). Neither of these species show any evidence of an androgen dependent decrease in plasma CBG during breeding and in addition they maintain plasma MCBC well in excess of the total plasma cortisol concentration.

In the wild, adult quoll show an increase in total and free cortisol concentration at the time when juvenile quoll are emerging from the den (November to February). Adult males have basal levels of testosterone during these months whereas juvenile males can produce sporadic, high levels. It has already been suggested in Chapter 4 that high testosterone levels in juveniles increase aggression which in turn aids dispersal, acquisition of den sites and food resources. The high levels of free cortisol in adult males at this time are approaching 'high stress' (5.8nM to 7.7nM) and may accurately reflect the social conflict within the population during this period.

During the breeding period adult male quoll experience levels of free cortisol suggesting 'moderate stress'. Godsell (1983) likewise found an increase in peripheral plasma corticosteroids in males during the breeding season and during summer when the population density was maximal. Godsell suggested that increased adrenocortical activity coincided with periods of decreased survivorship and with changes in the spacing patterns of quolls. The effect of stress during these times was to decrease the fitness of individuals and increase the mortality rate. In this study it was not possible to determine the duration of increased cortisol levels over the breeding period on the basis of monthly sampling, however, results from captive experiments suggest that free cortisol remains elevated for only a short time and is probably unlikely to cause immediate hardship to the animal.

The closely related Northern quoll, *Dasyurus hallucatus,* has a short breeding season and life history very similar to *Dasyurus viverrinus.* The pattern of cortisol secretion in these two species also appears similar. Northern quoll produce maximum free cortisol levels from November to

January, coinciding with dispersal of the young (Schmitt *et al.* 1988). Like the Eastern quoll, the Northern quoll shows no dramatic increase in free circulating cortisol during the breeding period when testosterone concentrations are high. Schmitt *et al.* (1988) suggest that the social hierachy of Northern quoll is established before the mating period and that the low free cortisol levels reflects fewer agonistic encounters during breeding. It could be that a similar endocrinological pattern occurs in the Eastern quoll. In captive animals, the highest testosterone and free cortisol levels are produced just before breeding. Testosterone and cortisol remain elevated for only a short time and both decline as mating proceeds. In the wild this could mean that males establish a dominance hierachy before the females enter oestrus (perhaps during the pro-oestrus phase). Once a male is successful in obtaining a female, they copulate without experiencing further challenges. Infact, the lengthy bouts of copulation observed in this species, as reported for other dasyurids (Lee & Cockburn 1985) would not be possible if males were continually challenged after obtaining a female. It may also be that diminishing levels of testosterone and free cortisol in the male during mating are manifested as aggression but, instead of inter-male rivalry, directed toward the female. This may account for the wounds sustained by the female during copulation.

This study found some differences in cortisol levels between wild and captive quoll. In captive animals, the mean total and free cortisol levels were consistently lower than those found in males in the wild (Table 7.3). In contrast, *Antechinus swainsonii* trapped in the wild before breeding had lower corticosteroids than laboratory held animals during the breeding period (McDonald *et al.* 1981). The authors suggest that the rise in cortisol in *A. swainsonii* is an intrinsic or 'programmed' cycle and that the high levels of free cortisol facilitate reproductive effort as a result of their gluconeogenic effects. It may be that a similar cycle occurs in the quoll although in this case ample food and refuge sites in captivity could have acted to reduce stress levels and hence cortisol secretion.

McDonald *et al.* (1981) also suggest that either the adrenal cortex of *Antechinus* has a low secretory capacity or that the hypothalamic-hypophyseal system is insensitive to environmental factors until just before the breeding period. These explanations may also be true for the Eastern quoll and have already been considered as influencing the male's response to a change in

photoperiod (Chapter 5). Nevertheless, they do not explain the fundamental reasons as to why some *Antechinus* species develop high concentrations of free cortisol that ultimately lead to factors causing death. Population regulation and life history strategy may be the underlying basis of the male *Antechinus* die-off as it appears to be in some other small microtine rodents (Christian 1950; Sadlier 1969; McDonald & Taitt 1982; Lee & Cockburn 1985; Lee & McDonald 1985). Selection is considered to favour males that maximise their fitness by investing most of their effort in reproduction; the smaller species therefore maximise their reproduction at the cost of longevity. The Eastern quoll experiences greater longevity by reducing reproductive effort.

The social grouping of male quoll housed with females over the breeding season, produced the highest free cortisol levels. Crowding and aggressive interactions are usually associated with an increase in adrenocortical activity and are known to adversly affect reproduction and survival (Archer 1979a; Dunbar 1985; Moberg 1985b). The nature and intensity of these responses often differ between species and sexes and are related to the age and social status of the individual. In Chapter 6 it was suggested that the low reproductive success of females over the breeding season could have been due to the stress arising from overcrowding or a change in social structure. The increase in the number of interactions between individuals in this grouping, whether due to increased density or behavioural changes associated with breeding activity, may have increased the levels of stress causing a reduction in reproductive success.

According to Moberg (1985b), subordinate males of most social groups experience stress stemming from their lowly social position and are subjected to attacks and threats from higher ranking animals. House mice, *Mus musculus,* show higher adrenocortical activity in mixed groups than when males are isolated from females (Archer 1970). Subordinate mice have the highest adrenocortical activity due to unsuccessful aggressive encounters while the successful dominant mice have low adrenocortical activity. The effect of competition for social dominance could have been to suppress the expression of sexual behaviour in the subordinate animal and disrupt the normal response of the pituitary-gonadal axis when in the presence of oestrus females. In this study, dominant quoll frequently had low testosterone and free cortisol levels during copulation. Archer (1979a) reported that dominant individuals in a hierachy may show little or no increase in adrenal weight and

yet are responsible for most of the reproduction in crowded populations. Similarly, some other marsupials in captivity, such as possums and bandicoots, experience stress levels of corticosteroids that are a result of the altered social behaviour imposed by confinement (Presidente 1978).

When males were housed near or isolated from females during oestrus, testosterone and free cortisol levels remained low. Although a significant rise in free cortisol occurred at the expected time of breeding, the levels probably were too low to produce any physiological signs of stress. Hypothetically, they may, however, represent some 'anticipation' of female contact similar to that which might occur in the wild. The increase in free cortisol levels were not accompanied by an increase in testosterone which highlights the existence of two discrete neurophysiological pathways leading to steroid secretion.

There may be many explanations as to why males isolated from females maintained low levels of free cortisol. Prolonged stress caused by the separation of animals may have led to an overstimulation of the pituitary-adrenocortical axis leading to adrenal exhaustion as proposed by Seyle's General Adaptation Syndrome (reviewed by Lee & McDonald 1985). Adrenal exhaustion, however, is thought to result from socially-induced stress of overcrowding (Christian 1950) and as group numbers in this experiment were small, overcrowding was unlikely to be a factor causing low cortisol levels. The reverse may have been true whereby restricted physical access between males probably resulted in a lowering of stress as expressed by the low free cortisol levels. The most likely explanation for the low free cortisol levels may be that the absence of females at breeding time has prevented the normal cycle of reproductive activity in males. This inturn has influenced cortisol secretion. It was suggested in Chapter 6 that a stimulus from the female quoll near mating may trigger a rise in androgens in the male. This may also be responsible, either directly or indirectly via testosterone, for inducing a rise in free cortisol levels in wild and captive males housed with females over the breeding season. No additional evidence is available on this as yet.

A noticable feature of all quoll profiles was the relatively short duration of any high free cortisol levels. High testosterone levels in these and other experiments were also noted to be of short duration. This is interesting because the Eastern quoll displays both aggressive mating behaviour and aggressive inter-specific male encounters during mating (Nelson & Smith

1971; Godsell 1983; pers. obs.). The hormonal basis of these aggressive responses is difficult to discern and the mechanisms involved in the secretion and expression of testosterone and cortisol require further exploration.

7.4.2 Biochemistry and Haematology

The ranges of plasma biochemical parameters measured in the Eastern quoll were similar to those of many other marsupial and eutherian mammals including man (Ganong 1965; Lewis *et al.* 1968; Parsons *et al.* 1970; Parsons *et al.* 1971a; Cheal *et al.* 1976; Turner & Bagnara 1976; Barnett *et al.* 1979; Whittington & Grant 1983). The quoll showed little change in protein and triglycerides throughout the year but cholesterol and glucose levels varied significantly. In the wild, the highest cholesterol levels were recorded during December and January. Males during this time also produce high total and free cortisol levels. This is an interesting association because in many animals the measure of cholesterol depletion has been used as an indicator of increased ACTH and cortisol secretion (Ganong 1965) and a indirect measure of adrenal and steroid activity. The results of this experiment, however, do not reflect cholesterol depletion in the quoll. Cholesterol levels were also found to vary significantly in captive animals over the short time period of analysis. This finding demonstrates that cholesterol production can be influenced by a number of factors such as the amount of fat in the diet and exercise and that any association between cholesterol and stress requires close inspection (Ganong 1965).

Glucose levels ranged between 4.0 and 8.0 nM in wild and captive male quoll. The normal range of glucose in humans is 3.9 to 5.9 mM whereas patients suffering disorders such as diabetes mellitus have values as high as 7.8 mM (Ganong 1965). Compared to humans, the glucose range in the quoll is quite high but whether this represents an abnormality is unlikely. The Brushtail and Mountain possum show a reduction in the level of plasma glucose and increase in plasma protein in summer to indicate a change in adrenal activity (Barnett *et al.* 1979). This trend occurred only for protein levels in wild Eastern quoll and was not apparent in captive animals. In contrast, Cheal *et al.* (1976) found no significant differences in plasma protein concentration between samples or sexes in *Antechinus stuartii* but males did show a significant drop in plasma glucose before mating (Barnett 1973). No

reduction in glucose was observed from the monthly sample periods for male quoll in the wild or during more frequent sampling of captive males over the breeding period. Wild and captive male quoll showed a decrease in triglycerides and cholesterol levels during March and April but these were not associated with any decrease in steroids or increase in total cortisol levels.

In conclusion while some biochemical parameters were found to change throughout the year in the quoll, these were more likely to reflect seasonal factors than related specifically to breeding activity. The seasonal cycle of weight loss is not apparently related to gluconeogenesis of protein in response to an increase in circulating adrenocortical hormones. Instead, weight loss is more likely to be related to behavioural and other physiological factors such as increased activity and mobility generated by increased testosterone levels. The association between cholesterol levels and adrenal activity, as reported for some other species, is not borne out in the results from this study and therefore any association between cholesterol and stress is not apparent.

The range of haematological values for the Eastern quoll are similar to those reported for other marsupials and monotremes (Bolliger & Backhouse 1960a,b; Parer & Metcalf 1967a,b; Lewis *et al.* 1968; Parsons *et al.* 1970; Parsons *et al.* 1971b; Nicol 1982; Whittington & Grant 1983) and comparable to some eutherian mammals (Sealander 1964; Dobrowolska & Gromadzka 1978; Dieterich & Feist 1980; Wintrobe *et al.* 1981). Direct comparison between species is difficult, however, as a number of haematological parameters such as haemoglobin and haematocrit are influenced by body size (Lee & Brown 1970).

The HB, PCV values and differential leukocyte counts obtained for the quoll in this study were comparable to those reported earlier by Parsons *et al.* (1971b). MCV values were lower and alkali resistant haemoglobin levels were considerably lower than those recorded by Parsons, however, this may reflect a difference in methodology and expression of results. The red cell parameters for the quoll generally were lower than those reported in the Brushtail possum, *Trichosurus vulpecula,* although white cell counts were similar (Presidente 1978; Presidente & Correa 1981).

The total leukocyte and eosinophil counts of the quoll were low compared to eutherian mammals although representative of marsupials in general (Parsons *et al.* 1971b). Presidente and Correa (1981) observed a shift from low to a higher percentage of neutrophils in male possums in poor

condition (neutrophilia) and they associated this with stress factors arising from captivity and handling. Although the quoll has a higher percentage of neutrophils in the blood than the possum, it is unlikely (on the basis of previous results in this chapter) that this reflects a degree of neutrophilia and stress.

A wide range of small mammals, including the quoll, show no significant sex differences in haematology (Sealander 1964; Cheal *et al.* 1976; Whittington & Grant 1983) whereas the Brushtail and Mountain possum, *T. vulpecula* and *T. caninus,* show marked sexual dimorphism in haemoglobin concentration, haematocrit and erythrocyte count (Barnett *et al.* 1979; Presidente & Correa 1981). These differences reflect a wide degree of species variation in haematology.

Some of the most interesting findings of this study were the red cell disorders such as 'Ring' leucocytes, Howell-Jolly bodies, Heinz bodies, spherocytes and spicule cells in the blood profiles of all Eastern quoll. While these disorders are often associated with abnormalities in humans they are sometimes typical of quite healthy individuals in other species. For example healthy koalas have nucleated erythrocytes and reticulocytes and large numbers of eosinophils and monocytes which would be considered to reflect an abnormal blood history in humans (Bolliger & Backhouse 1960b). Heinz bodies and Ring cells have also been reported in the blood of one long nosed bandicoot, *Isoodon nasuta,* (Parsons *et al.* 1971b) and Howell-Jolly bodies, leptocytes, microcytes and hypochromism increase in abundance in male *Antechinus stuartii* immediately prior to death (Cheal *et al.* 1976). Spherocytes and spicule cells are found in humans with red cell membrane or red cell metabolic defects and also are characteristic of several types of human anaemias (Ganong 1965). The haemoglobin content of the quoll's blood, however, is similar to many other small mammals and shows no evidence of anaemia (Lee & Brown 1970; Parsons *et al.* 1971b). Any evidence of anaemia in the quoll might be related to the heavy but seasonal parasite load carried by the animal (Pearse 1981; pers. obs.).

The blood picture of 20% of apparently healthy animals (Type 3) resembled the 'hyposplenic' picture found in humans suffering from either infections, lymphomas, sarcoid or lipid storage disease (Ganong 1965). The syndrome which develops in some male *Antechinus* at the time of mating is related to the involution of lymphoid tissue in the spleen as a result of stress

(Barker *et al.* 1978). The syndrome is evident pathologically by gastric and duodenal ulcers, neutrophilia and lymphopenia and the recrudescence of latent infections of parasitic *Babesia* species (Arundel *et al.* 1977; Cheal *et al.* 1976). Similarly, post mortems conducted on male phascogales, *Phascogale calura,* revealed hemorrhaging due to stomach and duodenal ulcers and also greatly reduced spleens (Bradley unpub.). Gastric ulcers are known to develop as a result of stress in a range of mammals (reviewed by Bradley 1987). Snowshoe hares, *Lepus americanus macfarlani,* develop gastric ulcers after only one week in captivity and display haemolytic neutrophilia, lymphopenia and eosinopenia (Dieterich & Feist 1980). Brushtail possums in poor condition develop gastric ulcers, involution of splenic lymphoid follicles, as well as neutropenia and changes in white blood cell parameters (Presidente & Correa 1981). Although it was not known whether the quoll develops gastric or duodenal ulcers, it is most likely that the red cell disorders present in 20% of quoll develop as a result of increasing age and that this is infact the 'normal' haematological profile for this species.

Sick or aged quolls in captivity were characterised by a loss of weight, wastage of hind limb muscle and loss of coordination. They also developed patchy hair loss, increased fluid intake and produced lower than normal haematocrit (~27%). Wounds sustained by animals during fighting remained infected for two to three months until they were treated with antibiotics. Two moribund animals captured in the wild were diagnosed (by the Haematology department) as having osteomyelitis, a disease causing inflammation of the bone marrow. All these events suggest poor immunological competence.

In summary, this study has presented preliminary evidence of some deterioration in health as indicated by the haematological profile of the quoll. Potentially, it would seem that the quoll's blood is one of low resistance to infection and latent disease. The relatively high levels of free cortisol which on occasions can be found in this species may act further to suppress the immune system of the quoll and increase the animal's susceptibility to infections and parasitism. Recent studies on the Short nosed bandicoot, *Isoodon macrourus,* have found a similar trend of low haematocrit in sick bandicoots, anaemia caused by heavy and seasonal parasite loads and a deterioration in the blood profile associated with environmental conditions, stress and suppression of the immune system (R. Gemmell, pers. comm.).

7.4.3 General Conclusion

This series of experiments have shown that there is no substantial endocrinological evidence to suggest that the Eastern quoll experiences a major stress response during the breeding season. The changes in partitioning of MCBC, CBG, Alb and free cortisol show no resemblence to the sequence of events that occur in some of the smaller Dasyuridae exhibiting a stress syndrome. Although this finding can only be qualified by the measure of total cortisol and cortisol partitioning and not from other indicators, such as catecholamines, the conclusion is consistent with the fact that the Eastern quoll is a long lived member of the Dasyuridae.

The male Eastern quoll is, however, sensitive to changes in its social and physical well-being. This finding was most evident in the cortisol profiles of male quoll that were isolated from or had restricted access to females during the breeding season. The finding reinforces earlier suggestions of this study that the female quoll plays an important part in initiating mating activity in the male. It appears that the aggressive behaviour and arousal exhibited by animals during breeding is not due to prolonged high testosterone or to excessively high free cortisol levels. The timing between high testosterone and free cortisol levels, male aggression and copulation will provide valuable information on the breeding strategy of the Eastern quoll in the future.

The red cell disorders observed in the haematology of this species, in addition to a seasonal weight loss and at times high free cortisol and testosterone levels, suggests that a combination of factors may act synergistically to lower the animal's auto-immune response and increase susceptibility to infection or disease. Parasite infestation is highest when food resources are limiting (Pearse 1981) and this may help reduce the fitness of adults in their second or third breeding season. These factors in addition to social pressures, may ultimately contribute to the natural causes of mortality in this species.

Collectively the findings of this study suggest that while 'stress' plays little part in the breeding biology of the quoll, 'stress' may be important in influencing longevity of the species.

CHAPTER 8

SYNTHESIS AND GENERAL DISCUSSION

The Eastern quoll, *Dasyurus viverrinus,* displays a pattern of reproduction similar to many seasonally breeding mammals. The short and intense breeding period is the culmination of a series of precisely timed events which ultimately form the basis of the social structure and life history of this species.

8.1 SEASONAL CYCLES

8.1.1 Adults

Male Eastern quoll exhibit pronounced changes in body weight and testes size throughout the year. Adult males gain in body weight and show an increase in testes size from late November, December. In the wild this period coincides with an abundance of spring insects, the major food item in the quoll's diet (Blackhall 1980). Body weight and testes reach maximum size by April and May, before the breeding period and both parameters decline before the onset of mating. This pattern of growth followed by regression has been documented in many of the smaller Dasyuridae (Taylor & Horner 1970; Inns 1976; Kerr & Hedger 1983) and is known to occur in other seasonally breeding male mammals (Sadlier 1969; Clarke 1981; Temple-Smith 1984).

Male quoll in captivity display cycles for body weight and testes size similar to those of quoll in the wild, despite there being no change in food supply. In most males, the testes attain maximum size before body weight and this implies the two parameters are not solely dependent on each other. It is likely that testes size and body weight involve inherent cycles stimulated by some seasonal cue.

For much of the year male Eastern quoll maintain basal concentrations of plasma LH and testosterone. Two to three months before breeding these hormones gradually increase. The major function of the testes

while under hormonal control is to initiate and maintain spermatogenesis and prime the accessory glands such as the prostate and Cowper's glands for reproduction (deKretser 1984). Sharpe (1987) claims that most investigations tend to grossly overestimate the levels of testosterone required to maintain spermatogenesis in the male mammal. Levels just above basal at between 2.0 and 4.0 ng per ml of LH and between 1.0 and 2.0 ng per ml of testosterone may be sufficient to stimulate and maintain spermatogenesis in the male quoll. A portion of the testosterone, however, could be directed toward increasing muscle tissue and body weight as well as increasing activity.

During breeding, LH levels range from 12.0 to 16.0 ng per ml and testosterone levels from 2.5 to 7.6 ng per ml in wild animals. These ranges are similar to other male mammals (see Table 4.4 and Clarke 1981). As Eastern quoll, like most other Dasyuridae, lack a high affinity system for binding sex hormones in the plasma (Sernia 1978; Bradley 1982), a large proportion of the testosterone produced during the breeding season is biologically active. It is unlikely that the androgen concentrations are related to increased testicular activity because the testes are undergoing a decline in size and cellular deterioration during this time (Fletcher 1977). The increased androgen levels are more likely to be associated with increased activity of the prostate and accessory glands, and in other male mammals are expressed through aggression, dominance and sexual display. Captive male quoll exhibit aggressive mating behaviour in the form of threats, ritualised aggressive displays and physical encounters. Males in the wild also become increasingly mobile and wide ranging reflecting an increase in metabolic activity. In the wild, some males maintain higher hormone levels until the end of the breeding season and this may be advantageous when competing for den sites, food resources or females that have returned to oestrus.

8.1.2 First Year Males

First year males show a large increase in body weight and testes size between March and April and it is likely the testes are producing mature sperm by this time. Maturity is reached at a body weight of between 900 and 1000 grams and a testes index of between 5.0 and 6.0. Although first year males show age related increases in body weight and scrotal size, the synchrony of these cycles with adult cycles confirms that body weight and

testes size are influenced by additional factors, probably reproductive.

Juvenile males produce a rise in LH and testosterone shortly after weaning. The levels are similar to those measured in adult males when approaching the breeding season and could be responsible for triggering the maturation of the gonads and initiation of spermatogenesis. In mammals, all the androgens, including testosterone, 5α-dihydrotestosterone and androstanediol, can stimulate the production of androgen binding protein by the Sertoli cells (Ramaley 1979). The high testosterone levels in first year males could also be associated with the aggressive behaviour required by the physically smaller animal to obtain den sites, food resources or to aid dispersal. After this short but sharp peak in hormones, the endocrine profiles of first year males appear similar to those of older animals.

No results were obtained concerning the reproductive success of first year males in the wild. However, the results of one experiment using animals in captivity proved that males (and females) can reproduce successfully in their first season.

8.1.3 Females

While this study was concerned primarily with seasonal breeding in the male, information was also obtained on the reproductive cycle of the female Eastern quoll. In captivity the female's gestation period was reported to be 19 ± 2 days (n=12) compared to 21 ± 2 days (n=5) reported by Fletcher (1977). Slight differences may well be due to interference during the gestation period and stresses imposed by confinement.

The female quoll shows only small fluctuations in body weight throughout the year. Females, like males, lose a large proportion of body weight during the mating period. There was no evidence of females continuing to lose weight throughout the lactation period and this is consistent with the strategy of 'denning' which enables the female to hunt for food unhindered by large and numerous young.

In the wild, the synchrony of mating was demonstrated by the close timing of births and the similar sized pouch young. Godsell (1983) considered that the mating pattern of the quoll implied that both sexes were promiscuous.

Observations from the current study indicate that females did not mate with more than one male although it was possible some males mated with several females. Promiscuity by the male mammal is considered to provide a genetic advantage by increasing biological fitness and parental investment in species where the male gives little or no assistance to rearing the young (Russell 1984).

The female quoll was confirmed to be polyoestrus although out of phase breeding was rare in the wild and confined to two oestrous cycles separated by less than a month. Some females returned to oestrus 5 to 6 days after losing their first litter of young but in other animals this period extended from 11 to 20 days. This time period is similar to that found in other marsupials (Tyndale-Biscoe 1984). In most cases a short delay means that if the young are lost early in lactation the female can still breed in the same season and hence maximise reproductive effort. The low breeding success of females in captivity highlights the fact that adverse factors arising from overcrowding or disruption to the social system can affect reproduction. Low reproductive success of the closely related Tiger quoll, *Dasyurus maculatus,* maintained in captivity has also been attributed to social disruption (Conway 1988).

This study found the hormonal profiles of the female quoll throughout the oestrous cycle and gestation period to be similar to other female marsupials eventhough species variation is apparent (Hinds & Tyndale-Biscoe 1985; Tyndale-Biscoe *et al.* 1986; Tyndale-Biscoe & Renfree 1987). The female quoll produces an increase in LH concentration near ovulation. Progesterone levels gradually increase throughout pregnancy and are maximal just prior to parturition. There was some evidence of a pulse in prolactin occurring just before parturition but generally, prolactin levels increased throughout the lactation period. The differences in prolactin concentration between wild and captive female quoll in this study and that of Hinds & Merchant (1986) suggest that diet and stress may effect the secretion of this hormone.

Females that did not produce young still underwent a period of pouch development while the progesterone, LH and prolactin profiles of pregnant and non-pregnant quoll appear similar in duration and synchrony. These observations agree with the findings of O'Donoghue (1912) that the growth and development of the corpus luteum is the same in non-pregnant as in pregnant female quoll. The findings also support Sharman's (1970)

hypothesis that the marsupial oestrous cycle is equivalent hormonally to gestation and that no endocrine recognition of pregnancy occurs. As recent evidence suggests subtle but significant endocrine, physiological and histological differences associated with pregnancy occur in some Macropodinae (Merchant 1979; Tyndale-Biscoe 1984; Tyndale-Biscoe & Renfree 1987), a more rigorous sampling procedure should be undertaken to either confirm or deny such a pattern in the Eastern quoll.

8.2 PHYSIOLOGICAL MECHANISMS

This study confirmed that testosterone secretion in the male quoll is controlled directly by LH and that a diurnal, and possibly seasonal, component can affect the intensity of secretion (Appendix E). A negative feedback mechanism involving the hypothalamus and anterior pituitary gland regulates the secretion of LH and testosterone in most other male mammals (Hearn 1975; McDonald & Waring 1979; Tyndale-Biscoe & Renfree 1987) and therefore it is likely that the maintenance of the reproductive system of the male quoll is also pituitary dependent.

The range of LH levels in the male quoll are much wider than those of testosterone but this comparison may be reversed in other marsupial and eutherian mammals and appears species specific (Lincoln & Short 1980; Lincoln 1985; Smith *et al.* 1985 and many others). The male quoll shows that an increase in LH is followed within hours by an increase in testosterone and that the secretion of LH and testosterone is episodic rather than occurring in pulses. Both these experiments were preliminary and the patterns certainly require closer inspection. There was no evidence in this study to suggest that repeated blood sampling causes a decline in testosterone secretion (as found by Lincoln 1978). However, testosterone concentrations did fluctuate considerably over a 24 hour period and this agrees with the findings of other workers who caution against the use of single samples to define the hormonal status of an animal.

ACTH appears to be the prime regulator of glucocorticoid production in the quoll. In other mammals a negative feedback mechanism regulates this secretion so that an increase in ACTH quickly produces a rise in cortisol which gradually inhibits ACTH release. Normally, stress factors

induce the release of pituitary ACTH and a rise becomes a necessary component in resistance to stress (Ganong 1965; Roth 1985). An increase in free cortisol in the male quoll was not associated with a decrease in plasma MCBC or CBG nor was there any high correlation between testosterone and cortisol levels. These findings confirm that the male Eastern quoll does not experience the same sequence of endocrine events that occur in many of the smaller Dasyuridae males after mating (McDonald *et al.* 1981) and is consistent with the long life span of this species.

8.3 FACTORS CONTROLLING BREEDING

These investigations have shown that body weight and LH secretion in the male quoll may be influenced by changes in light duration. Photoperiod therefore is a likely proximate factor controlling reproductive activity. In juvenile and adult animals, LH and testosterone levels normally increase during April so that the onset of breeding activity occurs during a time of decreasing daylength. Some component during the autumnal equinox in late March in Tasmania may be the mechanism that initiates the preparatory activity required for breeding. The mechanism that controls this stimulation in other male mammals is a change in sensitivity of the hypothalamus to feedback from the gonads (Turek & Campbell 1979; Lincoln & Short 1980). In the quoll, some signal in March may trigger the hypothalamus to increase its ouput of LH-RH that inturn activates the hypothalamic-pituitary pathway eventually leading to an increase in gonadal hormones. Although it is known that the hypothalamus may change its output of LH-RH independent of any final effect on gonadal hormones (Clarke 1981), the endocrine profiles and physiological cycles reported in this study are in accord with the strictly seasonal nature and synchrony of the quoll's breeding pattern.

Caution must be exercised against taking too simplistic a view of the role of photoperiod. Most southern species of Dasyuridae reach late lactation in early spring or summer but as the duration of lactation varies, the time of onset of breeding differs between species (Tyndale-Biscoe & Renfree 1987). If photoperiod is the proximate factor controlling reproduction, each species of Dasyuridae may be reponding in a different way to changing daylength. Responding to additional local cues would also allow the animal greater

control over the timing of breeding and the subsequent denning and emergence of young at the most favourable time of the year. While photoperiod may be a proximate factor regulating hormonal and body cycles, additional, more specific factors may well be influential during the breeding cycle.

Other dasyurids such as some *Antechinus* (Dickman 1982; Wilson & Bourne 1984) and *Dasyurus hallucatus* (Schmitt *et al.* 1988), as well as many eutherian mammals (Sadlier 1969; Bronson 1985) show that the timing of breeding can vary between different locations even when there is little change in photoperiod. Observations on the timing of breeding in two populations of Eastern quoll in this study found that there were several weeks difference in the stage of breeding activity. This variation may be due to a response to the food cycle which itself is influenced by local changes in temperature and rainfall. As the annual body weight cycle of the quoll is not directly related to food intake, it is likely that the factors controlling reproductive activity in the male quoll agree with Russell (1984) who concludes "...the best breeding situation probably is determined by a variety of events of which the availability of food is but one."

In addition to the food cycle, the sexual status of the female quoll may be an important influence on the male. In most experiments, a gradual rise in LH and testosterone occurred earlier in the year regardless of the degree of contact with the female. When male quoll were denied physical access to females near oestrus, LH, testosterone and cortisol levels remained low. This study proposes that a cue from the female quoll is responsible for the surge in LH and testosterone occurring in the male near mating.

Ovarian activity in the female is suggested to be the prime factor designating the breeding season in some other marsupials (Tammar wallaby, Catling & Sutherland 1980; Inns 1982; McConnell *et al.* 1984; Kowari, Fletcher 1983; Brushtail possum, Gemmell *et al.* 1986; *Antechinus stuartii, A. swainsonii, A. flavipes,* Dickman 1985) and the males of these species all show a rise in androgens prior to mating. It is advantageous for any female approaching oestrus to be attractive to the male for some time before she is sexually receptive and ready to copulate (Russell 1984). The female quoll has a short pro-oestrous phase followed by a period of up to 5 days before spontaneous ovulation (Hill & O'Donoghue 1913) and it is possible that a cue is liberated during this time.

Even though the Eastern quoll is a solitary animal the male is

sensitive to changes in its social environment and reflects this sensitivity with a change in cortisol levels. It is likely, therefore, that the male can detect social cues whether they be from young or transient males or from females. The distribution of olfactory secretions over the head and mouth become an important focus in social encounters (Ewer 1968; Eisenberg & Golani 1977; Aslin 1980; Fanning 1982) and in dasyurids, secretions of the mouth, chin region or cloaca, usually are deposited on the substrate, vegetation or are promoted by face washing and grooming (Croft 1982; Conway 1988). In the quoll most of these are directed towards anti- social behaviour and avoidance but behaviours such as communal and paired nesting, cloacal dragging and a range of postures including an upright stance, all enhance the receiving of olfactory signals at breeding time.

Experiments using captive animals demonstrate that the rise in LH, testosterone and free cortisol occur prior to mating and that the hormone levels decline immediately before and during copulation. This may mean that within the population the male's position in the social hierarchy and dominance are decided in advance of mating and that successful males can copulate without experiencing further challanges. Schmitt *et al.* (1988) have also suggested that the social hierarchy of the Northern quoll, *Dasyurus hallucatus*, was decided in advance of mating and that the moderately high free cortisol levels acted to prevent further agonistic behaviour between males.

A more detailed investigation into olfactory communication in the Eastern quoll is one that will provide valuable information on the synchronisation and reproductive strategy of this species. The factors controlling reproductive activity in the male Eastern quoll, as suggested by this project, are summarised in Fig. 8.1. While the pathways may be similar to those of many other male mammals, the fine tuning is species specific.

8.4 REPRODUCTIVE STRATEGY

Dasyurids have been classified according to six life history strategies, determined largely on components of the female's breeding biology (Lee *et al.* 1982; Lee & Cockburn 1985). In Table 8.1 the life history strategy of four Dasyurids has been presented according to a similar scheme but one with a slightly different perspective, namely the use of body size. This is not

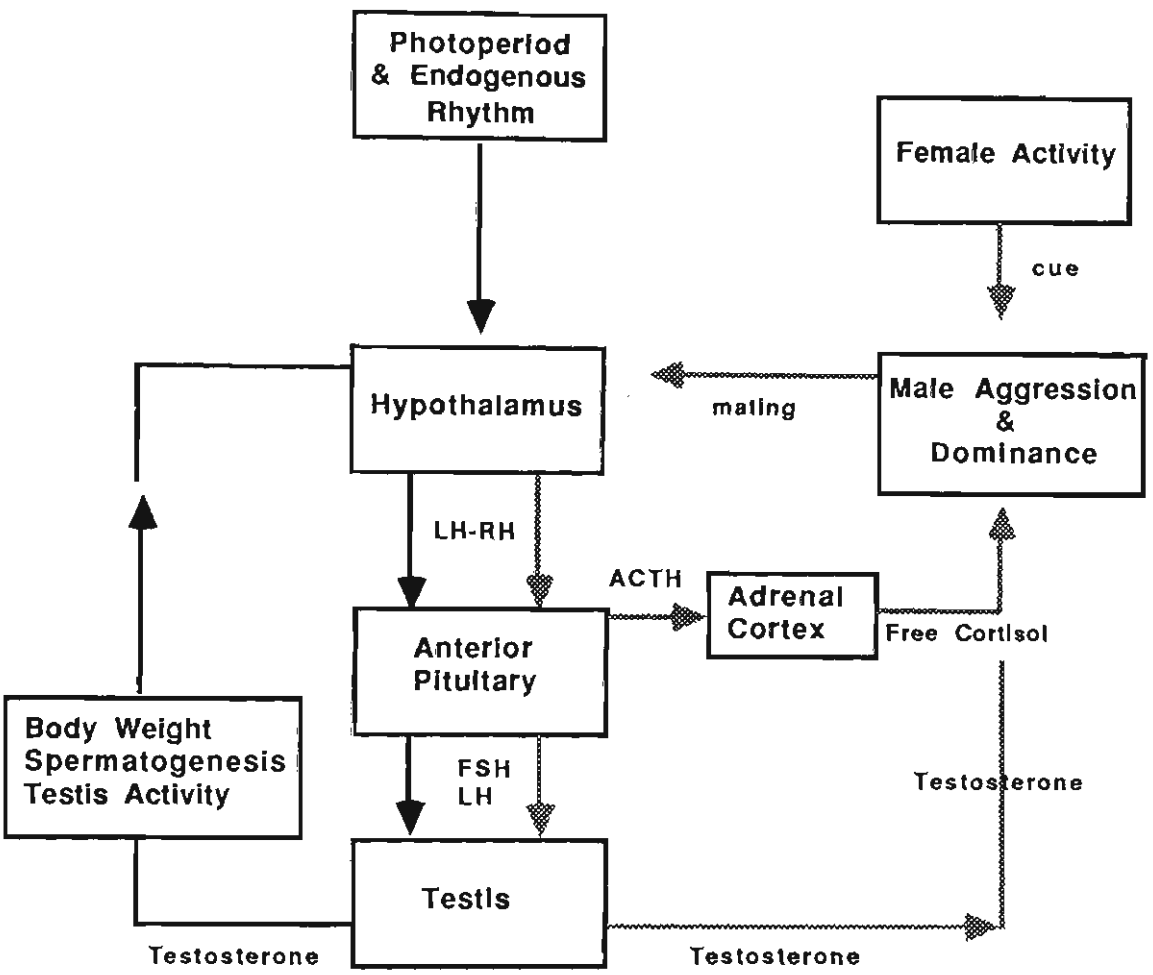

Fig. 8.1 Control of seasonal Breeding in the male Eastern Quoll.

intended as an alternate classification system but is used more to illustrate the role of the male and in particular the possible variation in testosterone and cortisol secretion in relation to the breeding season. The four species shown in Table 8.1 were selected because they represent a continuum in body size and because endocrinological information was available for all males. All the species are found in temperate regions and experience a highly synchronous and short breeding season with mating taking place during winter. The males display a high level of aggression during the breeding period and provide no parental assistance toward raising the young.

Table 8.1 shows that the position of the male in this stratagem is very important and, like the female, the male shows a transition in factors in its breeding pattern. Lee *et al.* (1982) recognised that body size has a significant influence on the life history strategy of Dasyurids and that often it is a compromise with other ecological benefits. In Table 8.1 the benefits or disadvantages are shown as a gradual shift in factors to either side and with them there is a shift between 'r and K' selected breeding strategies. Fleming (1979) has outlined the traits of each selection type for small mammals and has also emphasised the importance of birth and mortality rates and dispersal patterns which are not considered here.

In the female Dasyurid a shift from r to K selection represents a shift toward polyoestry coincident with an increase in reproductive life span and longevity. Fewer young are produced, there is a reduction in teat number and pouches tend to be larger and more developed. Mating takes place earlier in the year so that the young have a longer pouch and den life. They are all weaned by the end of the year when food resources are abundant. The absolute costs of reproduction become lower as the mass of the young produced by a larger female become smaller relative to her own size. The females tend to be more stable in their movements and during mating copulate with only one male.

In the male Dasyurid a similar shift in factors occurs. Moving from an r to a K selected breeding strategy there is an increase in longevity, delayed maturity and a general decrease in reproductive effort/season. The Eastern quoll appears to be a transition species between the extremes of r and K selection. As body size increases there is an increase in the sexual dimorphic ratio. The cycle of testes growth and regression becomes less pronounced and, interestingly, the profiles of testosterone and free cortisol tend also to shift.

Table 8.1 Components of reproductive strategy of four seasonally breeding Dasyurid species

Component of reproduction	*Antechinus stuartii* [1]	*Dasyurus hallucatus* [2]	*Dasyurus viverrinus* [3]	*Sarcophilus harrisii* [4]
Mature body weight				
Males	0.3 - 0.4 kg	0.6 - 0.9 kg	1.1 - 1.9 kg	5.0 - 11.8 kg
Females	0.26 - 0.3 kg	0.4- 0.5 kg	0.7 - 1.1 kg	4.0 -6.5 kg
Dimorphic Ratio	1.16	1.55	1.66	1.60
Longevity in wild				
Males	11.5 mnths	2 to 3 yrs	3 to 5 yrs	> 6 yrs
Females	1 to 2 yrs*	2 to 3 yrs	3 to 5 yrs	> 6 yrs
Residency Patterns				
Male	?	res. & trans.	res. & trans.	res & trans.
Female	?	res. & trans.	resident	?
Reproductive life span				
Males	1 yr	2 ± 1yrs	2 to 3 yrs	6 yrs
Females	1 yr	2 ± 1 yrs	2 to 3 yrs	4 to 6 yrs
Sexual promiscuity	Male & Fem.	Male & Fem.	Male ?	Male?
Breeding season	July - Aug.	early July	early June	Feb.- March
Age at first breeding season	11 mnths	11 mnths	11 mnths**	23 mnths
No. of births per year	1	1	1 - 2	1 - 2
Gestation length - days	27	<31?	19 ± 2	21 - 31?
Lactation period (days)	90 - 110	125	165 - 180	~240
Month of weaning	Dec - Feb	Nov - Jan	Dec - Feb	Nov - Dec
Range of litter size	6 to 10	6 to 8	4 to 6	2 to 4
Usual number of teats	6 to 10	6 to 8	6	4
% of breeding females	100 %	91%	84%	58 %
Neonate weight - mg	16.4	?	12.5	18
% of females body wt	0.057	?	0.013	0.003
ann. % change in scrotal width	?	75.4%	49 %	?
Testosterone prior to mating	high	high	high	moderate
Testosterone during mating	high	declining	declining	low
Free cortisol during breeding	high	moderate	moderate	low
Androgen dependent fall in MCBC	yes	no	no	?
Plasma components during breed.	changing	changing	stable	stable?
Reproductive effort	100 %	high	moderate	moderate ?
Reproductive strategy	r	r ->K	r -> K	K

* rarely 3 years
** probably does not breed until the second year

Sources
1 Barnett 1973; Bradley *et al.* 1980;Tyndale-Biscoe & Renfree 1987
2 Begg 1981b; Schmitt *et al.* 1988
3 this study; Hill & Hill 1955; Godsell 1983
4 Green 1967; Guiler 1970; Hughes 1982; this study (Appendix B).

Cortisol and testosterone concentrations increase prior to the mating period with a tendency to decline during mating. Plasma biochemistry including the levels of glucose, protein and cholesterol, tend to be more stable over the breeding period whereas in the smaller species they show greater fluctuation. These subtle but significant hormonal differences reflect a change in breeding strategy.Some qualification should be made regarding the smaller species of Dasyurid (body weight ~100 grams). Not all small Dasyurids show the same pattern of cortisol and androgen secretion as male *Antechinus stuartii* as not all small species exhibit the phenomenon of male die off after mating. Most species, however, still tend to be short lived and as more endocrine information becomes available it will be interesting to see how this classification changes. It is suspected that the endocrine changes will also correlate with the life histories and, as Lee and McDonald (1985) point out they may provide an excellent basis for the study of the mechanisms behind semelparous and iteroparous strategies.

As a comparison, the Fat tailed dunnart, *Sminthopsis crassicaudata,* is a small desert dwelling dasyurid that experiences a lengthy breeding season (Morton 1978). The females are polyoestrus and capable of rearing two litters in succession. Males are relatively short lived (18 months) but do not die after mating. Endocrinological information shows that the male does not experience any androgen dependent decrease in MCBC at breeding time and that plasma cortisol levels never exceed MCBC (McDonald *et al.* 1981). The males produce an increase in androgens prior to breeding with the greatest number of litters born while androgen levels are decreasing. It may be that this smaller species has a reproductive strategy in the continuum of the Eastern quoll (Table 8.1), and like other species, the pattern has been modified in this case to account for a lengthy breeding season and the seasonal insect cycle.

Although the natural selection processes operating on the male and female are different, the end result is one of reproductive cohesion.

8.5 The Eastern Quoll

The Eastern quoll breeds during winter at a time of lowest temperatures, least food resources (Blackhall 1980) and highest population densities. The animals are undergoing a decline in body weight and carrying the

greatest parasite load (Pearse 1981). While mating occurs at an unfavourable time, the denning of the young coincides with the resumption of the insect cycle so that the young are weaned when food supplies are abundant. Conditions are therefore optimum for lactating females and for weaned young. Braithwaite & Lee (1979) consider that the need to associate late lactation and weaning with the peak in food accessibility is probably the single most important factor favouring a high degree of synchrony in mating. While this may be so, it is interesting that the endocrine and physiological profiles of captive quoll, where diet did not vary, were similar in timing and duration to males in the wild. This suggests that, while the insect or food cycle may be important in the breeding pattern, it probably is not the single most important cue.

The synchrony of the breeding season and the short duration of high androgen and free cortisol levels means that the quoll condenses its reproductive effort into a short period. In doing so it avoids the deleterious effects of prolonged high steroid levels and declining resources. With increasing age it is likely that factors such as poor immunological competence, parasite load, high androgen and free cortisol levels all act synergistically to cause a gradual deterioration in health of males after breeding.

There are definite sex-specific roles for male and female quoll and these are reflected in the social structure of the population and the endocrinological and physiological cycles of each sex. Godsell (1983) and Russell (1984) describe sex specific roles as a conflict of interests arising from the differing parental investment contributed by each sex.

The male Eastern quoll makes little contribution towards raising the young. The male's energetic costs are primarily behavioural and his investment in the offspring ends at insemination (Bronson 1985). A large proportion of first year males become transient and although the relationship between body weight, dominance and mating success has not been determined, it is likely that smaller males are out-competed by larger animals during the breeding season. The role of the first year male is to increase competetion and intra- male aggression among resident males during the breeding season. First year females are an important component of the breeding population because they have the highest reproductive success and are comparatively stable in their movements. Sedentary behaviour in oestrous females may have favoured scent marking, small home ranges and reduced activity levels.

Bronson (1985) concluded that at present we have no broad theoretical understanding of the way the energy partitioning processes relate to the endocrine system and to the neuroendocrine controls of reproduction,

"...we have but pieces of the puzzle derived from laboratory studies of isolated challenges and isolated responses."

The endocrinological information presented on *Dasyurus viverrinus* in this project has complemented the ecological understanding of this species and provided additional pieces to the puzzle.

REFERENCES

ABRAHAM, G.E. (1980). Reliability criteria for steroid Radioimmunoassay. *In* Radioimmunoassay of Steroid Hormones, ed. D. Gupta, pp. 9-19. Verlag & Chemie: Germany.

ALLEN, N.T. & BRADSHAW, S.D. (1980). Diurnal variation in plasma concentrations of testosterone, 5α-dihydrotestosterone, and corticosteroids in the Australian brush-tailed possum, *Trichosurus vulpecula* (Kerr). *Gen. Comp. Endocrinol.* 40: 455-458.

ALMEIDA, O.F.X. & LINCOLN, G.A. (1984). Reproductive photorefractoriness in rams and accompanying changes in the patterns of melatonin and prolactin secretion. *Biol. Reprod.* 30: 143-158.

ARCHER, J. (1970). Effects of aggressive behaviour on the adrenal cortex in male laboratory mice. *J. Mammal.* 51: 327-332.

ARCHER, J. (1979a). Animals under stress. The institute of biology's studies in biology, no. 108. Edward Arnold: Cambridge.

ARCHER, M. (1979b). The status of Australian Dasyurids, Thylacinids and Myrmecobiids. *In* The status of endangered Australasian wildlife, ed. M.J. Tyler, pp. 29-44. Royal Zoological Society of South Australia.

ARNOLD, J.E. & SHIELD, J. (1970). Growth and development of the chuditch (*Dasyurus geoffroii*). *Bull. Aust. Mammal. Soc.* 2: 198.

ARUNDEL, J.H., BARKER, I.K. & BEVERIDGE, I. (1977). Diseases of marsupials. *In* The Biology of Marsupials, eds. B. Stonehouse & D. Gilmore, pp. 141-154. Macmillan: London.

ASLIN, H.J. (1980). Biology of a laboratory colony of *Dasyuroides byrnei* (Marsupialia : Dasyuridae). *Aust. Mammal.* 5: 253-259.

BAIRD, D.T. (1984). The Ovary. *In* Reproduction in Mammals, Bk 3. Hormonal control of Reproduction, 2nd edn, eds. C.R. Austin & R.V. Short, pp. 91-115. Cambridge University Press: London.

BALLARD, P.L. (1979). Delivery and transport of glucocorticoids to target cells. *In* Glucocorticoid Hormone Action, eds. J.D. Baxter & G.G. Rousseau, pp. 25-48. Springer-Verlag: Berlin.

BALTHAZART, J., REBOULLEAU, C. & CHENG, M.F. (1981). Diurnal variations of plasma FSH, LH, and testosterone in male ring doves kept under different photoperiods. *Gen. Comp. Endocrinol.* 44: 202-206.

BARKER, I.K., BEVERIDGE, I., BRADLEY, A.J. & LEE, A.K. (1978). Observations on spontaneous stress-related mortality among males of the Dasyurid marsupial *Antechinus stuartii* Macleay. *Aust. J. Zool.* 26: 435-447.

BARNES, A. & GEMMELL, R.T. (1984). Correlations between breeding activity in the marsupial bandicoots and some environmental variables. *Aust. J. Zool.* 32: 219-226.

BARNETT, C.H. & BRAZENOR, C.W. (1958). The testicular rete mirabile of marsupials. *Aust. J. Zool.* 6: 27-32.

BARNETT, J.L. (1973). A stress response in *Antechinus stuartii* Macleay. *Aust. J. Zool.* 21: 501-513.

BARNETT, J.L., HOW, R.A. & HUMPHREYS, W.F. (1979). Blood parameters in natural populations of *Trichosurus* species (Marsupialia: Phalangeridae). 1. Age, sex and seasonal variation in *T. caninus* and *T. vulpecula*. *Aust. J. Zool.* 27: 913-926.

BARTELS, H., RIEGEL, K., KLEIHAUER, E. & LANG, E.M. (1966). Comparative studies of the respiratory functions of mammalian blood. II Marsupialia: great grey kangaroo and Tasmanian devil. *Respir. Physiol.* 1: 145-150.

BEGG, R.J. (1981a). The small mammals of Little Nourlangie Rock, N.T. II. Ecology of *Antechinus bilarni*, the sandstone antechinus (Marsupialia : Dasyuridae). *Aust. Wildl. Res.* 8: 57-72.

BEGG, R.J. (1981b). The small mammals of Little Nourlangie Rock, N.T. III. Ecology of *Dasyurus hallucatus,* the northern quoll (Marsupialia: Dasyuridae). *Aust. Wildl. Res.* 8: 73-85.

BETKE, K., MARTI, H.R. & SCHLICHT, I. (1959). Estimation of small percentages of foetal haemoglobin. *Nature (Lond.),* 184: 1877-1878.

BIGGERS, J.D. (1966). Reproduction in male marsupials. *Symp. Zool. Soc. Lond.* 15: 251-280.

BIGGINS, J.G. (1984). Communication in possums : a review. *In* Possums and Gliders, eds. A.P. Smith & I.D. Hume, pp. 35-57. Australian Mammal Society: Sydney.

BLACKHALL, S. (1980). Diet of the Eastern native-cat, *Dasyurus viverrinus* (Shaw), in southern Tasmania. *Aust. Wildl. Res.* 7: 191-197.

BOLLIGER, A. & BACKHOUSE, T.C. (1960a). Blood studies on the echidna *Tachyglossus aculeatus. Proc. Zool. Soc. Lond.* 135: 91-97.

BOLLIGER, A. & BACKHOUSE, T.C. (1960b). The blood of the koala (*Phascolarctos cinereus). Aust. J. Zool.* 8: 363-370.

BOYD, I.L. (1986). Photoperiodic regulation of seasonal testicular regression in the wild European rabbit (*Oryctolagus cuniculus*). *J. Reprod. Fertil.* 77: 463-470.

BRADLEY, A.J. (1982). Steroid binding proteins in the plasma of dasyurid marsupials. *In* Carnivorous Marsupials, ed. M. Archer, pp. 651-657. Royal Zoological Society of New South Wales: Sydney.

BRADLEY, A.J. (1985). Steroid binding proteins and life history in marsupials. *In* Current Trends in Comparative Endocrinology, eds. B. Loft & W.N. Holmes, pp. 269-270. Hong Kong University Press: Hong Kong.

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., McDONALD, I.R. & LEE, A.K. (1976). 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.

BRAITHWAITE, R.W. (1974). Behavioural changes associated with the population cycle of *Antechinus stuartii* (Marsupialia). *Aust. J. Zool.* 22: 45-62.

BRAITHWAITE, R.W. & LEE, A.K. (1979). A mammalian example of semelparity. *Am. Nat.* 113: 151-156.

BRONSON, F.H. (1985). Mammalian reproduction : an ecological perspective. *Biol. Reprod.* 32: 1-26.

BRYANT, S.L. (1986). Seasonal variation of plasma testosterone in a wild population of male Eastern quoll, *Dasyurus viverrinus* (Marsupialia : Dasyuridae) from Tasmania. *Gen. Comp. Endocrinol.* 64: 75-79.

BRYANT, S.L. (1988). Maintenance and captive breeding of the marsupial Eastern quoll *Dasyurus viverrinus*. *Int. Zoo. Yearb.* 27:119-124.

BRYANT, S.L. & ROSE, R.W. (1985). Effect of cadmium on the reproductive organs of the male potoroo *Potorous tridactylus* (Macropodidae). *Aust. J. Biol. Sci.* 38: 305-311.

BUCHMANN. O.L.K. & GUILER, E.R. (1977). Behaviour and ecology of the Tasmanian devil, *Sarcophilus harrisii*. *In* The Biology of Marsupials, eds. B. Stonehouse & D. Gilmore, pp. 155-168. Macmillan: London.

CAKE, M.H., OWEN, F.J. & BRADSHAW, S.D. (1980). Difference in concentration of progesterone in plasma between pregnant and non-pregnant quokkas (*Setonix brachyurus*). *J. Endocrinol.* 84: 153-158.

CALABY, J.H. & TAYLOR, J.M. (1981). Reproduction in two marsupial-mice, *Antechinus bellus* and *A. bilarni* (Dasyuridae), of tropical Australia. *J. Mammal.* 62: 329-341.

CANFIELD, P.J. & WHITTINGTON, R.J. (1983). Morphological observations on the erythrocytes, leukocytes and platelets of free-living platypuses, *Ornithorhynchus anatinus* (Shaw) (Monotremata: Ornithorhynchidae). *Aust. J. Zool.* 31: 421-432.

CARRICK, F.N. & COX, R.I. (1973). Testosterone concentrations in the spermatic vein plasma of marsupials. *J. Reprod. Fertil.* 32: 338.

CARRICK, F.N. & COX, R.I. (1977). Testicular endocrinology of marsupials and monotremes. *In* Reproduction and Evolution, eds. J.H. Calaby & C.H. Tyndale-Biscoe,pp. 137-141. Australian Academy of Science: Canberra.

CATLING, P.C. & SUTHERLAND, R.L. (1980). Effect of gonadectomy, season and the presence of female tammar wallabies (*Macropus eugenii*) on concentrations of testosterone, luteinizing hormone and follicle-stimulating hormone in the plasma of male tammar wallabies. *J. Endocrinol.* 86: 25-33.

CHARD, T. (1982). An introduction to radioimmunoassay and related techniques. Elsevier Biomedical Press: New York.

CHEAL, P.D., LEE, A.K. & BARNETT, J.L. (1976). Changes in the haematology of *Antechinus stuartii* (Marsupialia), and their association with male mortality. *Aust. J. Zool.* 24: 299-311.

CHESTER-JONES, I.C., VINSON, G.P., JARRETT, I.G. & SHARMAN, G.R. (1964). Steroid components in the adrenal venous blood of *Trichosurus vulpecula. J. Endocrinol.* 30: 149-150.

CHRISTIAN, J.J. (1950). The adreno-pituitary system and population cycles in mammals. *J. Mammal.* 31: 247-259.

CLARKE, J.R. (1981). Physiological problems of seasonal breeding in eutherian mammals. *In* Oxford Reviews of Reproductive Biology, ed. C.A. Finn, pp. 244-313. Clarendon Press: Oxford.

CONWAY, K. (1988). Captive management and breeding of the Tiger quoll *Dasyurus maculatus. Int. Zoo Yearb.* 27: 108-119.

COOK, B., McDONALD, I.R. & GIBSON, W.R. (1978). Prostatic function in the brush-tailed possum, *Trichosurus vulpecula. J. Reprod. Fertil.* 53: 369-375.

COOLEY, H. & JANSSENS, P.A. (1977). Metabolic effects of infusion of cortisol and adrenocorticotrophin in the tammar wallaby (*Macropus eugenii* Desmarest). Gen. Comp. Endocrinol. 33: 352-358.

CORRIE, J.E.T., RATCLIFFE, W.A. & MACPHERSON, J.S. (1982). The provision of ^{125}I labelled tracers for radioimmunoassay of haptens: A general approach? *J. Immunol. Methods.* 51: 159-166.

COWIE, A.T. (1984). Lactation. *In* Reproduction in mammals. Bk. 3, Hormonal control of reproduction 2nd edn, eds. C.R. Austin & R.V. Short, pp. 195-231. Cambridge University Press: London.

COWPER, W. (1704). The anatomy of a male opossum: in a letter to Dr Edward Tyson from Mr William Cowper. *Philosophical Transactions of the Royal Society,* 24: 1565-1590.

CROFT, D.B. (1982). Communication in the Dasyuridae (Marsupialia) : a review. *In* Carnivorous Marsupials, ed. M. Archer,pp. 291-309. Royal Zoological Society of New South Wales: Sydney.

CURLEWIS, J.D., AXELSON, M. & STONE, G.M. (1985). Identification of the major steroids in ovarian and adrenal venous plasma of the brush-tail possum *(Trichosurus vulpecula)* and changes in the peripheral plasma levels of oestradiol and progesterone during the reproductive cycle. *J. Endocrinol.* 105: 53-62.

CURLEWIS, J.D. & STONE, G.M. (1985a). Peripheral androgen levels in the male brush-tail possum (*Trichosurus vulpecula*). *J. Endocrinol.* 105: 63-70.

CURLEWIS, J.D. & STONE, G.M. (1985b). Some effects of breeding season and castration on the prostate and epididymis of the brushtail possum, *Trichosurus vulpecula. Aust. J. Biol. Sci.* 38: 313-326.

CUTTLE, P. (1982). Life history strategy of the dasyurid marsupial *Phascogale tapoatafa. In* Carnivorous Marsupials. ed. M. Archer, pp. 13-22. Royal Zoological Society of New South Wales: Sydney.

DACIE, J.V. & LEWIS, S.M. (1984). Practical Haematology. 6th ed. Churchill Livingston: Edinburgh.

DICKMAN, C.R. (1982). Some ecological aspects of seasonal breeding in *Antechinus* (Dasyuridae, Marsupialia). *In* Carnivorous Marsupials, ed. M. Archer, pp. 139-150. Royal Zoological Society of New South Wales: Sydney.

DICKMAN, C.R. (1985). Effects of photoperiod and endogenous control on timing of reproduction in the marsupial genus *Antechinus. J. Zool. (Lond.).* 206: 509-524.

DICKMAN, C.R., HAYSSEN, V. & KING, D.H. (1987). Effects of seasonal reversal of photoperiod on the reproductive rhythm of a small marsupial. *J. Zool. (Lond).* 213: 766-768.

DIETERICH, R.A. & FEIST, D.D. (1980). Hematology of Alaskan snowshoe hares (*Lepus americanus macfarlani*) during years of population decline. *Comp. Biochem. Physiol.* 66: 545- 547.

DOBROWOLSKA, A. & GROMADZKA, J. (1978). Relationship between haematological parameters and progesterone blood concentration in different stages of estrous cycle in common vole, *Microtus arvalis*. *Comp. Biochem. Physiol.* 61A: 483-485.

D'OCCHIO, M.J, SCHANBACHER, B.D. & KINDER, J.E. (1984). Profiles of luteinizing hormone, follicle-stimulating hormone, testosterone and prolactin in rams of diverse breeds : Effects of contrasting short (8L:16D) and long (16L:8D) photoperiods. *Biol. Reprod.* 30: 1039-1054.

DUNBAR, R. (1985). Stress is a good contraceptive. *New Scientist.* 17: 16-18.

EINSENBERG, J.F. & GOLANI, I. (1977). Communication in metatheria. *In* How animals communicate, ed. T.A. Sebeok, pp. 575-579. Indiana University Press: Bloomington.

EPPLE, G. (1973). The role of pheromones in the social communication of marmoset monkeys (Callithricidae). *J. Reprod. Fertil. (Supp.),* 19: 447-454.

EVANS, S.M., TYNDALE-BISCOE, C.H. & SUTHERLAND, R.L. (1980). Control of gonadotrophin secretion in the female tammar wallaby (*Macropus eugenii*). *J. Endocrinol.* 86: 13-23.

EWER, R.F. (1968). A preliminary survey of the behaviour in captivity of the dasyurid marsupial, *Sminthopsis crassicaudata* (Gould). *Z. Tierpsychol.* 25: 319-365.

FADEM, B.H. (1985). Evidence for the activation of female reproduction by males in a marsupial, the grey short-tailed opossum (*Monodelphis domestica*). *Biol. Reprod.* 33: 112-116.

FADEM, B.H. & RAYVE, R.S. (1985). Characteristics of the oestrous cycle and influence of social factors in grey short-tailed opossums (*Monodelphis domestica*). *J. Reprod. Fertil.* 73: 337-342.

FADEM, B.H. & COLE, E.A. (1985). Scent-marking in the grey short-tailed opossum (*Monodelphis domestica*). *Anim. Behav.,* 33: 730-738.

FANNING, F.D. (1982). Reproduction, growth and development in *Ningaui* sp. (Dasyuridae, Marsupialia) from the Northern Territory. *In* Carnivorous Marsupials, ed. M.Archer, pp. 23-37. Royal Zoological Society of New South Wales: Sydney.

FARNER, D.S. & FOLLET, B.K. (1979). Reproductive periodicity in birds. *In* Hormones and Evolution, vol. 2. ed. E.J.W. Barrington, pp. 829-872. Academic Press: N.Y.

FLEAY, D. (1935). Breeding of *Dasyurus viverrinus* and general observations of the species. *J. Mammal.* 16: 10-16.

FLEAY, D. (1940). Breeding of the tiger cat. *Victorian Nat.* 56: 159-163.

FLEMING, T.H. (1979). Life history strategies. *In* Ecology of small mammals, ed. D.M. Stoddart,pp 1-52. Chapman & Hall: London.

FLETCHER, T.P. (1977). Reproduction in the native cat *Dasyurus viverrinus* (Shaw). unpubl. Honours Thesis, University of Tasmania, Hobart.

FLETCHER, T.P. (1983). Endocrinology of reproduction in the dasyurid marsupial *Dasyuroides byrnei* (Spencer). Ph.D Thesis, La Trobe University, Melbourne.

FLETCHER, T.P. (1985). Aspects of reproduction in the male eastern quoll, *Dasyurus viverrinus* (Shaw) (Marsupialia : Dasyuridae), with notes on polyoestry in the female. *Aust. J. Zool.* 33: 101-110.

FLYNN, T.T. (1930). The uterine cycle of pregnancy and pseudopregnancy as it is in the diprotodont marsupial *Bettongia cuniculus* with notes on other reproductive phenomena in this marsupial. *Proc. Linn. Soc. N.S.W.* 55: 506-531.

FOWLER, J. & COHEN, L. (1987). Statistics for ornithologists. Guide 22, British Trust for Ornithology: Britain.

GANONG, W.F. (1965). Review of medical physiology Lange Medical Publications, Los Altos: California.

GANSLOSSER, U & MEISSNER, K. (1984). Behavioural signs of oestrus in *Dasyuroides byrnei* (Marsupialia: Dasyuridae). *Aust. Mammal.* 7: 223-224.

GEMMELL, R.T. (1979). The fine structure of the luteal cells in relation to the concentration of progesterone in the plasma of the lactating bandicoot, *Isoodon macrourus* (Marsupialia: Paramelidae). *Aust. J. Zool.* 27: 501-510.

GEMMELL, R.T. (1986). Sexual maturity in the male bandicoot, *Isoodon macrourus. Aust. Mammal. Soc. (Abstract).* 9(1): 6.

GEMMELL, R.T. (1987). Influence of melatonin on the initiation of the breeding season of the marsupial bandicoot, *Isoodon macrourus. J. Reprod. Fertil.* 79: 261-265.

GEMMELL, R.T., CEPON, G. & BARNES, A. (1986). Weekly variations in body weight and plasma testosterone concentrations in the captive male possum, *Trichosurus vulpecula. Gen. Comp. Endocrinol.* 62: 1-7.

GEMMELL, R.T., JENKIN, G. & THORBURN, G.D. (1980). Plasma concentrations of progesterone and 13,14-dihydro-15-keto-prostaglandin F2α at parturition in the bandicoot, *Isoodon macrourus*. *J. Reprod. Fertil.* 60: 253-256.

GEMMELL, R.T., JOHNSTON, G. & BARNES, A. (1985). Seasonal variations in plasma testosterone concentrations in the male marsupial bandicoot, *Isoodon macrourus* in captivity. *Gen. Comp. Endocrinol.* 59: 184-191.

GEMMELL, R.T., McFARLANE, J. & TODHUNTER, R. (1987). The effect of the frequency of blood sampling on the plasma concentrations of testosterone and cortisol in the male marsupial. *Abstract, Proceedings of the nineteenth annual conference, Australian Society for Reproductive Biology,* p73.

GILMORE, D.P. (1969). Seasonal reproductive periodicity in the male Australian brush-tailed possum *Trichosurus vulpecula*. *J. Zool. (Lond.).* 157: 75-98.

GODFREY, G.K. (1969). The influence of increased photoperiod on reproduction in the dasyurid marsupial, *Sminthopsis crassicaudata*. *J. Mammal.* 50: 132-133.

GODSELL, J. (1982). The population ecology of the eastern quoll *Dasyurus viverrinus* (Dasyuridae, Marsupialia), in southern Tasmania. *In* Carnivorous Marsupials, ed. M.Archer, pp. 199-207. Royal Zoological Society of New South Wales: Sydney.

GODSELL, J. (1983). Ecology of the Eastern quoll. Ph.D Thesis, Australian National University, Canberra.

GOWER, D.B. (1979). Steroid Hormones. Croom Helm: London.

GREEN, B. & EBERHARD, I. (1983). Water and sodium intake, and estimated food consumption, in free-living eastern quolls, *Dasyurus viverrinus*. *Aust. J. Zool.* 31: 871-880.

GREEN, B., MERCHANT, J. & NEWGRAIN, K. (1987). Milk composition in the eastern quoll, *Dasyurus viverrinus* (Marsupialia : Dasyuridae). *Aust. J. Biol. Sci.* 40: 379-387.

GREEN, R.H. (1967). Notes on the devil (*Sarcophilus harrisii*) and the quoll (*Dasyurus viverrinus*) in north-eastern Tasmania. *Records of the Queen Victoria Museum,* 27: 1-13.

GREENWOOD, A. (1923). Marsupial spermatogenesis. *Q. J. Microsc. Sci.* 67: 203-218.

GREENWOOD, F.C., HUNTER, W.M. & GLOVER, J.S. (1963). The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* 89: 114-123.

GRUBB, P. & JEWELL, P. A. (1973). The rut and the occurrence of oestrus in the Soay sheep on St. Kilda. *J. Reprod. Fertil. (Supp).* 19: 491-502.

GUILER, E.R. (1970). Observations on the Tasmanian devil, *Sarcophilus harrisii* (Marsupialia: Dasyuridae). II. Reproduction, breeding, and growth of pouch young. *Aust. J. Zool.* 18: 63-70.

GUSTAFSON, A.W. & SHEMESH, M. (1976). Changes in plasma testosterone levels during the annual reproductive cycle of the hibernating bat *Myotis lucifugus lucifugus* with a survey of plasma testosterone levels in adult male vertebrates. *Biol. Reprod.* 15: 9-24.

HALL-MARTIN, A.J. (1987). Role of musth in the reproductive strategy of the African elephant (*Loxodonta africana*). *South African Journal of Science.* 83:616-623.

HAMMOND, G.L., VIINIKKA, L. & VIHKO, R. (1977). Automation of radioimmunoassay for some sex steroids with use of both iodinated and tritiated ligands. *Clin. Chem.* 23: 1250-1257.

HARDER, J.D. & FLEMING, M.W. (1981). Estradiol and progesterone profiles indicate a lack of endocrine recognition of pregnancy in the opossum. *Science,* 212: 1400-1402.

HARDER, J.D. and FLEMING, M.W. (1986). Annual pattern of circulating testosterone relative to the breeding season in male opossums (*Didelphis virginiana*). *Biol. Reprod. (Supp)* 34: 203.

HARDER, J.D., HINDS, L.A., HORN, C.A. & TYNDALE-BISCOE, C.H. (1985). Effects of removal in late pregnancy of the corpus luteum, Graafian follicle or ovaries on plasma progesterone, oestradiol, LH, parturition and post-partum oestrus in the tammar wallaby, *Macropus eugenii. J. Reprod. Fertil.* 75: 449-459.

HARDING, H.R., CARRICK, F.N. & SHOREY, C.D. (1977). Spermatozoa of Australian marsupials: ultrastructure and epididymal development. *In* Reproduction and Evolution, eds. J.H. Calaby & C.H. Tyndale-Biscoe, pp. 151-152. Australian Academy of Science: Canberra.

HARDING, H.R., WOOLLEY, P.A., SHOREY, C.D. & CARRICK, F.N. (1982). Sperm ultrastructure, spermiogenesis and epididymal sperm maturation in dasyurid marsupials: phylogenetic implications. *In* Carnivorous Marsupials, ed. M. Archer, pp. 659-673. Royal Zoological Society of New South Wales: Sydney.

HEARN, J.P. (1972). The development of a radioimmunoassay for gonadotrophin in the Tammar wallaby, *Macropus eugenii. J. Reprod. Fertil.* 28: 132.

HEARN, J.P. (1975). The role of the pituitary in the reproduction of the male tammar wallaby, *Macropus eugenii. J. Reprod. Fertil.* 42: 399-402.

HEDDLE, R.W.L. & GUILER, E.R. (1970). The form and function of the testicular rete mirabile of marsupials. *Comp. Biochem. Physiol.* 35: 415-425.

HEINSOHN, G.E. (1966). Ecology and reproduction of the Tasmanian bandicoots *(Perameles gunnii* and *Isoodon obesulus). Univ. Calif. Publ. Zool.* 80: 1-107.

HILL, J.P. (1900). On the foetal membranes, placentation and parturition, of the native cat (*Dasyurus viverrinus*). *Anat. Anz.* 18: 364-373.

HILL, J.P. & HILL, W.C.O. (1955). The growth stages of the pouch young of the native cat (*Dasyurus viverrinus*) together with observations on the anatomy of the new born young. *Trans. Zool. Soc. Lond.* 28: 349-452.

HILL, J.P. & O'DONOGHUE, C.H. (1913). The reproductive cycle in the marsupial *Dasyurus viverrinus. Q. J. Microsc. Sci.* 59: 133-174.

HINDS, L.A. (1983). Progesterone and prolactin in marsupial reproduction. Ph.D Thesis, Australian National University, Canberra.

HINDS, L.A. and JANSSENS, P.A. (1986). Changes in prolactin in peripheral plasma during lactation in the brushtail possum *Trichosurus vulpecula. Aust. J. Biol. Sci.* 39: 171-178.

HINDS, L.A. & MERCHANT, J.C. (1986). Plasma prolactin concentrations throughout lactation in the eastern quoll, *Dasyurus viverrinus* (Marsupialia : Dasyuridae). *Aust. J. Biol. Sci.* 39: 179-186.

HINDS, L.A. & den OTTOLANDER, R.C. (1983). Effect of changing photoperiod on peripheral plasma prolactin and progesterone concentrations in the tammar wallaby (*Macropus eugenii). J. Reprod. Fertil.* 69: 631-639.

HINDS, L.A. & TYNDALE-BISCOE, C.H. (1982). Plasma progesterone levels in the pregnant and non-pregnant tammar, *Macropus eugenii. J. Endocrinol.* 93: 99-107.

HINDS, L.A. & TYNDALE-BISCOE, C.H. (1985). Seasonal and circadian patterns of circulating prolactin during lactation and seasonal quiescence in the tammar, *Macropus eugenii. J. Reprod. Fertil.* 74: 173-183.

HOCHEREAU-de REVIERS, M.T., PERREAU, C. & LINCOLN, G.A. (1985). Photoperiodic variations of somatic and germ cell populations in the Soay ram testis. *J. Reprod. Fertil.* 74: 329-334.

HOCKING, G.J. & GUILER, E.R. (1983). The mammals of the Lower Gordon River region, south-west Tasmania. *Aust. Wildl. Res.* 10: 1-23.

HORN, C.A., FLETCHER, T.P. & CARPENTER, S. (1985). Effects of oestradiol-17ß on peripheral plasma concentrations of LH and FSH in ovariectomized tammars (*Macropus eugenii*). *J. Reprod. Fertil.* 73: 585-592.

HUGHES, R.L. (1965). Comparative morphology of spermatozoa from five marsupial families. *Aust. J. Zool.* 13: 533-543.

HUGHES, R.L. (1982). Reproduction in the Tasmanian devil *Sarcophilus harrisii* (Dasyuridae, Marsupialia). *In* Carnivorous Marsupials. ed. M. Archer, pp. 49-63. Royal Zoological Society of New South Wales: Sydney.

HUMPHREYS, W.F., BRADLEY, A.J., HOW, R.A. & BARNETT, J.L. (1984). Indices of condition of Phalanger populations: a review. *In* Possums and Gliders, eds. A.P. Smith & I.D. Hume, pp 59-77. Australian Mammal Society: Sydney.

INNS, R.W. (1976). Some seasonal changes in *Antechinus flavipes* (Marsupialia : Dasyuridae). *Aust. J. Zool.* 24: 523-531.

INNS, R.W. (1982). Seasonal changes in the accessory reproductive system and plasma testosterone levels of the male tammar wallaby, *Macropus eugenii*, in the wild. *J. Reprod. Fertil.* 66: 675-680.

IRBY, D.C., KERR, J.B., RISBRIDGER, G.P.& de KRETSER, D.M. (1984). Seasonally and experimentally induced changes in testicular function of the Australian bush rat (*Rattus fuscipes*). *J. Reprod. Fertil.* 70: 657-666.

JANSSENS, P.A. & HINDS, L.A. (1981). Long-term effects of corticosteroid administration in the tammar wallaby, *Macropus eugenii*. *Gen. Comp. Endocrinol.* 45: 56-60.

JEFFCOATE, S., GILBY, E.D. & EDWARDS, R. (1973). The preparation and use of [^{125}I] steroid-albumin conjugates as tracers in steroid radioimmunoassays. *Clin. Chim. Acta.* 43: 343-349.

JEWELL, P.A. (1973). Population density and social factors affecting reproduction. *J. Reprod. Fert (Supp.).* 19: 455-456.

KELLEY, K.W. (1985). Immunological consequences of changing environmental stimuli. *In* Animal Stress, ed. G.P. Moberg, pp. 193-223. American Physiological Society, Bethseda: Maryland.

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.

KERR, J.B., KEOGH, E.J., HUDSON, B., WHIPP, G.T. & deKRETSER, D.M. (1980). Alterations in spermatogenic activity and hormonal status in a seasonally breeding rat, *Rattus fuscipes*. *Gen Comp. Endocrinol.* 40: 78-88.

KIRSCH, J.A.W. & MURRAY, M.D. (1969). A scheme for the identification of the blood of Australasian marsupials. *Aust. J. Zool.* 17: 799-816.

KITCHENER, D.J., COOPER, N. & BRADLEY, A. (1986). Reproduction in male *Ningaui* (Marsupialia : Dasyuridae). *Aust. Wildl. Res.* 13: 13-25.

KRATZING, J.E. (1984). The structure and distribution of nasal glands in four marsupial species. *J. Anat.* 139: 553-564.

de KRETSER, D.M. (1984). The testis. *In* Reproduction in Mammals, Bk. 3, Hormonal Control of Reproduction, 2nd edn, eds. C.R.Austin & R.V. Short, pp. 76-90. Cambridge University Press: London.

LEE, A.K., BRADLEY, A.J. & BRAITHWAITE, R.W. (1977). Corticosteroid levels and male mortality in *Antechinus stuartii*. *In* The Biology of Marsupials, eds. B. Stonehouse & D. Gilmore, pp. 209-220. Macmillan: London.

LEE, A.K. & BROWN, J.H. (1970). Variations in the hemoglobin concentrations of Great Basin rodents. *J. Mammal.* 51: 669-674.

LEE, A.K. & COCKBURN, A. (1985). Evolutionary Ecology of Marsupials. Cambridge University Press.

LEE, A.K. &McDONALD, I.R. (1985). Stress and population regulation in small mammals. *In* Oxford Reviews of Reproductive Biology, ed. J.R. Clarke, pp. 261-304. Clarendon Press: Oxford.

LEE, A.K., WOOLLEY, P. & BRAITHWAITE, R.W. (1982). Life history strategies of dasyurid marsupials. *In* Carnivorous Marsupials, ed. M. Archer, pp. 1-11. Royal Zoological Society of New South Wales: Sydney.

LEMON, M. (1972). Peripheral plasma progesterone during pregnancy and the oestrous cycle in the tammar wallaby *Macropus eugenii*. *J. Endocrinol.* 55: 63-71.

LEVINE, S. (1985). A definition of stress? *In* Animal Stress, ed. G.P. Moberg, pp. 51-69. American Physiological Society, Bethseda: Maryland.

LEWIS, J.H., PHILLIPS, L.L. & HANN, C. (1968). Coagulation and hematological studies in primitive Australian mammals. *Comp. Biochem. Physiol. (short communication).* 25: 1129-1135.

LINCOLN, G.A. (1976). Secretion of LH in rams exposed to two different photoperiods. *J. Reprod. Fertil.* 47: 351-353.

LINCOLN, G.A. (1978). Plasma testosterone profiles in male macropodid marsupials. *J. Endocrinol.* 77: 347-351.

LINCOLN, G. A. (1985). Seasonal breeding in deer. *Biology of Deer Production. The Royal Society of New Zealand, Bulletin* 22: 165-179.

LINCOLN, G.A. & GUINNESS, F.E. (1973). The sexual significance of the rut in red deer. *J. Reprod. Fertil. (Supp).* 19: 457-489.

LINCOLN, G.A. & KAY, R.N.B. (1979). Effects of season on the secretion of LH and testosterone in intact and castrated red deer stags (*Cervus elaphus*). *J. Reprod. Fertil.* 55: 75-80.

LINCOLN, G.A. & SHORT, R.V. (1980). Seasonal breeding: nature's contraceptive. *Recent Prog. Horm. Res.* 36: 1-52.

McALLAN, B.M. & DICKMAN, C.R. (1986). The role of photoperiod in the timing of reproduction in the dasyurid marsupial *Antechinus stuartii. Oecologia.* 68: 259-264.

McCONNELL, S.J., FLETCHER, T.P. & ELLENDORFF, F. (1984). The effect of olfactory bulbectomy on plasma testosterone levels in male tammar wallabies (*Macropus eugenii*) exposed to females undergoing an oestrous cycle. *Bull. Aust. Mammal. Soc. (Abstract).* 8(2): 143.

McCONNELL, S.J. & TYNDALE-BISCOE, C.H. (1985). Response in peripheral plasma melatonin to photoperiod change and the effects of exogenous melatonin on seasonal quiescence in the tammar, *Macropus eugenii. J. Reprod. Fertil.* 73: 529-538.

McDONALD. I.R. (1977). Adrenocortical function in marsupials. *In* The Biology of Marsupials, eds. B. Stonehouse & D. Gilmore, pp. 345-379. Macmillan: London.

McDONALD, I.R. (1986). Episodic steroid secretion in koalas *Phascolarctos cinereus. Bull. Aust. Mammal Society (Abstract).* 9(1): 6.

McDONALD, I.R., LEE, A.K., BRADLEY, A.J. & THAN, K.A. (1981). Endocrine changes in dasyurid marsupials with differing mortality patterns. *Gen. Comp. Endocrinol.* 44: 292-301.

McDONALD, I.R. & TAITT, M.J. (1982). Steroid hormones in the blood plasma of Townsend's vole (*Microtus townsendii*). *Can. J. Zool.* 60(10): 2264-2269.

McDONALD, I.R. & WARING, H. (1979). Hormones of marsupials and monotremes. *In* Hormones and Evolution, ed. E.J.W. Barrington, pp. 873-92. Academic Press: London.

McDOUGALL, S. (1985). Androgen and behavioural response to females and the effect of vasectomy on the prostate in the male tammar wallaby *Macropus eugenii.* BSc.(Vet.). Thesis, University of Sydney, Sydney.

McFARLANE, J.R. & CARRICK, F.N. (1987). Episodic secretion of androgens and cortisol in the brushtail possum (*Trichosurus vulpecula*). *Abstract, Proceedings of the nineteenth annual conference, Australian Society for Reproductive Biology,* p. 74.

McFARLANE, J.R., CARRICK, F.N. & BROWN, A.S. (1986a). Seasonal changes in the size of the prostate in the brushtail possum *Trichosurus vulpecula*. *Bull. Aust. Mammal. Soc. (Abstract).* 9(1): 56.

McFARLANE, J.R., CARRICK, F.N., GEMMELL, R.T. & MacDONALD, G. (1986b). Androgen secretion in the male bandicoot. *Bull. Aust. Mammal. Soc. (Abstract).* 9(1): 16.

McGUCKIN, M.A. & BLACKSHAW, A.W. (1987). Seasonal changes in spermatogenesis (including germ cell degeneration) and plasma testosterone concentration in the grey-headed fruit bat, *Pteropus poliocephalus*. *Aust. J. Biol. Sci.* 40: 211-220.

McLEOD, B.J. & CRAIGON, J. (1985). Time series analysis of plasma LH and FSH concentrations as a method of assessing episodic secretion. *J. Reprod. Fertil.* 74(2): 575-587.

MAHONEY, J.A. & RIDE, W.D.L. (1984). The identity of Captain Cook's quoll *Mustela quoll* Zimmermann, 1783 (Marsupialia: Dasyuridae). *Aust. Mammal.* 7(2): 57-62.

MELROSE, W.D, PEARSE, A.M., JUPE, D.M.D., BAIKIE, M.J., TWIN, J.E. & BRYANT, S.L. (1987). Haematology of the Australian Eastern quoll, *Dasyurus viverrinus*. *Comp. Biochem. Physiol.* 88A(2): 239-241.

MERCHANT, J.C. (1979). The effect of pregnancy on the interval between one oestrus and the next in the tammar wallaby, *Macropus eugenii*. *J. Reprod. Fertil.* 56: 459-463.

MERCHANT, J.C., NEWGRAIN, K. & GREEN, B. (1984). Growth of the eastern quoll, *Dasyurus viverrinus* (Shaw), (Marsupialia) in captivity. *Aust. Wildl. Res.* 11: 21-29.

MOBERG, G.P. (1985a). Biological responses to stress : key to assessment of animal well-being. *In* Animal Stress, ed. G.P. Moberg, pp. 27-49. American Physiological Society, Bethseda: Maryland.

MOBERG, G.P. (1985b). Influence of stress on reproduction: measure of well-being. *In* Animal Stress, ed. G.P. Moberg, pp. 245-267. American Physiological Society, Bethseda: Maryland.

MOLDEN, D.P., ALEXANDER, M.M. & NEELEY, W.E. (1982). Fetal haemoglobin: optimum conditions for its estimation by alkali denaturation. *Am. J. Clin. Pathol.* 77: 568-572.

MORITZ, C. (1982). Some aspects of the ecological and genetical structure of a natural population of *Antechinus stuartii* (Dasyuridae, Marsupialia). *In* Carnivorous Marsupials, ed. M. Archer,pp. 685-694. Royal Zoological Society of New South Wales: Sydney.

MORTON, S.R. (1978). An ecological study of *Sminthopsis crassicaudata* (Marsupialia : Dasyuridae). III. Reproduction and life history. *Aust. Wildl. Res.* 5: 183-211.

MYKYTOWYCZ, R. (1973). Reproduction of mammals in relation to environmental odours. *J. Reprod. Fertil. (Supp.).* 19: 433-446.

NARS, P.W. & HUNTER, W.M. (1973). A method for labelling oestradiol-17ß with radioiodine for radioimmunassays. *J. Endocrinol.* 57: xlvii-xlviii.

NELSON, J.E. & SMITH, G. (1971). Notes on growth rates in native cats of the family Dasyuridae. *Int. Zoo. Yearb.* 11: 38-41.

NICOL, S.C. (1982). Physiology of the Tasmanian devil *Sarcophilus harrisii* (Dasyuridae, Marsupialia). *In* Carnivorous Marsupials, ed. M. Archer, pp. 273-278. Royal Zoological Society of New South Wales: Sydney.

ODDIE, C.J., BLAINE, E.H., BRADSHAW, S.D., COGHLAN, J.P., DENTON, D.A., NELSON, J.F. & SCOGGINS, B.A. (1976). Blood corticosteroids in Australian marsupial and placental mammals and one monotreme. *J. Endocrinol.* 69: 341-348.

O'DONOGHUE, C.H. (1912). The corpus luteum in the non-pregnant *Dasyurus* and polyovular follicles in *Dasyurus*. *Anat. Anz.* 41: 356-368.

O'REILLY, H.M., ARMSTRONG, S.M. & COLEMAN, G.J. (1984). Response to variations in lighting schedules on the circadian activity rhythms of *Sminthopsis macroura froggatti* (Marsupialia: Dasyuridae). *Aust. Mammal.* 7: 89-99.

ORTAVANT, R., DAVEAU, A., GARNIER, D.H., PELLETIER, J., de REVIERS, M.M. & TERQUI, M. (1982). Diurnal variation in release of LH and testosterone in the ram. *J. Reprod. Fertil.* 64: 347-353.

PARER, J.T. & METCALFE, J. (1967a). Respiratory studies of monotremes. I. Blood of the platypus (*Ornithorhynchus anatinus*). *Respir. Physiol.* 3: 136-142.

PARER, J.T. & METCALFE, J. (1967b). Respiratory studies of monotremes. II. Blood of the echidna (*Tachyglossus setosus*). *Respir. Physiol.* 3: 143-150.

PARSONS, R.S., ATWOOD, J., GUILER, E.R. & HEDDLE, R.W.L. (1971b). Comparative studies on the blood of monotremes and marsupials. 1. Haematology. *Comp. Biochem. Physiol.* 39B: 203-208.

PARSONS, R.S., GUILER, E.R. & HEDDLE, R.W.L. (1971a). Comparative studies on the blood of monotremes and marsupials. II. Electrolyte organic constituents, proteins, gas analysis and enzymes. *Comp. Biochem. Physiol.* 39B: 209-217.

PARSONS, R.S., HEDDLE, R.W.L., FLUX, W.G. & GUILER, E.R. (1970). Studies on the blood of the Tasmanian devil. *Comp. Biochem. Physiol.* 32: 345-351.

PEARSE, A.M. (1981). Aspects of the biology of *Uropsylla tasmanica* Rothschild (Siphonaptera). MSc. Thesis, University of Tasmania, Hobart.

PELLETIER, J. (1986). Contribution of increasing and decreasing daylength to the photoperiodic control of LH secretion in the Ile-de-France ram. *J. Reprod. Fertil.* 77: 505-512.

PELLETIER, J., GARNIER, D.H., de REVIERS, M.M., TERQUI, M. & ORTAVANT, R. (1982). Seasonal variation in LH and testosterone release in rams of two breeds. *J. Reprod. Fertil.* 64: 341-346.

PELLIS, S.M. & NELSON, J.E. (1984). Some aspects of predatory behaviour of the Quoll, *Dasyurus viverrinus* (Marsupialia: Dasyuridae). *Aust. Mammal.* 7: 5-15.

PERRET, M. (1985). Diurnal variations in plasma testosterone concentrations in the male lesser mouse lemur (*Microcebus murinus*). *J. Reprod. Fertil.* 74: 205-213.

PRESIDENTE, P.J.A. (1978). Diseases in free-ranging marsupials and those held in captivity. *Uni. of Sydney Post. Grad. Comm. in Vet. Sci. Proc. no.* 36: 457-471.

PRESIDENTE, P.J.A. & CORREA, J. (1981). Haematology, plasma electrolytes and serum biochemical values of *Trichosurus vulpecula* (Kerr) (Marsupialia : Phalangeridae). *Aust. J. Zool.* 29: 507-517.

RAMALEY, J.A. (1979). Development of gonadotropin regulation in the prepubertal mammal. *Biol. Reprod.* 20: 1-31.

READ, D.G. (1984). Reproduction and breeding season of *Planigale gilesi* and *P. tenuirostris* (Marsupialia : Dasyuridae). *Aust. Mammal.* 7: 161-173.

RENFREE, M.B. & TYNDALE-BISCOE, C.H. (1973). Intra-uterine development after diapause in the marsupial *Macropus eugenii*. *Dev. Biol.* 32: 28-40.

RODGER, J.C. & HUGHES, R.L. (1973). Studies of the accessory glands of male marsupials. *Aust. J. Zool.* 21: 303-320.

ROSE, R.M., BERNSTEIN, I.S. & GORDON, T.P. (1975). Consequences of social conflict on plasma testosterone levels in Rhesus monkeys. *Psychom. Med.* 37: 50-61.

ROTH, J.A. (1985). Cortisol as mediator of stress-associated immunosuppression in cattle. *In* Animal Stress, ed. G.P. Moberg, pp. 225-243. American Physiological Society, Bethseda: Maryland.

RUSSELL, E.M. (1984). Social behaviour and social organisation of marsupials. *Mammal Rev.* 14: 101-154.

SADLEIR, R.M.F.S. (1969). The ecology of reproduction in wild and domestic mammals. Methuens: London.

SADLEIR, R.M.F.S. & TYNDALE-BISCOE, C.H. (1977). Photoperiod and the termination of embryonic diapause in the marsupial *Macropus eugenii*. *Biol. Reprod.* 16: 605-608.

SANDES, F.P. (1903). The corpus luteum of *Dasyurus viverrinus* with observations on the growth and atrophy of the Graaffian follicle. *Proc. Linn. Soc. N.S.W.* 28: 364-405.

SCHMITT, L.H., BRADLEY, A.J., KEMPER, C.M., KITCHENER, D.J., HUMPHREYS, W.F. & HOW, R.A. (1988). Population biology of the northern native cat, *Dasyurus hallucatus*, in tropical north-western Australia. draft manuscript prepared for *Aust. J. Zool.*

SCOTT, M.P. (1986). The timing and synchrony of seasonal breeding in the marsupial, *Antechinus stuartii* : interaction of environmental and social cues. *J. Mammal.* 67: 551-560.

SEALANDER, J.A. (1964). The influence of body size, season, sex, age and other factors upon some blood parameters in small mammals. *J. Mammal.* 45(4): 598-616.

SELWOOD, L. (1983). Factors influencing pre-natal fertility in the brown marsupial mouse, *Antechinus stuartii*. *J. Reprod. Fertil.* 68: 317-324.

SELYE, H. (1946). The general adaptation syndrome and diseases of adaptation. *J. Clin. Endocrinol. & Metab.* 6: 117-230.

SERNIA, C. (1978). Steroid-binding proteins in the plasma of the echidna, *Tachyglossus aculeatus*, with comparative data for some marsupials and reptiles. *Aust. Zool.* 20: 87-98.

SERNIA, C., BRADLEY, A.J. & McDONALD, I.R. (1979). High affinity binding of adrenocortical and gonadal steroids by plasma proteins of Australian marsupials. *Gen. Comp. Endocrinol.* 38: 496-503.

SERNIA, C., HINDS, L.A. & TYNDALE-BISCOE, C.H. (1980). Progesterone metabolism during embryonic diapause in the tammar wallaby, *Macropus eugenii*. *J. Reprod. Fertil.* 60: 139-147.

SETCHELL, B.P. (1974). Secretions of the testis and epididymis. *J. Reprod. Fertil.* 37: 165-177.

SETCHELL, B.P. (1977). Reproduction in male marsupials. *In* The Biology of Marsupials, eds. B. Stonehouse & D. Gilmore, pp. 411-459. Macmillan: London.

SETCHELL, B.P. & CARRICK, F.N. (1973). Spermatogenesis in some Australian marsupials. *Aust. J. Zool.* 21: 491-499.

SHARMAN, G.B. (1970). Reproductive physiology of marsupials. *Science.* 167: 1221-1228.

SHARMAN, G.B., FRITH, H.J. & CALABY, J.H. (1964). Growth of the pouch young, tooth eruption and age determination in the red kangaroo, *Megaleia rufa*. *C.S.I.R.O. Wildl. Res.* 9: 20-49.

SHARPE, R.M. (1987). Testosterone and spermatogenesis. *J. Endocrinol.* 113: 1-2.

SHAW, G. & RENFREE, M.B. (1986). Uterine and embryonic metabolism after diapause in the tammar wallaby, *Macropus eugenii*. *J. Reprod. Fertil.* 76: 339-347.

SHOREY, C.D. & HUGHES, R.L. (1973). Cyclical changes in the uterine endometrium and peripheral plasma concentrations of progesterone in the marsupial *Trichosurus vulpecula*. *Aust. J. Zool.* 21: 1-19.

SHORT, R.V. (1975). Roles of hormones in sex cycles. *In* Reproduction in Mammals. Bk.3. Hormones in Reproduction, eds. C.R. Austin & R.V. Short, pp. 42-73. Cambridge University Press: London.

SMITH, M.J., BENNETT, J.H. & CHESSON, C.M. (1978). Photoperiod and some other factors affecting reproduction in female *Sminthopsis crassicaudata* (Gould) (Marsupialia : Dasyuridae) in captivity. *Aust. J. Zool.* 26: 449-463.

SMITH, A.J., MONDAIN-MONVAL, M., MOLLER, O.M., SCHOLLER, R. & HANSSON, V. (1985). Seasonal variations of LH, prolactin, androstenedione, testosterone and testicular FSH binding in the male blue fox (*Alopex lagopus*). *J. Reprod. Fertil.* 74: 449-458.

SPEARS, N. & CLARKE, J.R. (1986). Effect of male presence and of photoperiod on the sexual maturation of the field vole (*Microtus agrestis*). *J. Reprod. Fertil.* 78: 231-238.

STATHAM, H.L. (1982). Trappability of *Antechinus stuartii* (Dasyuridae, Marsupialia) at Petroi, northeastern New South Wales. *In* Carnivorous Marsupials, ed. M.Archer,pp. 333-344. Royal Zoological Society of New South Wales: Sydney.

STEWART, F., SUTHERLAND, R.L. & TYNDALE-BISCOE, C.H. (1981). Macropodid marsupial testicular gonadotrophin receptors and their use in assays for marsupial gonadotrophins. *J. Endocrinol.* 89: 213-223.

STEWART, F. & TYNDALE-BISCOE, C.H. (1982). Prolactin and luteinizing hormone receptors in marsupial corpora lutea: relationship to control of luteal function. *J. Endocrinol.* 92: 63-72.

SUTHERLAND, R.L., EVANS, S.M. & TYNDALE-BISCOE, C.H. (1980). Macropodid marsupial luteinizing hormone: validation of assay procedures and changes in concentrations in plasma during the oestrous cycle in the female tammar wallaby (*Macropus eugenii*). *J. Endocrinol.* 86: 1-12.

SUNDQVIST, C., LUKOLA, A. & VALTONEN, M. (1984). Relationship between serum testosterone concentrations and fertility in male mink (*Mustela vison*). *J. Reprod. Fertil.* 70: 409-412.

TAYLOR, J.M. & HORNER, B.E. (1970). Gonadal activity in the marsupial mouse, *Antechinus bellus,* with notes on other species of the genus (Marsupialia : Dasyuridae). *J. Mammal.* 51: 659-668.

TAYLOR, R.J., BRYANT, S.L., PEMBERTON, D & NORTON, T.W. (1985). Mammals of the Upper Henty River region, western Tasmania: effects of disturbance. *Pap. Proc. R. Soc. Tasmania.* 119: 7-14.

TAYLOR, R.J. & ROSE, R.W. (1987). Comparison of growth of pouch young of the Tasmanian bettong, *Bettongia gaimardi,* in captivity and in the wild. *Aust. Wildl. Res.* 14: 257-262.

TEMPLE-SMITH, P.D. (1984). Reproductive structures and strategies in male possums and gliders. *In* Possums and Gliders, eds. A.P. Smith & I.D. Hume, pp. 89-106. Australian Mammal Society: Sydney.

THAN, K.A. & McDONALD, I.R. (1973). Adrenocortical function in the Australian brush-tailed possum *Trichosurus vulpecula* (Kerr). *J. Endocrinol.* 58: 97-109.

TUREK, F.W. & CAMPBELL, C.S. (1979). Photoperiodic regulation of neuroendocrine-gonadal activity. *Biol. Reprod.* 20: 32-50.

TURNER, C.D. & BAGNARA, J.T. (1976). General Endocrinology. 6th ed, W.B. Saunders Company: Philadelphia.

TYNDALE-BISCOE, C.H. (1955). Observations on the reproduction and ecology of the brush tailed possum *Trichosurus vulpecula* (Marsupialia). *Aust. J. Zool.* 3: 162-184.

TYNDALE-BISCOE, C.H. (1980). Photoperiod and the control of seasonal reproduction in marsupials. *In* Proceedings of the V1 International Congress of Endocrinology, eds. I.A. Cumming, J.W. Funder & F.A. O. Mendelsohn, pp.277-232. Australia Academy of Science: Canberra.

TYNDALE-BISCOE, C.H. (1984). Mammals: Marsupials. *In* Marshall's Physiology of Reproduction, 4th edn, vol.1, Chapter 5, ed. G.E. Lamming, pp 386-454. Churchill Livingstone: Edinburgh.

TYNDALE-BISCOE, C.H. & HEARN, J.P. (1981). Pituitary and ovarian factors associated with seasonal quiescence of the tammar wallaby, *Macropus eugenii*. *J. Reprod. Fertil.* 63: 225-230.

TYNDALE-BISCOE, C.H. & HINDS, L.A. (1984). Seasonal patterns of circulating progesterone and prolactin and response to bromocriptine in the female tammar *Macropus eugenii*. *Gen. Comp. Endocrinol.* 53: 58-68.

TYNDALE-BISCOE, C.H., HINDS, L.A., HORN, C.A. & JENKIN, G. (1983). Hormonal changes at oestrus, parturition and post-partum oestrus in the tammar wallaby (*Macropus eugenii*). *J. Endocrinol.* 96: 155-161.

TYNDALE-BISCOE, C.H., HINDS, L.A. & McCONNELL, S.J. (1986). Seasonal breeding in a marsupial: opportunities of a new species for an old problem. *Recent Prog. Horm. Res.* 42: 471-512.

TYNDALE-BISCOE, C.H. & RENFREE, M.B (1987). Reproductive Physiology of Marsupials. Cambridge University Press: London.

ULLMANN, S.L. (1984). Early differentiation of the testis in the native cat, *Dasyurus viverrinus* (Marsupialia). *J. Anat.* 138: 675-688.

VALENTE, A. (1984). The effect of photoperiod on the reproductive cycle of *Antechinomys laniger* (Spenceri form). *Bull. Aust. Mammal. Soc. (Abstract).* 8: 177.

VINSON, G.P., PHILLIPS, J.G., CHESTER-JONES, I & TSANG, W.N. (1971). Functional zonation of adrenocortical tissue in the brushpossum *Trichosurus vulpecula*. *J. Endocrinol.* 49: 131-140.

VINSON, G.P. & RENFREE, M.G. (1975). Biosynthesis and secretion of testosterone by adrenal tissue in the North American possum *Didelphis virginiana* and the effects of tropic stimulation. *Gen. Comp. Endocrinol.* 27: 214-222.

WAITES, G.M.H., SPEIGHT, A.C. & JENKINS, N. (1985). The functional maturation of the Sertoli cell and Leydig cell in the mammalian testis. *J. Reprod. Fertil.* 75: 317-326.

WALKER, M.T. & GEMMELL, R.T. (1983). Plasma concentrations of progesterone, oestradiol-17ß and 13,14-dihydo-15-oxo-prostaglandin F2∞ in the pregnant wallaby (*Macropus rufogriseus rufogriseus*). *J. Endocrinol.* 97: 369-377.

WARD, K.L. & RENFREE, M.B. (1984). Effects of progesterone on parturition in the tammar, *Macropus eugenii*. *J. Reprod. Fertil.* 72: 21-28.

WARING, H., MOIR, R.J. & TYNDALE-BISCOE, C.H. (1966). Comparative physiology of marsupials. *Adv. Comp. Physiol. Biochem.* 2: 237-376.

WEBER, E. (1975). Notes on breeding the eastern native cat at Melbourne Zoo. *In* Breeding endangered species in captivity, ed. R.D. Martin, pp. 183-186. Academic Press: London.

WEINER, C., SCHLECHTER, N. & ZUCKER, I. (1984). Photoperiodic influences on testicular development of deer mice from two different altitudes. *Biol. Reprod.* 30: 507-513.

WEISS, M. & McDONALD, I.R. (1966). Corticosteroid secretion in the Australian phalanger (*Trichosurus vulpecula*.) *Gen. Comp. Endocrinol.* 7: 345-351.

WEISS, M. & McDONALD, I.R. (1967). Corticosteroid secretion in kangaroos (*Macropus canguru major* and *M. (megaleia) rufus*). *J. Endocrinol.* 39: 251-261.

WEISS, M. & RICHARDS, P.G. (1971). Adrenal steroid secretion in the Tasmanian devil (*Sarcophilus harrisii*) and the eastern native cat (*Dasyurus viverrinus)*. A comparison of adrenocortical activity of different Australian marsupials. *J. Endocrinol.* 49: 263-275.

WHITTEN, W.K. (1956). Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *J. Endocrinol.* 13: 399.

WHITTINGTON, R.J. & GRANT, T.R. (1983). Haematology and blood chemistry of the free-living platypus, *Ornithorhynchus anatinus* (Shaw) (Monotremata : Ornithorhynchidae). *Aust. J. Zool.* 31: 475-482.

WILLIAMS, R. and WILLIAMS, A. (1982). The life cycle of *Antechinus swainsonii* (Dasyuridae, Marsupialia). *In* Carnivorous Marsupials, ed. M. Archer, pp. 89-95. Royal Zoological Society of New South Wales: Sydney.

WILSON, B.A. & BOURNE, A.R. (1984). Reproduction in the male dasyurid *Antechinus minimus maritimus* (Marsupialia : Dasyuridae). *Aust. J. Zool.* 32: 311-318.

WINTROBE, M.M., LEE, R.G., BOGGS, D.R., BITHELL, T.C., FOERSTER, J., ATHENS, J.W. & LUKENS, J.N. (1981). Clinical Hematology, 8th ed. Lea & Febiger: Philidelphia.

WOOD, D.H. (1970). An ecological study of *Antechinus stuartii* (Marsupialia) in a south-east Queensland rainforest. *Aust. J. Zool.* 18: 185-207.

WOOLLEY, P. (1966). Reproduction in *Antechinus* species and other dasyurid marsupials. *Symp. Zool. Soc. Lond.* 15: 281-294.

WOOLLEY, P. (1973). Breeding patterns, and the breeding and laboratory maintenance of dasyurid marsupials. *Exp. Anim.* 22: 161-172.

WOOLLEY, P. (1974). The pouch of *Planigale subtilissima* and other dasyurid marsupials. *J. R. Soc. West. Aust.* 57: 11-15.

WOOLLEY, P. (1975). The seminiferous tubules in dasyurid marsupials. *J. Reprod. Fertil.* 45: 255-261.

WOOLLEY, P.A. (1988). Reproduction in the Ningbing antechinus (Marsupialia : Dasyuridae) : field and laboratory observations. *Aust. Wildl. Res.* 15: 149-156.

WOOLLEY, P.A. & AHERN, L.D. (1983). Observations on the ecology and reproduction of *Sminthopsis leucopus* (Marsupialia : Dasyuridae). *Proc. R. Soc. Vict.* 95: 169-180.

WOOLLEY, P.A. & VALENTE, A. (1986). Reproduction in *Sminthopsis longicaudata* (Marsupialia: Dasyuridae): laboratory observations. *Aust. Wildl. Res.* 13: 7-12.

ZAR, J.H. (1984). Biostatistical Analysis. 2nd ed. Prentice-Hall International: London.

APPENDIX A

PREPARATION OF IODINATED TESTOSTERONE, LH AND PROLACTIN

A.1 Preparation of Iodinated Testosterone

Testosterone was iodinated with sodium-^{125} Iodide using chloramine-T and a method similar that described by Nars and Hunter (1973).

A.1.1 Materials

Reagents

1 : 2 mCi Na ^{125}I (20 µl)
2 : 2.22 µg histamine (10 µl $NaHPO_4$ buffer, 0.5 M / L, pH 8.0)
3 : 50 µg chloramine -T (10 µl in H_2O)
4 : 300 µg Na - metabisulphite reducing agent (10 µl in H_2O)
5 : 2.5 mg Testosterone-3-O-Carboxymethyloxime in 50 µl of dioxane
6 : 10 µl tri-n-butylamine in dioxane (1:5 v/v)
7 : 10 µl isobutylchloroformate in dioxane (1:10 v/v)
8 : 50 µl (34.5 µg oxime)
9 : HCL (0.9 ml of 0.1 mol / L)
10: NaOH (0.9 ml, 0.1 mol / L)
11: Na-metabisulphite (1.0 mg) in Na Phosp. buffer (1.0 ml, 0.5 mol / L, pH 7.0)

Buffers, Solutions and preparation.

1 : NaH_2PO_4 buffer (100 ml of buffer at 0.5 mol / L)
7.8 gms NaH_2PO_4 in 100 ml distilled H_2O
2 : Chloramine-T (need 50 µg / 10 µl H_2O)
dissolve 50 mg chloramine-T in 10 ml distilled H_2O (~5mg / ml)
3 : $Na_2S_2O_5$
dissolve 30 mg $Na_2S_2O_5$ in 1 ml distilled H_2O
4 : NaOH (0.9 ml, 0.1 mol / L)
Add 2 ml NaOH to 8 ml distilled H_2O
5 : Sodium metabisulphite pH 7.0
dissolve 10 mg Na metabisulphite in 10 ml sodium buffer
6 : Sodium metabisulphite (30 µg / 10 µl, pH 8.0)
dissolve 30.0 mg Na metabisulphite in 10 ml sodium buffer
7 : Histamine (need 0.222 mg / ml)
dissolve 22.2 mg in 100 ml buffer

8 : HCL
0.9 ml of 0.1 mol / l (if used 1.2 M HCL then 1 pt acid to 11 pts H_2O)
make up to 0.85 ml HCL

9 : Testosterone 3-O-Carboxymethyloxime
needed 2.4 mg but used 2.5 mg (0.0025 g)

10: Thin Layer Chromatography Plates (Art 5554, DC Alufolien)
Kieslgel 60 F254, 25 Folien 20 x 20 cm, thickness 2.0 mm, silicon coated.

A.1.2 Method

1 : T(3-CMO) in 50 µl dioxane
2 : Add 10 µl tributylamine (1:5)
3 : Add 10 µl isobutylchloroformate (1:10 v/v)
Stir 20 minutes at 10°C
4 : Add 3,000 ml (can try extra 0.5 ml) of dioxane
5 : Remove 50 µl and add to iodinated mixture (see below)

Unseparated Iodinated Reaction *

A : Histamine solution 10 µl
B : Add Na ^{125}I (2 m Ci)** 20 µl
C : Add chloramine-T 10 µl
Wait 15 seconds (precise timing critical)
D : Add $Na_2S_2O_5$ 10 µl

---

6 : Add 10 µl NaOH
Leave for 1 hr at 0°C (ice bath) for Coupling Stage
7 : Add 0.9 ml HCL in 1ml toluene
8 : Add NaOH 0.9 ml and 1 ml sodium metabisulphite pH 7.0
9 : Extract for 2 minutes with 0.5 ml toluene
10: Apply the organic phase (top layer) to TLC plates
11: Put plates in 100 ml of solvent (75 ml benzene and 25 ml ethanol)
12: Radioactive band photographed and cut from TLC plate into small pieces and eluted overnight in ethanol (shaking gently).

* Fume Cabinet, Protective Clothing and Caution required at all times.
** This procedure was repeated successfully using 1.5 m Ci Na ^{125}I.

A.2 Preparation of Iodinated LH

Rat LH-I6 was iodinated by the chloramine-T method described by Greenwood *et al.* (1963).

A.2.1 Materials

Buffers

1 : 0.05 M NaP pH 7.4
5.68 g Na_2HPO_4 (anhydrous), 1.56 g $NaH_2PO_4.2H_2O$ in 100 ml distilled H_2O.

2 : 0.2 %, 2 %, 5 % BSA
0.2 g, 2.0 g, 5.0 g BSA in 100 ml distilled water
Bovine serum albumin, bovine fraction V, powder (Sigma Chem. Co.)

Reagents

1 : Dilution of Rat-LH-I6 (obtained from NIADDK-NIH Bethesda, M.D. AFP-6833C) diluted 113 µg LH-I-6 with 1.13 ml PBS (0.05 M pH 7.4) to give 100 mg / ml. Used at 2 µg / 20 µl for iodination.

2 : Chloramine-T (need 2.2 µl C-T in 20 µl or 1 mg / ml)
dilute 75 mg Chloramine-T in 10 ml PBS-BSA then dilute 1:10 or 1 ml into 10 ml PBS-BSA. Variation of this concentration allows control of the final specific activity and is vital for the success of the iodination.

3 : Sodium metabisulphite
60 mg Na meta. in 10 ml PBS-BSA then 100 µl of this in 10 ml PBS-BSA.

4 : Potassium Iodide 1 µg / 1 ml
dilute by (a) 0.1 g KI in 10 ml PBS-BSA (b) 100 µl of (a) in 10 ml PBS-BSA then (c) 100 µl of (b) in 10 ml PBS-BSA to give 100 ng KI / 100 µl

Column G-25

1 : Sephadex G-25 (fine), particle size 20 - 80 µm (obtained from Pharmacia Fine Chemicals)

2 : G-25 (fine) column volume ~10 ml, gel volume ~ 7 ml (packed)

3 : Swell gel overnight in distilled water. Rinse column with decon and clean with distilled water. Set on clamps and flush with distilled water to remove air bubbles.

4 : Degas swollen G-25 under vacuum for ~ 15 min.

5 : Pack column to 3 ml mark (gives column bed 0.7 x 12.5 cm) and rinse with ~ 20 ml 0.2 % BSA. Do not allow column to dry out.

6 : Add 500 µl of 5 % BSA to prime column then run through with PBS. Count the number of drops to give ~ 500 µl volume (usually 17 - 18 drops).

A.2.2 Method

1 : degas G-25 column and prime with ~20 ml 0.2 % BSA (as above).

2 : prepare collection vials (eppendorf) with 100 µl of 5 % BSA

3 : Iodination *

2 µg LH + 20 µl PBS
+ 10 µl 0.05 M NaP
+ 1 mCi ^{125}I
+ 20 µl chloramine-T solution
mix for 30 seconds reaction time (maximum of 45 sec)
+ 500 µl $Na_2S_2O_5$ solution
+ 100 µl KI solution

4 : mix and layer onto column. Elute with PBS and do not allow to dry out.

5 : collect 500 µl fractions in each collection tube.

6 : count 10 µl from each to locate radioactivity. Keep the 2 most reactive fractions (usually fraction 9 and 10). Dilute only 1 reactive fraction with 800 µl PBS and 100 µl 5 % BSA for immediate use and store the second fraction with 200 µl BSA.

* Lead shields, protective clothing and specially designated radioactive area are required.

A.3 Iodination of Prolactin OPRL (NIH-P-S14)

Prolactin NIH-OPRL-14 was iodinated as outlined by Hinds (1983)

A.3.1 Materials

Buffers

1 : 0.05 M NaP pH 7.4 for G-25 column
2.271 g Na_2HPO_4 (anhydrous), 0.624 g $NaH_2PO_4.2H_2O$ in 400 ml dist. water.

2 : 0.05 M NaP + 0.1 % BSA pH 7.4 for G-100 column
2.271 g Na_2HPO_4 (anhydrous), 0.624 g $NaH_2PO_4.2H_2O$, 0.4 g BSA in 400 ml distilled water.

3 : 5 % BSA
0.5 g BSA in 10 ml distilled water.

4 : 0.088 mM H_2O_2 prepare fresh just before reaction.
0.088 mM = 113 µl H_2O_2 in 10 ml dist. water (0.1 M) then 9 µl of this in 10 ml distilled water.

Columns

1 : G-25 column.

Degas G25 fine sephadex (see Appendix A.2) for 15 mins. Pack column and run through with 1 volume of 0.05 M NaP. Condition column with 0.5 ml 5 % BSA and follow with 2 volumes of buffer.

2 : G-100 column.

Degas G100 sephadex. Pack column and run through with 100 ml 0.05 M NaP + 0.1 % BSA (degassed).

The G-25 column is required for the iodination process which involves lactoperoxidase. The enzymatic activity of the lactoperoxidase is not halted during the process and therefore destroys the tracer's binding ability within about 3 days. The G-100 column is used to separate out the lactoperoxidase and produce a purer tracer with a half life of around three weeks.

A.3.2 Method

1 : Prepare eppendorf tubes and count drops as for LH iodination (App. A.2)

2 : One day prior to iodination, pack and equilibrate G-100 column with 100 ml 0.05 M NaP pH 7.4 + 0.1 % BSA. Degas gel and buffer. Turn off overnight.

For G-25 column

5 µg PRL NIH-OPRL-14 (25 µl) + 10 µl 0.5 M Na P pH 7.4
+ 5 µl Na ^{125} I (0.5 mCi) note activity date.
+ 10 µl lactoperoxidase (10 µg in 25 µl)
+ 10 µl 0.088 mM H_2O_2

React for 50 - 60 sec (depending on ^{125} I activity date).

+ 500 µl 0.05 M NaP pH 7.4
+ 100 µl 0.05 M NaP pH 7.4

3 : Load on G-25 column. Collect 0.5 ml fractions into 100 µl 5 % BSA. Count 10 µl of each fraction to locate peak radioactivity.

For G-100 column Separation of ^{125}I-OPRL-14

4 : Pool 3 highest fractions from the G-25 column and load on G100 column. Have column and fraction collector at room temperature and a flow rate of 1 drop every 10 sec. (25 drops / fraction $\simeq$ 1ml).

5 : Collect 350 drops (~14 ml) then 1 ml fractions (15 - 52)

6 : Count 20 µl for 1 min.

7 : Pool highest fractions (eg 39, 40, 41, 42)

8 : Add 500 µl 5 % BSA and store aliquots of 500 µl or 1000 µl at 4 °C.

APPENDIX B

TESTOSTERONE LEVELS AND PLASMA COMPONENTS IN THE TASMANIAN DEVIL, *Sarcophilus harrisii*.

The aim of this work was to compare testosterone levels and plasma components in the closely related Tasmanian devil, to the Eastern quoll. Blood samples were obtained from devils in the wild by a fellow student David Pemberton (Dept. of Zoology, Uni. of Tas.). The samples were collected from an ear vein using a collection method similar to the quoll and plasmas were stored at -20°C until analysed. This testosterone assay was validated using blood samples collected from 4 devils maintained in captivity at the Medical Animal House, University of Tasmania (29.7.86).

B.1 TESTOSTERONE ASSAY AND VALIDATIONS

The assay used to measuring testosterone in the Eastern quoll was validated for the Tasmanian Devil and the standards, buffer, iodinated testosterone, antisera, charcoal and assay procedure have been described in Chapter 3. The status of the 4 captive devils used in assay validations were as follows : devils A and B were juvenile males, devil C was an old female with a reduced pouch and devil D was suckling 4 pouch young aged between 3.5 and 4 months.

B.1.1 Validations

(1) The Need for Extraction

Plasma from devils A and B were stripped of endogenous steroid using the method described in 3.2.2.6.

Five standard curves were prepared (as in section 3.2.4.1) using testosterone standards equilibrated in (a) buffer and extracted prior to assay (b) buffer but not extracted (c) stripped devil plasma and extracted prior to assay (d) stripped devil plasma and not extracted (e) stripped sheep plasma and extracted prior to assay.

The results in Fig.B.1 show that devil plasma required extraction to eliminate cross reactions between the plasma and the antibody which caused an over estimation of the hormone concentration. Devil curves (c) and (d) do not superimposed the 2 buffer curves (a) and (b) at high concentrations of testosterone. The devil curves are aligned with curve (e) which suggests that a standard curve equilibrated in stripped sheep plasma may be substituted in this testosterone assay.

(2) Cold Recovery

A standard curve was prepared using ethanol standards that had been dried and reconstituted in 200 µl of plasma from male devil B. This curve was compared to a standard curve prepared in stripped sheep plasma. Fig. B.2 shows the devil curve is parallel to the sheep standard curve but is displaced to the right. This displacement is possibly due to endogenous testosterone but indicates unlabelled testosterone can compete with endogenous testosterone in the devil plasma.

(3) Extraction Efficiency

The extraction efficiency of devil plasma (devil A) is presented in Table 3.1 in Chapter 3. Although there was a high recovery after a single extraction (greater than 90%), each devil plasma was double extracted to increase effeciency and eliminate the need to apply a correction factor.

(4) Dose Response

Serial dilutions of plasma (devil B) were extracted and duplicates measured on a standard curve. The %B/B0 for each volume produced a curve that was approximately parallel to the standard curve and when converted to pg per ml, closely corresponded to the dose measured (Table B.1).

Table B.1 Conversion of serial dilutions of male devil plasma.

Volume of devil plasma	pg Testosterone per tube	pg Testosterone per ml
50 µl	< Sensitivity	< Sensitivity
100 µl	28.1, 33.0	0.315, 0.370
150 µl	51.0, 67.0	0.383, 0.503
200 µl	99.0, 94.0	0.554, 0.526

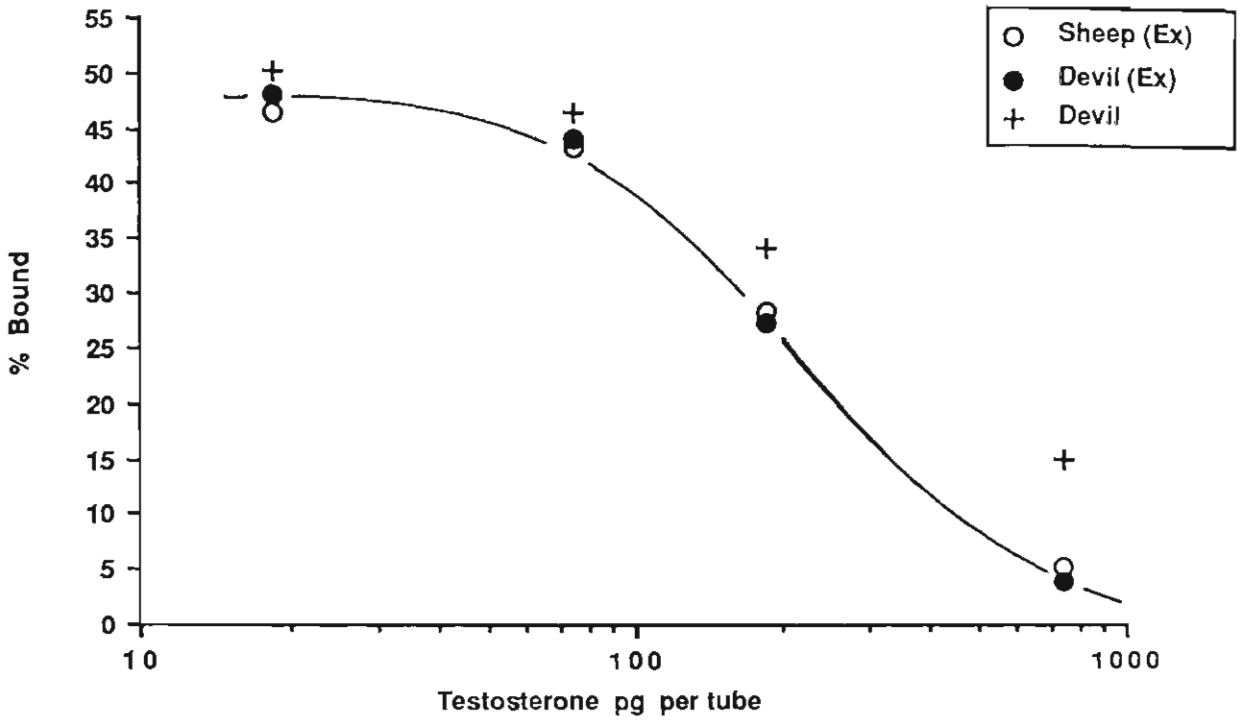

Fig. B.1 Testosterone standard curve using sheep (curve e) and devil plasma (curves c,d). Buffer curve not shown.

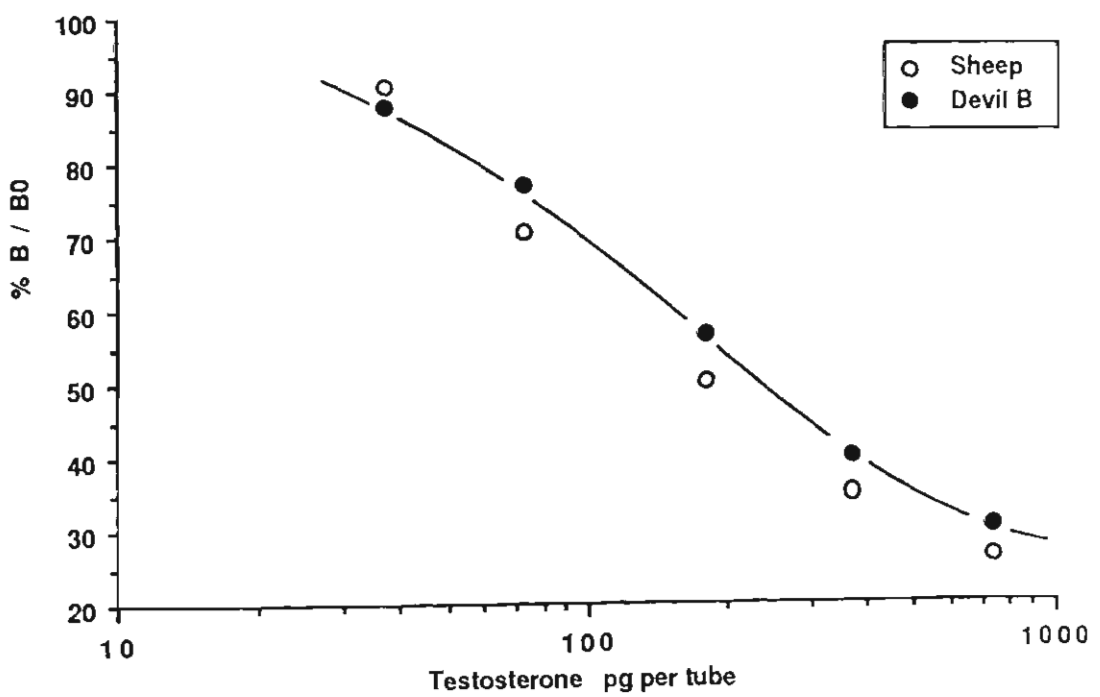

Fig. B.2 Recovery of unlabelled testosterone in sheep and devil plasma.

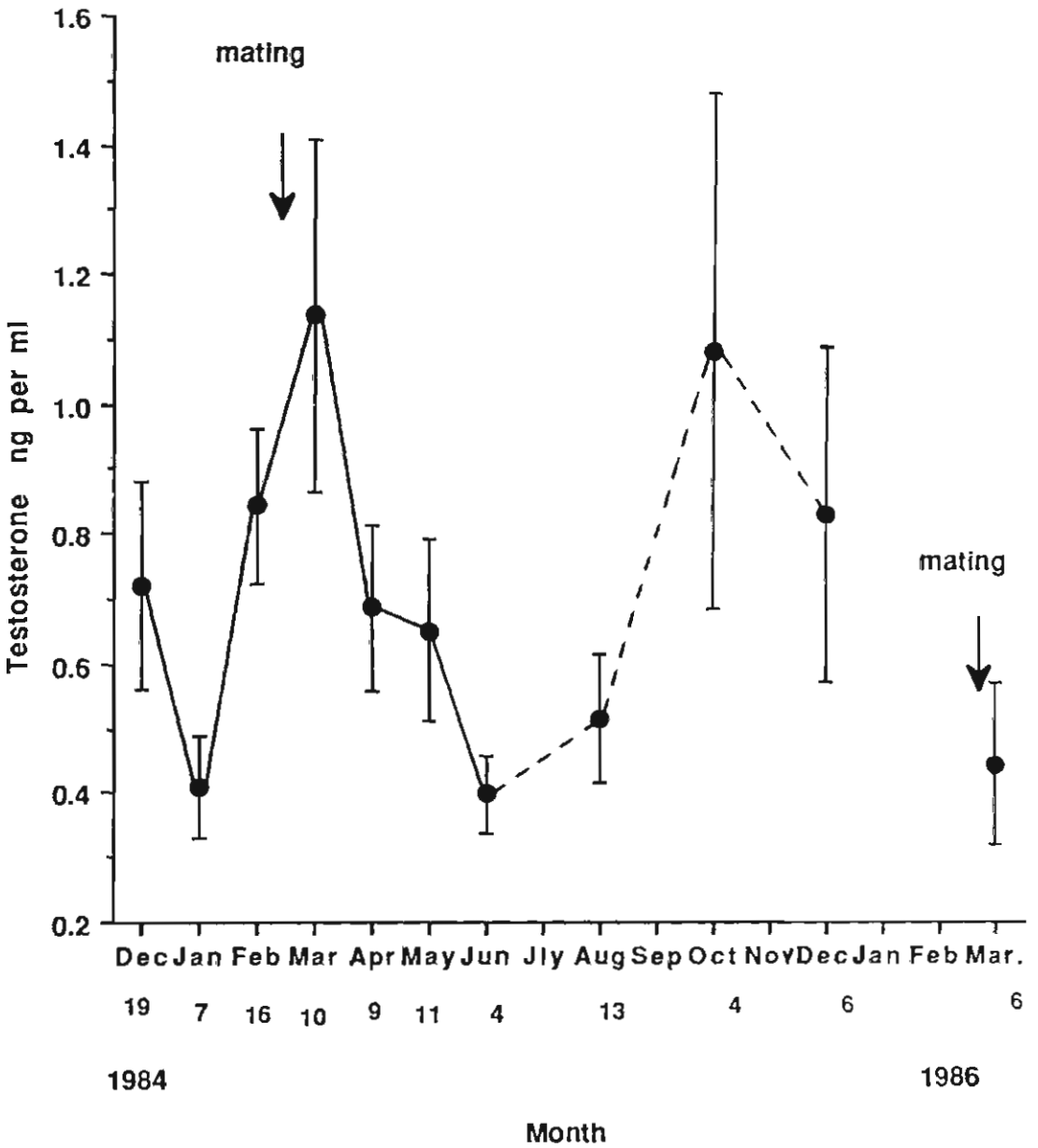

Fig. B.3 Mean (± s.e.) testosterone levels in the male Tasmanian devil, Sarcophilus harrisii. Mating occurred between February and March. Sample size is indicated below each month

B.1.2 Procedure

Devil plasmas were assayed using the same technique as used for the Eastern quoll. Initially aliquots of 200 µl of devil plasma were measured as independent duplicates, however, as this resulted in many males registering below sensitivity, all samples were assayed as 200 µl singletons and double extracted prior to assay.

B.1.3 Results

For most of the year male devils had levels of testosterone that were similar to the basal levels found in the male Eastern quoll. A small seasonal trend was apparent and some elevation occurred near the 1985 breeding season (Fig. B.3). This was well below that reported for the quoll (Bryant 1986) and individual variation was high. Although a variety of reasons may account for this, a number of spare male devil plasmas were kindly re-assayed by Dr. Dan Irby (Anatomy Department, Monash University) using an non-validated assay. His results are included in Table B.2 below. An analysis of corticosteroid levels in the future may indicate whether testosterone is being suppressed or inhibited by stress related activity during the breeding period.

B.2 PLASMA COMPONENTS

Devil plasmas were measured for a number of biochemistry components at the Commonwealth Public Health Department, Hobart. The techniques and tests were the same as those described for the Eastern quoll in Chapter 7. The results are presented in Table B.2

Table B.2 Levels of Testosterone and Plasma Components in the blood of wild caught Tasmanian Devils *Sarcophilis harrisii.*

Table Key:

Testost. : testosterone concentration
Gluc. : plasma glucose
Prot. : plasma protein
Alb. : plasma albumin
Trig. : plasma triglycerides
Chol. : plasma cholesterol.

Date	Devil Code	Testost. ng/ml	Dr Irby ng/ml	Gluc. mmol/l	Prot. g/l	Alb. g/l	Trig. g/l	Chol. mmol/l
16/11/84	R246	0.390	-	5.4	70.9	37.0	1.40	3.1
16/11/84	R246	0.305	-	5.7	70.5	38.3	1.52	3.2
17/11/84	L91	0.830	-	3.3	91.1	38.9	1.40	2.8
17/11/84	L32	0.685	-	2.2	74.8	37.2	1.65	2.9
18/11/84	L47	1.850	-	2.6	78.1	36.3	1.02	2.7
18/11/84	R224	0.340	-	0.8	79.9	38.1	1.10	2.8
21/11/84	R203	0.284	-	6.7	78.6	42.0	1.31	4.4
22/11/84	R24	0.314	-	5.0	78.8	44.1	1.03	3.4
23/11/84	L84	1.800	-	3.1	90.4	36.3	1.35	3.3
23/11/84	R208	1.525	-	4.9	79.5	40.9	1.59	4.9
23/11/84	R37	0.190	-	4.7	95.8	45.4	0.96	3.6
24/11/84	L177a	0.415	0.610	5.3	83.1	40.9	1.25	4.2
24/11/84	R73	0.515	-	5.5	92.0	45.2	1.60	4.0
24/11/84	L177b	0.298	-	5.6	88.9	42.1	1.57	4.6
24/11/84	L32	0.165	-	5.9	84.6	40.3	1.06	4.1
28/11/84	R75	2.500	-	3.7	81.3	43.7	2.46	3.6
29/11/84	L36	0.500	0.390	5.4	87.7	40.2	1.02	3.7
30/11/84	L84	0.398	-	2.1	67.6	31.7	1.66	2.6
30/11/84	R78	0.348	-	6.5	90.2	41.9	1.22	3.6
01/01/85	R236	0.386	-	-	-	-	-	-
02/01/85	R262	0.710	-	5.1	68.3	40.7	2.42	2.8
02/01/85	R264	0.760	-	7.0	67.6	40.1	2.34	2.9
03/01/85	R102	0.380	-	3.8	70.1	37.2	2.17	4.1
03/01/85	L183	0.140	-	4.3	79.9	41.0	1.14	3.5
03/01/85	L36	0.218	-	5.6	76.8	35.8	1.27	3.0
06/01/85	L118	0.285	0.430	5.5	77.2	37.0	1.46	3.7

Date	Devil Code	Testost. ng/ml	Dr Irby ng/ml	Gluc. mmol/l	Prot. g/l	Alb. g/l	Trig. g/l	Chol. mmol/l
Mating during February and March								
04/02/85	R47	0.870	-	-	-	-	-	-
04/02/85	L36	1.200	-	5.1	86.3	44.3	1.57	2.0
04/02/85	R272	1.875	-	6.1	69.3	37.2	0.62	4.4
04/02/85	R275	0.610	-	3.2	73.6	41.5	1.61	2.5
06/02/85	L47	0.395	-	5.7	70.4	33.7	0.91	2.3
07/02/85	L183	0.430	0.580	6.4	71.4	39.1	1.47	3.8
07/02/85	R304	0.565	-	6.0	71.4	38.7	1.24	5.3
09/02/85	R236	1.630	-	5.2	79.5	40.6	1.26	4.2
09/02/85	R47	1.060	-	-	-	-	-	-
10/02/85	R318	0.390	-	6.1	83.9	41.7	1.59	5.1
10/02/85	L36	0.600	-	4.3	62.9	32.7	1.14	2.8
10/02/85	R25	0.570	-	-	-	-	-	-
22/02/85	R82	1.090	-	-	-	-	-	-
23/02/85	R208	0.160	-	3.8	82.1	40.9	1.74	3.5
24/02/85	L84	0.900	-	0.3	75.9	34.5	1.45	2.9
24/02/85	L36	1.175	-	1.0	78.6	39.8	1.26	2.4
06/03/85	R262	0.810	-	-	-	-	-	-
06/03/85	R52	0.940	-	-	-	-	-	-
07/03/85	R345	0.185	-	5.1	58.6	33.5	1.01	2.9
07/03/85	R346	0.750	-	4.2	72.2	43.2	1.24	4.0
07/03/85	R75	2.850	-	2.7	68.1	37.7	2.65	2.4
08/03/85	R275	1.010	-	1.9	71.1	39.8	1.13	2.4
08/03/85	R282	0.790	-	-	-	-	-	-
08/03/85	R285	0.100	0.400	5.0	70.8	40.2	0.87	5.5
09/03/85	R355	-	0.280	-	-	-	-	-
09/03/85	R317	1.780	-	6.0	67.6	38.1	1.22	3.6
13/03/85	R224	2.230	-	4.0	71.8	39.6	1.01	3.4
08/04/85	L183	0.470	0.430	1.8	82.5	40.4	0.96	3.1
08/04/85	R23?	0.935	-	2.7	82.4	42.0	1.51	3.4
08/04/85	L47	0.198	-	1.4	72.3	34.3	1.48	2.0
09/04/85	R47	0.725	-	1.4	80.4	38.3	1.47	3.8
09/04/85	R355	1.390	0.970	2.1	72.3	40.9	1.32	3.4
09/04/85	R224	0.355	-	1.8	74.0	38.5	1.16	3.1
09/04/85	L84	0.665	-	1.6	78.8	34.4	1.48	2.4
20/04/85	R246	1.150	-	-	-	-	-	-
28/04/85	L43	0.345	-	0.1	67.0	33.3	1.91	2.86

Date	Devil Code	Testost. ng/ml	Dr Irby ng/ml	Gluc. mmol/l	Prot. g/l	Alb. g/l	Trig. g/l	Chol. mmol/l
02/05/85	R275	0.380	0.370	3.9	74.1	39.2	1.92	4.2
02/05/85	L144	1.180	-	2.4	86.8	41.8	2.35	1.9
02/05/85	R370	1.710	-	4.6	74.7	39.2	1.57	2.9
02/05/85	R282	0.405	-	1.8	93.8	51.9	1.08	3.0
03/05/85	R246	0.855	-	2.8	76.6	40.0	1.17	3.3
03/05/85	L187	0.600	-	2.5	70.7	36.8	1.25	3.7
03/05/85	R203	0.418	-	1.5	81.5	43.3	0.01	3.1
04/05/85	R260	0.335	-	2.4	77.1	35.0	1.11	2.3
04/05/85	R412	<S	-	2.6	72.4	40.7	1.71	2.9
04/05/85	R282	0.530	-	-	-	-	-	-
06/05/85	R350	0.069	-	1.7	84.2	45.1	1.23	3.6
09/05/85	R224	0.690	0.330	4.6	70.8	37.2	1.49	3.9
09/05/85	R23?	<S	-	4.8	78.6	39.4	1.22	3.7
12/06/85	R52	0.375	-	2.8	76.3	36.4	1.17	2.8
20/06/85	L84	0.450	-	0.3	73.8	34.9	1.48	3.8
29/06/85	R?	0.555	-	0.5	84.9	42.6	1.17	3.9
29/06/85	R368	<S	-	7.1	68.8	39.2	0.81	3.9
30/06/85	R435	0.235	-	-	-	-	-	-
03/08/85	R436	0.281	-	9.3	75.9	38.7	1.17	3.6
04/08/85	R246	0.425	-	4.1	75.9	39.3	1.05	3.5
04/08/85	R438	1.350	0.670	5.9	76.6	39.2	1.08	2.6
04/08/85	R325	0.505	-	4.7	70.4	37.8	1.52	4.0
05/08/85	R451	0.285	-	3.7	70.7	37.0	1.78	4.2
05/08/85	R453	-	-	0.2	75.3	43.6	1.55	2.5
06/08/85	R236	1.250	-	7.0	77.6	38.4	1.20	4.4
07/08/85	R387	0.505	-	2.7	75.6	40.5	1.14	3.3
07/08/85	R457	<S	-	-	-	-	-	-
08/08/85	R374	0.395	-	6.0	76.9	40.1	1.37	2.7
08/08/85	R458	0.100	-	-	-	-	-	-
08/08/85	R275	0.240	-	-	-	-	-	-
09/08/85	R463	<S	-	-	-	-	-	-
09/08/85	R372	0.510	-	4.2	74.2	36.9	1.44	2.9
11/08/85	L123	0.610	-	-	-	-	-	-
12/08/85	R246	0.293	-	5.7	73.5	38.8	1.42	4.2
24/10/85	R354	2.230	-	4.1	78.9	42.2	1.50	4.2
24/10/85	R236	0.340	-	3.6	76.8	39.0	1.31	2.7
26/10/85	R325	0.950	-	1.6	78.3	42.2	1.23	3.8
29/10/85	R405	0.840	-	-	-	-	-	-

Date	Devil Code	Testost. ng/ml	Dr Irby ng/ml	Gluc. mmol/l	Prot. g/l	Alb. g/l	Trig. g/l	Chol. mmol/l
12/12/85	R436	1.800	-	0.3	80.7	44.4	2.30	5.0
12/12/85	R451	1.470	-	-	-	-	-	-
12/12/85	R458	0.735	-	0.5	80.5	41.0	1.51	4.5
14/12/85	R282	0.294	-	-	-	-	-	-
14/12/85	R224	0.373	-	4.7	78.0	39.9	2.88	4.3
14/12/85	R236	0.373	-	-	-	-	-	-
05/03/86	L66	0.300	-	-	-	-	-	-
06/03/86	R487	0.875	-	3.0	69.7	38.8	1.43	4.0
12/03/86	L88	0.100	-	-	-	-	-	-
16/03/86	L177	0.100	-	-	-	-	-	-
17/03/86	R273	0.775	-	0.4	65.4	39.0	1.06	3.3
19/03/86	L123	<S	-	-	-	-	-	-
20/03/86	R354	0.548	-	-	-	-	-	-

APPENDIX C

STAGES OF POUCH DEVELOPMENT AND AGE ESTIMATION

The stages of pouch development in the Eastern quoll are similar to those described by Begg (1981b) for the Northern quoll, *Dasyurus hallucatus*. The following description is based on captive and wild females and includes data from Green (1967).

Stages of pouch development and associated measurements.

(1) Immature: a shallow depression of bare skin (diameter 12 to 16 mm) with 6 exposed small teats (teat height 2 to 4 mm). No discoloration of surrounding fur.

(2) Parous but not currently breeding (January to late March): pouches are formed but shallow with a wide, loose rim of skin. The teats appear dirty and some may still be elongated. Pouches range in diameter from 15 to 25 mm and teats 5 to 12 mm in height. The fur surrounding the pouch is brown-orange in colour.

(3) Entering the breeding condition (April to June):

(a) the pouch begins to deepen and the rim loosens.

(b) the pouch continues to deepen, thicken and widen with a red interior.

(c) 1 to 2 weeks prior to oestrus, the pouch appears fully developed, thick and with a red interior.

(d) during mating the pouch remains deep and thick with a crimson interior. Pouches range in diameter from 19 to 26 mm. The urogenetial sinus becomes red, distended and produces a mucous discharge. Teats are small. Visual signs of copulation include matting of the hair on the neck and back, bite and scratch marks along the neck and down the back.

(4) Prior to birth (late June): the pouch remains fully developed after copulation but the interior changes from crimson to red in colour. One to two days prior to birth the pouch interior becomes very moist and small glands are

visible beneath the surface of the skin.

(5) Birth (early July): on the day of birth the interior of the pouch is glandular, very moist and has a noticeably raised temperature.

(6) Early lactation (July to August): during the first two months of lactation pouches increased in size to accomodate and tightly envelop the young. Mammary glands were visible but not swollen. Green (1967) recorded a teat length of 2 mm with a tip of 0.5 mm in the mouth of a newborn young and a teat length of 8 mm and diameter of 2 mm with a young approximately 1 month old attached.

(7) Full lactation (August to late November): pouches gradually increased in size (diameter 30 to 40 mm, depth 25 mm, Green 1967). Individual mammary glands ranged in diameter from 27 to 34 mm and protruded from the pouch (Plate 4a). Some teats were elongated with lengths between 6 and 10 mm and, when massaged, would readily secrete milk. Non-lactating glands appeared flaccid. This period also coincided with the time when the young were no longer permanently attached to the teat.

(8) Weaning (December to February): pouch and mammary glands gradually decline in size and fullness. Pouch diameters were about 24 to 30 mm and lactating mammary glands were 20 mm or less. Teats remained elongated (6 to 10 mm in length) and occasionally were observed protruding from the contracted entrance of the pouch. By March, pouches had returned to stage (2) in appearance.

Pouch Development in Non-Lactating Females

Females not observed with pouch young or signs of a lactating pouch also underwent a period of pouch development. Two wild and one captive female appeared in this condition and all were classified as old aged on the basis of body weight, tooth wear and date of first capture. The following stages of pouch development were observed in these females:

December until March : stage (2)
April : stage (3a), pouch diameter ~13 mm.
May : stage (3b), pouch diameter ~11 mm.
July : stage (3c), other females with ~1 week old pouch young.
August : stage (3d) pouch.
September : stage (3c) pouch.
October to January : stage (3b) pouch.

Figure C.1 shows the age estimation curves from birth to pouch vacation using a number of body parameters. The measurements were based on the criteria outlined in Sharman *et al.* (1964).

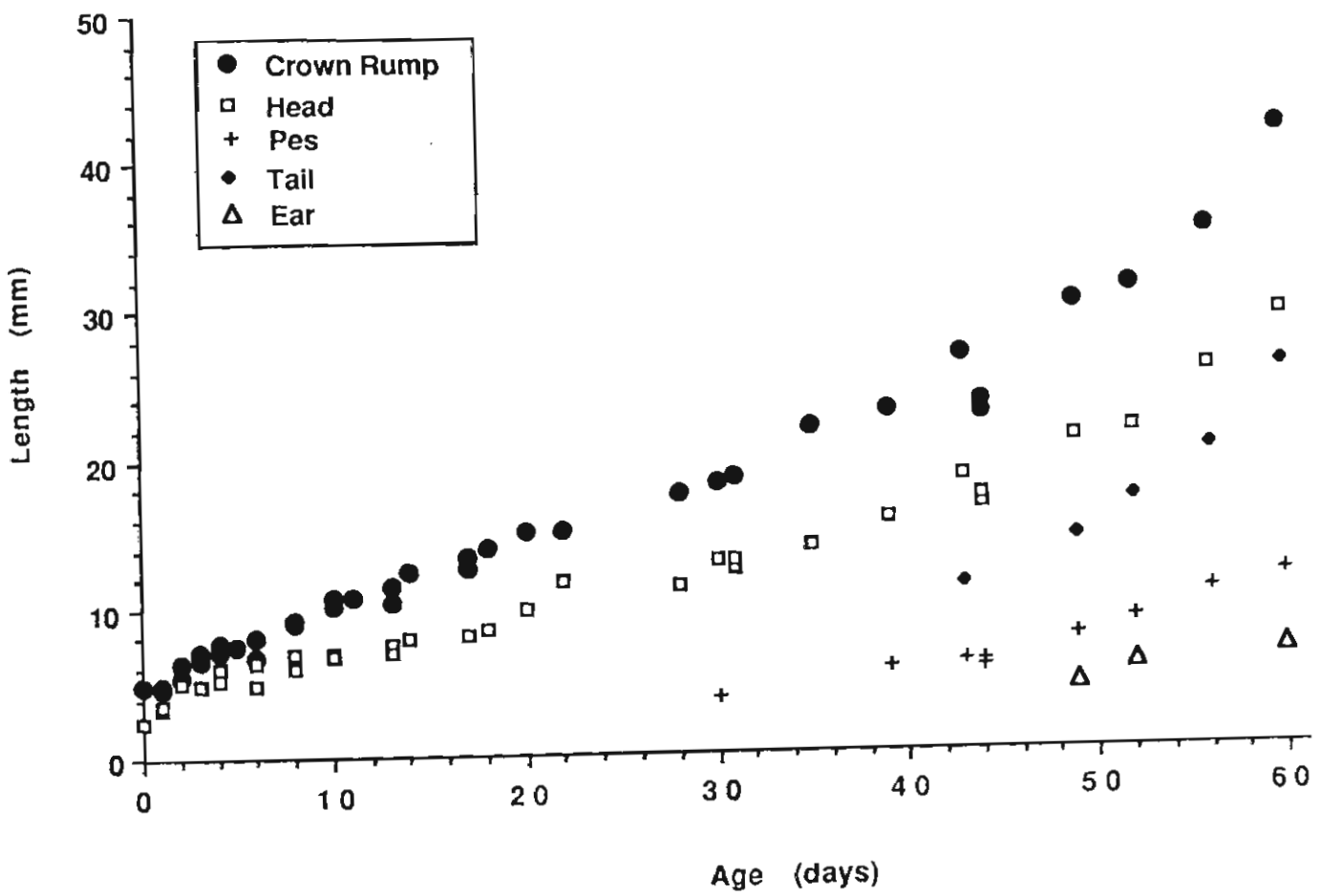

Fig. C.1 Body measurements for aging quoll pouch young up to 60 days. (measures from 7 litters)

APPENDIX D

REGIONAL VARIATION IN THE TIMING OF BREEDING

A comparison was made between the timing of breeding in populations from the north (Ringarooma) and south (Cradoc) of Tasmania. The latitude of Ringarooma is 42°30' and Cradoc is 22°30' with a linear distance between of approximately 250 kilometers. Table D.1 shows the variation in body weight, scrotal size and hormonal levels between animals at the two sites.

Table D.1 Mean (+ s.e.) variation in parameters of males trapped at two locations.

Parameter	Body weight km	Scrotal width m m	Scrotal height mm	Testosterone ng / ml	LH ng / ml
Cradoc 5.3.84	1.34 ± 0.3 n = 7	29.3 ± 2.1 n = 7	22.9± 2.2 n = 7	0.94± 0.4 n = 7	1.05 ± 0.9 n = 6
Ringarooma 19.3.84	1.21 ± 0.2 n = 10	28.9 ± 1.5 n = 10	22.3± 2.1 n = 10	0.89± 0.3 n =10	0.86 ±0.6 n = 9
Cradoc 2.4.84	1.35± 0.2 n = 11	30.0 ± 0.8 n = 11	23.8 ± 2.1 n = 11	1.63 ± 0.3 n = 11	1.54± 0.5 n = 9
Ringarooma 29.5.84	1.16 ± 0.2 n = 9	28.6 ± 1.8 n = 9	22.8± 2.7 n = 9	1.98 ± 0.7 n = 9	3.62± 1.9 n = 8
Cradoc 5.6.84	1.32 ± 0.2 n = 16	28.3 ± 1.9 n = 16	21.7 ± 1.7 n = 16	5.00 ± 1.3 n = 15	13.0 ± 6.4 n = 11

A sample collected prior to March from Ringarooma was necessary to determine the phase of the weight and scrotal cycles. The Ringarooma males showed a loss in weight between March and May. The most obvious difference between the two sites was in the LH and testosterone levels. By mid- March, the Ringarooma males had basal levels of LH and testosterone compared to rising levels at Cradoc. When animals were mating at Cradoc, males in the north had LH and testosterone levels equivalent to those which occurred at Cradoc one month previously. There appeared to be a difference of approximately three to four weeks in the hormonal levels and body cycles of males between the two locations.

Female quoll collected from Ringarooma and returned to the laboratory mated and gave birth three weeks after females that had been maintained in captivity. This difference in the timing of mating also supports the suggestion that there is about a three week lag in the onset of breeding between animals in the north and south of Tasmania.

APPENDIX E

TESTOSTERONE - LH REGULATION & DIURNAL CYCLES

E.1 LH - TESTOSTERONE REGULATION

E.1.1 Aim

This experiment was conducted to test whether a negative feedback mechanism regulated the secretion of LH and testosterone in the male quoll.

E.1.2 Procedure

The testes were removed from 6 mature male quoll. Three males were castrated in April, prior to the breeding season and 3 were castrated during the mating period in June. Castration involved complete removal of the scrotal sac and suturing the 2 spermatic cords. The animals were fully sedated and the details of anaesthetics are provided in Chapter 2.

Males were caged individually in the laboratory for the first 14 days after castration. They were then re-housed in larger concrete pens or released into an outside enclosure. A blood sample was collected prior to surgery and then daily for 14 days after castration. Blood samples continued to be collected for the next 60 days and then periodically for up to 2 years. Two assays were required to measure the testosterone samples and 1 needed for LH.

E.1.3 Results

Endocrine results from the 6 castrated males demonstrated that removal of the testes enhanced LH secretion. In the April males, a decline in testosterone and a rise in LH occurred within 2 days of castration (Fig. E.1). LH levels increased from 1.5 ng per ml before the operation to between 11.0 and 17.0 ng per ml, 2 days after castration. Maximal levels of around 80.0 ng LH per ml were reached near day 10 although 1 animal recorded a LH level of 122.4 ng per ml on day 7 after castration. From 14 (to 400) days after castration, LH concentrations fluctuated between 5.0 and 35.0 ng per ml. Testosterone levels were near or below the sensitivity of the assay within 4 days of castration and remained at this level throughout the experiment.

The 3 males castrated in June also showed a decline in testosterone

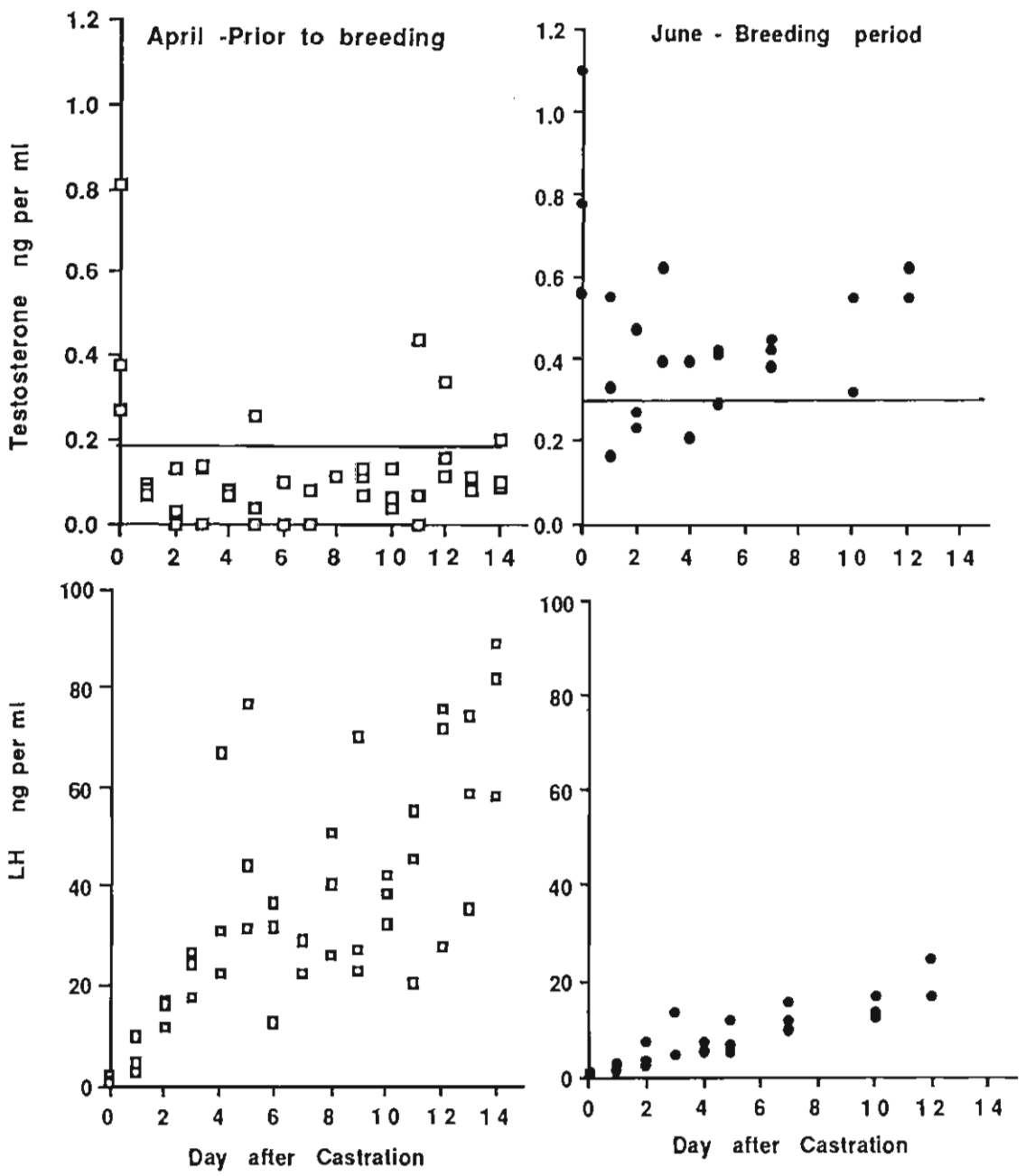

Fig. E.1 LH and testosterone levels in males castrated prior to breeding (n=3) or during the breeding season (n=3). The line indicates the sensitivity of the testosterone assay.

and rise in LH concentration immediately following castration. Although LH increased steadily, by day 10 levels were below 20.0 ng per ml which compared to 20.0 to 80.0 ng per ml in April males at the same time. Testosterone concentrations fluctuated around 0.40 ng per ml for up to 60 days after castration. These levels were similar to basal concentrations of intact males.

Linear regressions were fitted to the data points from zero to 14 days after castration. The regression of the line for testosterone in April males produced a negative slope (testost.= -0.18 x day after cast. + 0.29, r^2=0.14) but a near horizontal slope in June (testost.= 0.09 x day after cast. + 0.49, r^2=0.06). The regression lines for LH were similar to each other but the slope was much steeper with a higher intercept in April (LH= 3.70 x day after cast. + 11.44, r^2=0.37) than in June (LH= 2.54 x day after cast. + -2.64, r^2=0.62).

None of the six males gained excessive weight after castration other than that which could be accounted for by seasonal weight changes. Two animals sacrificed approximately two years after castration had prostate glands weighing 0.34 grams and 0.61 grams. These contrast to prostate weights ranging from 1.30 to 3.70 grams in intact males of similar body weight at the same time of the year (Fletcher 1977).

E.1.4 Discussion

An inverse relationship occurs between the secretion of LH and testosterone in the Eastern quoll. This result supports the hypothesis that a negative feedback mechanism exists between the gonads and the pituitary gland in male marsupials and eutherian mammals (Hearn 1975; Lincoln 1978; Catling & Sutherland 1980; Stewart *et al.* 1981; Inns 1982; Irby *et al.* 1984; deKretser 1984). The feedback mechanism is probably similar to that found in female marsupials between the ovary and pituitary LH and FSH release (Evans *et al.* 1980). The observations of greatly reduced prostate glands in two castrate males agrees with other workers who state the prostate gland is under androgen control (Cook *et al.* 1978; Inns 1982; Fletcher 1983; Wilson & Bourne 1984; Gemmell *et al.* 1986; McFarlane *et al.* 1986a).

In the quoll, testosterone concentrations declined within 2 days of castration and LH levels were maximal after day 10. This pattern is similar to the tammar wallaby, *M. eugenii,* and kowari, *Dasyuroides byrnei,* where testosterone levels were undetectable 2 to 5 days after gonadectomy and LH

concentrations were maximal after 10 to 14 days (Catling & Sutherland 1980; Fletcher 1983). The quoll has a higher range of LH levels compared to the tammar (20 to 90 ng per ml compared to 4 to 8 ng per ml) but both species have considerably lower ranges of LH than the kowari (350 to 500 ng per ml). The blue fox, *Alopex lagopus,* produces LH levels fluctuating from 10 to 100 ng per ml for up to one year after castration (Smith *et al.* 1985) which demonstrates considerable species variation in LH secretion.

Quolls castrated in June continued to produce testosterone levels around 0.40 ng per ml after castration. These were above the sensitivity of the assay and similar to the basal levels found in intact males. It is possible that the testosterone in these animals was being produced at another site, most likely the adrenal cortex. The adrenal gland is a source of androgenic steroids in other marsupials (Vinson *et al.* 1971; Vinson & Renfree 1975; Gemmell 1979; Allen and Bradshaw 1980) and stimulation of adrenal testosterone secretion may be a long term consequence of castration. Other animals can produce an elevation in testosterone after castration with levels in long term castrate males being similar to intact females (Allen cited by McDonald & Waring 1979; McDonald & Taitt 1982). The mammalian adrenal gland is known to secrete a wide range of steroids (Gower 1979) all of which cross react with the testosterone antibody in the RIA (Chapter 3). Any of these factors may have assisted in inflating testosterone levels.

Two assays were required to measure testosterone levels in this experiment therefore inter- assay varation combined with a natural episodic secretory pattern, may have accounted for some of the variation between groups. However, as the LH samples were measured in only one assay, the differences between the groups in LH cannot be due to assay variation. It may be that the anticipation of the breeding season and or castration itself, has exerted some effect on the LH-RH response and the adrenal gland. This suggestion would need to be substantiated by a more detailed investigation.

E.2 DIURNAL CYCLES

E.2.1 Aim

This work examined whether changes in hormone concentration could be accounted for, at least in part, by an inherent diurnal secretory pattern.

Samples were collected at different times of the year to test whether proximity to the breeding season influenced the intensity of secretion.

E.2.2 Procedure

Blood samples were collected from 6 adult males over a 24 hour period. Three males (nos. 80, 102, 101) were examined during September with blood samples collected every 4 hours for 24 hours. Three males (nos. 210, 214, 216) were examined during the breeding period in July and were sampled every 3 hours for 24 hours.

Animals were restrained in sacks during the daytime sampling to minimise interference from capture and handling. During the night they were released in their cages and observed under red light. Every animal had been handled in captivity for some weeks prior to the start of this experiment.

The blood samples were centrifuged and the plasma stored at -20°C until assayed for LH and testosterone.

E.2.3 Results

The secretion of LH and testosterone fluctuated in a cyclic manner over a 24 hour period (Fig. 6.2). Males in July produced testosterone levels ranging from 0 (or below sensitivity of the assay) to 1.0 ng per ml. LH levels ranged from 0 to 2.2 ng per ml. During September, males had testosterone concentrations also ranging from 0 to 1.0 ng per ml and LH from 0 to 1.5 ng per ml. There was no significant variation between the testosterone or LH levels produced in either period (ANOVA, LH, $F_{[1,12]}=1.81$, $0.1<p<0.25$; testosterone, $F_{[1,12]}=1.31$, $p>0.25$).

There were no significant variation between the concentrations of testosterone secreted during the day or night hours (7 am to 3 pm in September, 8 am to 5 pm in July) (ANOVA, day, $F_{[1,5]}=0.28$, $p>0.25$; night, $F_{[1,5]}=1.20$, $p>0.25$) or between LH secretion during the day or night (day, $F_{[1,5]}=1.78$, $0.1<p<0.25$; night, $F_{[1,5]}=0.29$, $p>0.25$). Animal no. 216 was not included in these calculations because samples could not be obtained during the night hours.

E.2.4 Discussion

It appears that at least two peaks in testosterone and LH occur over a 24 hour period in the male quoll. The hormone releases could be in the form of

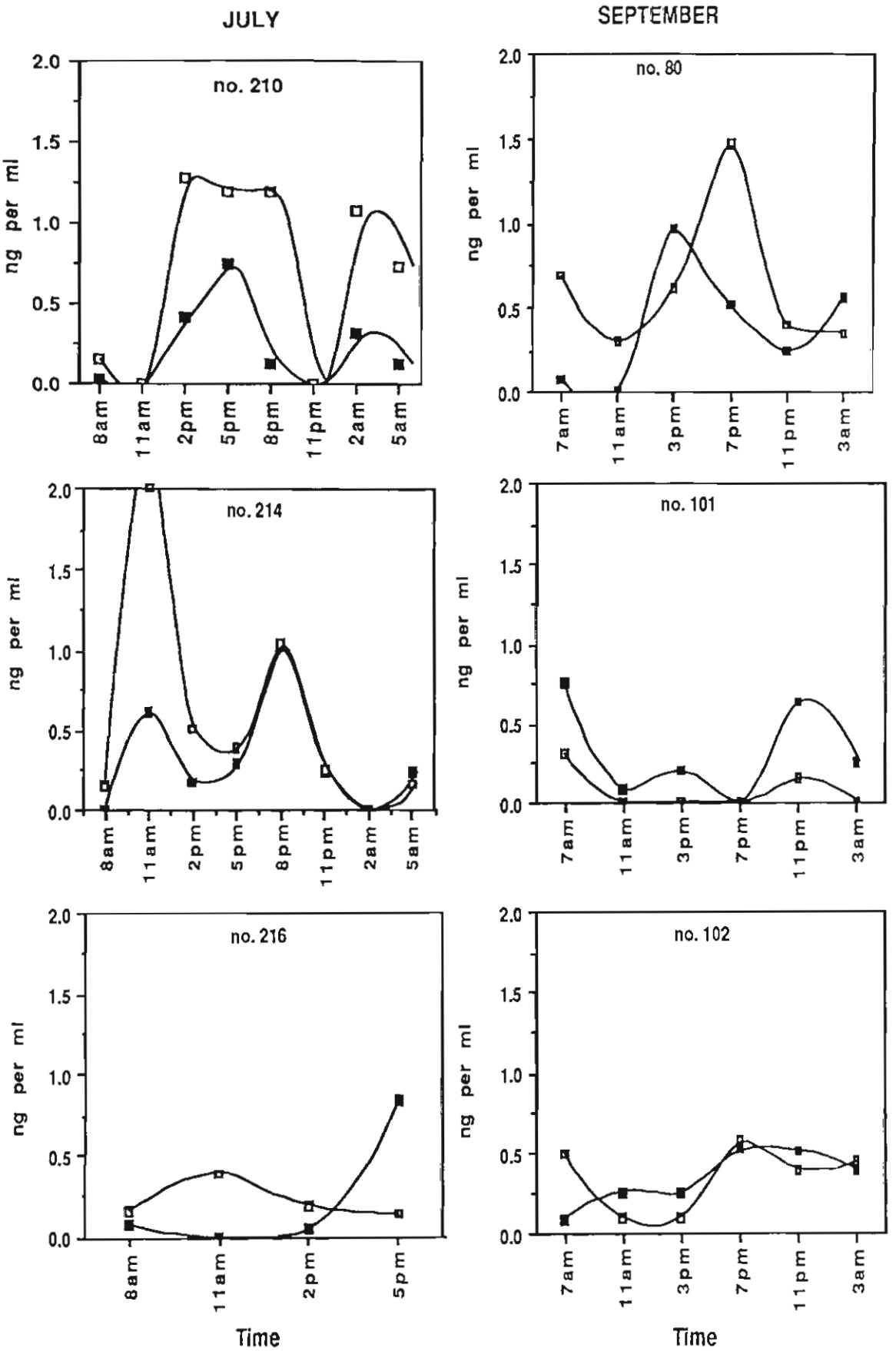

Fig. E.2 Fluctuations in LH [] and testosterone ▮ over 24 hours in 3 individual male quoll in July and 3 in September.

diurnal cycles, rather than surges or pulses, but the sampling interval and small number of animals made this difficult to confirm. In a later experiment (Chapter 7) where males were injected with synthetic ACTH and sampled at more frequent intervals, testosterone secretion showed an episodic fluctuation.

Diurnal variation in the release of testosterone and LH has been shown to occur in many mammals, including man (Short 1975; Gower 1979; Lincoln 1985). In the Soay ram for example, episodic surges in LH and testosterone show transitory peaks approximately every 2 hours (Lincoln & Short 1980). This pattern is similar to the bandicoot, *Isoodon macrourus,* which releases a range of hormones in an episodic fashion (McFarlane *et al.* 1986b). The breeding koala, *Phascolarctos cinereus,* can produce spontaneous pulses of plasma testosterone ranging from 0.5 to 8.0 ng per ml over two to three hourly periods (McDonald 1986). 5α-dihydrotestosterone and testosterone vary diurnally in the nocturnal brushtail possum,*Trichosurus vulpecula,* with significantly higher peaks occurring in early morning than evening (Allen & Bradshaw 1980; McFarlane & Carrick 1987). This is similar to the lesser mouse lemur, *Microcebus murinus,* where the highest levels of testosterone occur in the day during the period of least activity day (Perret 1985).

In some mammals, testosterone and LH release vary over the year; the peaks per unit time increasing in frequency and magnitude during the breeding season (Lincoln & Short 1980; Clarke 1981; Ortavant *et al.* 1982; Pelletier *et al.* 1982; Lincoln 1985; Perret 1985). In the bandicoot, testosterone ranges between 2.0 to 10.0 ng per ml in the breeding season compared to 0 to 2.0 ng per ml out of breeding (Gemmell *et al.* 1985; McFarlane *et al.* 1986b). The brushtail possum also exhibits low amplitude cycles of androgens when sampled outside the breeding season (Curlewis & Stone 1985a) and *Microcebus murinus* produces significant daily changes in plasma testosterone levels throughout the year except during the sexual rest period in Autumn (Perret 1985). In the present experiment, seasonal differences in LH or testosterone secretion were not detected. It could be males in July had already reached basal levels of LH and testosterone which in turn may have reduced the amplitude of the cycles.

Although some marsupials exhibit marked changes in peripheral androgen concentration over a 24 hour period, other species show a progressive decline in androgens over the sampling period (Lincoln 1978; Curlewis & Stone 1985a; Gemmell *et al.* 1987). Workers have attributed this decline to the stress of

sampling and experimental procedures and caution that this effect should always be a major consideration when analysing diurnal secretory patterns. In this and a study in Chapter 7, serial blood sampling did not cause a decrease in testosterone levels. It seems unlikely therefore that the frequency and technique of blood collection in this study imposed significant stress factors on the quoll.