

The influence of gender on thermoregulation in pouched mice,

Saccostomus campestris

Submitted in fulfilment of
the requirements for the degree of
Master of Science
University of Natal
December 2000

Nomakwezi Mzilikazi
School of Botany and Zoology
University of Natal
Private Bag X 01
Scottsville
3209
South Africa

PREFACE

The experimental work described in this dissertation was carried out in the School of Botany and Zoology, University of Natal, Pietermaritzburg, from February 1999 to November 2000, under the supervision of Dr Barry G. Lovegrove.

These studies present original work by the author and have not been submitted in any form for any degree or diploma to any other University. Where use has been made of the work of others it is duly acknowledged in the text.

All procedures used in this study complied with the “Principles of animals care”, publication no.86-23, revised 1986 (National Institute of Health) and the “Code of ethics for animal experimentation” manual adopted by the University of Natal.

A handwritten signature in blue ink, appearing to read 'NMz', is written above a horizontal dotted line.

Nomakwezi Mzilikazi

Pietermaritzburg

December 2000

ABSTRACT

Saccostomus campestris display sexual disparity in the use of summer daily torpor in response to energy stress. The hypothesis that males may compensate for a limited heterothermic capacity with lower normothermic body temperatures by maintaining lower resting metabolic rates relative to females was tested. Furthermore, the influence of testosterone on torpor incidence in males was investigated.

Body temperature (T_b) and oxygen consumption (VO_2) were measured at various ambient temperatures (T_a) and were compared between the sexes under food ad libitum and food restriction treatments. There were no significant differences in T_b and VO_2 between sexes under food ad libitum treatment. Under food restriction there were pronounced sex differences in the employment of heterothermy. Females defended a lower setpoint T_b for torpor (*ca.* 25°C), than males (*ca.* 29°C), and also employed torpor more frequently than males. Non-torpid males did, however show slight reductions in VO_2 under food restriction.

The effect of testosterone on daily torpor was investigated by comparing minimum T_b and torpor frequency of castrated mice implanted with testosterone-filled (experimental) and saline-filled (control) silastic capsules in response to food ad libitum and food restriction treatments. Testosterone inhibited torpor in males. The majority of control animals employed torpor under both food ad libitum and food restriction diets.

It was concluded that although the animals were capable of shallow, summer torpor, it was confined to moderate ambient temperatures and was not used at low T_a 's where several animals became pathologically hypothermic. Females derive energetic benefits from the use of torpor whereas males may partially compensate for their limited

heterothermic capacity by a reduction in resting metabolic rates, accompanied by moderate reductions in body temperature during energetically stressful periods. The difference in the capacity for daily heterothermy between sexes was attributed to differences in their reproductive physiology.

ACKNOWLEDGEMENTS

Special thanks go to Dr Barry Gordon Lovegrove for his supervision. Dr Colleen Downs co-supervised the study. The study was partly funded by a core-rolling grant from the National Research Foundation (NRF) to BGL. My sincere thanks to Dr Lovegrove and the NRF who generously paid for me to attend the 11th International Hibernation Symposium held in Jungholz, Austria, in August 2000.

Several people assisted in different ways during the course of my studies and to all I extend my sincere thanks and gratitude. The KwaZulu-Natal Nature Conservation Services granted us permission to trap study animals and the staff at the Coastal Forest Reserve, South of Kozi Bay assisted with trapping and kindly provided accommodation. To Barry, thanks for making my “real camping” experience most enjoyable. Jaishree Raman, Khanyi Mbatha and Ms Heather Dempster fed my animals during my absences from the School. Dr Colleen Downs, Mark Brown and Prof. Mike Perrin assisted with cardiac puncture. Andrew McKechnie helped with animal trapping, surgery and feeding. Dr Gerhard Körtner patiently explained the working of the respirometry equipment and also helped with surgery. Prof. Stephan Steinlechner kindly supplied the testosterone, silastic tubing and advice on how to prepare the capsules. Pokazi Tetyana, Khanyi Mbatha, Mark Brown, Jaishree Raman, Megan McMaster, Michelle Tilley, Laura Forrest and Catherine Ampoogonian all provided small and big favors during the course of the study. Various staff members of the Risk Management Services, University of Natal, walked me home after working late in the department. Finally, I would like to thank my parents for their support and encouragement.

Thesis structure

Chapter 1 provides a general introduction and outlines the major paradox investigated in this study, that is, the conflict between the use of summer torpor and the maintenance of reproductive activity in male *S. campestris*. It also gives a detailed description of the study species and the site from which the study animals were collected. The rationale for using F1-generation laboratory bred animals in this study as well as the breeding technique used is presented. The maintenance conditions are also described. The objectives of the study are also outlined in Chapter 1. Chapters 2 and 3 are written as individual papers. In Chapter 2 the hypothesis that males may compensate for a limited heterothermic capacity with lower normothermic body temperatures resulting from maintenance of lower resting and basal metabolic rates relative to females is tested. Furthermore, daily torpor parameters are compared between sexes. In Chapter 3, the hormonal influence of testosterone on daily torpor is investigated. Chapter 4 outlines the general conclusions reached in this study.

Throughout the text the terms daily torpor and hibernation are used as defined by Geiser and Ruf (1995). Daily torpor refers to the proximate physiological response whereby an animal decreases minimum oxygen consumption (VO_2) and body temperature (T_b) below normothermic levels for periods not exceeding 24 hours. Hibernation refers to a state where this response lasts for more than 24 hours. In addition, the mean minimum T_b is lower in hibernators than in daily heterotherms (5.8°C vs. 17.4°C), as is mean metabolic reduction (5.1 vs. 29.5% of BMR).

Table of contents

Preface.....	(i)
Abstract.....	(ii)
Acknowledgements.....	(iv)
Thesis structure.....	(v)
Chapter 1. General introduction.....	1
Study animals and breeding programme.....	4
Literature cited.....	5
Chapter 2. Sex differences in resting metabolic rates and patterns of summer torpor in pouched mice, <i>Saccostomus campestris</i>	11
Introduction.....	11
Materials and methods.....	12
Study animals and general maintenance.....	12
Experimental procedures.....	13
Measurement of T_b	13
Metabolic measurements.....	14
Experimental protocol.....	15
Data and statistical analysis.....	15
Results.....	17
Body mass.....	17
Torpor frequency.....	18
Minimum body temperature.....	18
Metabolic rates.....	20
Torpor bout length.....	22
Discussion.....	24
Summary.....	27
Literature cited.....	27

Chapter 3. The influence of testosterone on torpor in male pouched mice, <i>Saccostomus campestris</i>	32
Introduction.....	32
Materials and methods.....	34
Study animals and general maintenance.....	34
Experimental procedures.....	34
Gonadectomy.....	35
Preparation and implantation of silastic capsules.....	35
Blood samples and hormone analysis.....	35
Measurement of T_b	36
Experimental protocol.....	37
Data and statistical analysis.....	37
Results.....	39
Body mass.....	39
Torpor frequency.....	40
Blood testosterone and exhibition of daily torpor.....	42
Daily minimum T_b	44
Torpor bout duration.....	46
Discussion.....	47
Summary.....	50
Literature cited.....	51
Chapter 4. Conclusions.....	57

Chapter One

Introduction

Hibernation and daily torpor are widely employed by many mammals and birds in response to energy stress (Lyman et al. 1982; Geiser and Ruf 1995). To date, the majority of laboratory studies have measured the energetics of torpor in winter-acclimated Nearctic and Palaeartic small mammals and birds (Lyman et al. 1982). In these species, torpor is usually associated with predictable cold and low food availability during winter. Moreover, torpor patterns are strongly influenced by photoperiod and hence season (Heldmaier and Steinlechner 1981).

Because both cold exposure and food shortage occur predominantly during winter, it is perhaps not surprising that the use of daily torpor and hibernation is usually associated with cold (Geiser and Baudinette 1987). However it is questionable whether the few non-Holarctic species that hibernate do so in response to cold alone. For example, whereas species such as the marsupial mountain pygmy possum clearly hibernate seasonally in response to predictable cold and snow (Körtner et al. 1998; Körtner and Geiser 1998), Nicol and Andersen (1996) have questioned whether hibernation in the echidna is an adaptation to cold. They proposed that hibernation is used in anticipation of food shortage, and hence may represent an adaptation to the energy-poor and climatically unpredictable Australasian region.

Many species from tropical and subtropical areas also enter spontaneous and induced torpor, both under winter and summer conditions (Geiser and Baudinette 1985; Geiser and Baudinette 1987; Ellison and Skinner 1992; Audet and Thomas 1996; Webb and Skinner 1996; Lovegrove and Raman 1998). Daily torpor in the marsupial *Sminthopsis macroura* has been observed both during winter and summer, at ambient temperatures as high as 25°C when the

animals were food restricted (Geiser and Baudinette 1985; Geiser and Baudinette 1987). Similarly, tropical mouse lemurs, (*Microcebus* spp.) employ torpor at moderate ambient temperatures, suggesting that daily torpor may be used to balance energy requirements when food and water availability is poor, even in species that may never experience extreme cold (Ortmann et al. 1996). In the majority of studies, the use of summer torpor has been linked to unpredictable food availability, suggesting that torpor may be used for energy conservation by species experiencing periodic food shortages even in warm climates (Coburn and Geiser 1998).

Although daily torpor has been observed in mammals from five geographic zones (no data for Indomalaya), Lovegrove (2000) reported that the occurrence of summer torpor is most common in the Australasian and Afrotropical zones and has yet to be recorded in Neotropical and Palaearctic species. Only one species uses summer torpor in the Nearctic zone and it is a desert species. Lovegrove (2000) provides a simple model that predicts that summer torpor, and possibly hibernation, are most likely to be used by animals that display risk-averse foraging behavior and that also have a limited capacity for fat storage. The model further predicts that daily torpor is likely to be most common in areas where winters are moderate and seasonality is less pronounced. Moreover, summer torpor should occur most frequently in habitats with high climatic variability and hence where resource availability is highly variable in time and space (Lovegrove 2000).

In tropical and subtropical areas resource unpredictability is generated mostly as a consequence of summer rainfall anomalies related to the El Niño Southern Oscillations (ENSO) (Philander 1983; Stone et al. 1996), especially in the Australasian, Afrotropical and Indomalayan zoogeographical regions and parts of the Neotropics. In ENSO afflicted areas summer rainfall fails periodically (Tyson 1986), resulting in decreased productivity. ENSO is thus potentially an important evolutionary force, and should select for physiological traits that conserve energy

(Meserve et al. 1995; Lovegrove and Raman 1998; Nicol and Anderson 2000; Lovegrove 2000), such as use of daily torpor.

Like their Nearctic and Palearctic counterparts, tropical and subtropical small mammals have been observed to breed during the warm, wet summer months normally associated with abundant food resources (Bronson 1989). Summer breeding is thought to be controlled by photoperiod and presumably ensures that prevailing climatic conditions optimize breeding success (Berry and Bronson 1992). However, in a number of southern hemisphere species, reproduction is not photoresponsive. Moreover, reproduction tends to be aseasonal and opportunistic, with animals breeding at any time of the year provided there are enough food resources to support a breeding effort (Bernard and Hall 1995; White et al. 1996; White et al. 1997). This opportunistic breeding pattern is presumably adaptive in unpredictable regions and is likely to optimize fitness. Furthermore, small mammals are usually short-lived, and must continuously reproduce to counterbalance their short life expectancy (Bronson 1989). As a result, short-lived mammals tend to be more opportunistic and less seasonal in their reproductive efforts.

However, reproductive activity is known to restrict the use of daily torpor in male rodents. Complete gonadal quiescence and low circulating testosterone concentrations are required before these animals can enter torpor (Hall and Goldman 1980; Goldman et al. 1986; Lee et al. 1990). Thus, since reproductive activity and daily torpor appear to be mutually exclusive, it seems that males face a conflict between the need to conserve energy through the use of summer torpor, and their capacity to optimize fitness by opportunistic breeding.

The main objective of this study was to test certain predictions concerning gender differences in energy-saving physiological traits. Specifically, the Afrotropical pouched mouse, *Saccostomus campestris*, was used as a model animal (See Lovegrove and Raman 1998) to investigate conservative energy-saving traits such as low basal metabolic rate (BMR) and

employment of summer torpor in response to resource unpredictability. Furthermore, the influence of testosterone on the display of summer torpor by male *S. campestris* was investigated.

Study animals and breeding programme

Saccostomus campestris is a nocturnal rodent widely distributed throughout southern Africa. It has a wide habitat tolerance, although sandy substrates are generally preferred (Skinner and Smithers 1990). *Saccostomus campestris* has been classified as primarily herbivorous (Kerley et al. 1990) or omnivorous (Kerley 1989), and individuals are also known to carry seeds in their cheek pouches (Skinner and Smithers 1990). In the field, reproduction generally occurs during the warm, wet summer months (Skinner and Smithers 1990), although breeding may occur independently of photoperiod control (Bernard and Hall 1995). The capacity for aseasonal breeding has been reported for this species and its breeding pattern has been described as opportunistic (Bernard and Hall 1995).

Garland and Adolph (1991) have emphasized that a number of physiological traits are influenced by environmental factors such as ambient temperature, season, nutritional status and photoperiod. Generally these factors can be controlled by acclimation in the laboratory prior to physiological measurements. However, not all environmental effects on physiological traits are reversible as some may occur during early development. Garland and Adolph (1991) therefore suggest that the use of laboratory-bred animals may alleviate these problems because developmental effects of age, nutrition, photoperiod and ambient temperature can be controlled and standardized. In this study, F1-generation *S. campestris* bred in captivity from adult mice trapped with Sherman traps at Mabibi Coastal Forest Reserve, South of Kozi Bay, on the

KwaZulu-Natal north coast, South Africa in April 1999 were used (Permit number 613/1999, KwaZulu-Natal Nature Conservation Services).

The site was chosen because of its close proximity to an epicenter of high rainfall variability, namely Mozambique. Mozambique is known to have a strong association between rainfall variability and a high Southern Oscillation Index (Stone et al. 1996). For example, the coefficient of variation (CV) of mean annual precipitation (MAP) at Maputo is 33.5% (mean: 771mm; SD: 259mm), with MAP ranging from 278-1456mm over a 87-year period. Rainfall data were obtained online from the National Climate Data Centre, (NCDC) in Asheville, NC (<http://ingrid.ldgo.columbia.edu/SOURCES/.NOAA/.NCDC>). This high rainfall variability is similar to that typically recorded in the arid western regions of southern Africa where MAP < 250mm (Desmet and Cowling 1999), and is double that of Holarctic regions of similar MAP.

Two weeks after capture, adult animals were paired based on bodyweight, and housed in glass terraria. They were fed an *ad libitum* diet of rodent pellets, apple, lettuce and restricted quantities of sunflower seed. Water was also available *ad libitum*. The animals were maintained at an ambient temperature of 25°C and a 14L: 10D photoperiod.

The gestation period was 23 days. Soon after birth, the male was removed and the pups remained with the dam for 45 days. The diet of the lactating females was supplemented with ProNutro, a commercial high protein cereal (22% protein, 59% carbohydrate, and 6% fat) mixed with water. At three months of age all the animals had attained reproductive maturity, males with well developed testes, and females being clearly perforate.

Literature cited

Audet D, Thomas DW (1996) Evaluation of the accuracy of body temperature measurement using external radio transmitters. *Can J Zool* 74: 1778-1781

- Bernard RTF, Hall J (1995) Failure of the estrous cycle and spermatogenesis to respond to day length in a subtropical African rodent, the pouched mouse (*Saccostomus campestris*). Biol Reprod 52: 1291-1295
- Berry R, Bronson FH (1992) Life history and bioeconomy of the house mouse. Biol Rev 67: 519-550
- Bronson FH (1989) Mammalian Reproductive Biology. London, The University of Chicago Press
- Coburn DK, Geiser F (1998) Seasonal changes in energetics and torpor patterns in the subtropical blossom-bat *Syconycteris australis* (Megachiroptera). Oecologia 113: 467-473
- Desmet P, Cowling RM (1999) The climate of the Karoo- a functional approach. In: Dean W, Milton S (eds) The Karoo- Ecological patterns and processes. Cambridge, Cambridge University Press, pp 3-16
- Ellison GTH, Skinner JD (1992) The influence of ambient temperature on spontaneous daily torpor in pouched mice *Saccostomus campestris*: Rodentia-Cricetidae from southern Africa. J therm Biol 17: 25-31
- Garland T, Adolph SC (1991) Physiological differentiation of vertebrate populations. Ann Rev Ecol Syst 22: 193-228

- Geiser F, Baudinette RV (1985) The influence of temperature and photophase on daily torpor in *Sminthopsis macroura* (Dasyuridae : Marsupalia). *J Comp Physiol B* 156: 129-134
- Geiser F, Baudinette RV (1987) Seasonality of torpor and thermoregulation in three dasyurid marsupials. *J Comp Physiol* 157B: 335-344
- Geiser F, Ruf T (1995) Hibernation versus daily torpor in mammals and birds: Physiological variables and classification of torpor patterns. *Physiol Zool* 68: 935-966
- Goldman BD, Darrow JM, Duncan MJ, Yogeve L (1986) Photoperiod, Reproductive hormones and winter torpor in three hamster species. In: Heller HC, Mussachia XJ, Wang LCH (eds) *Living in the Cold: Physiological and Biochemical adaptations*. New York, Elsevier Science Publishing Co., pp 341-351
- Hall V, Goldman BD (1980) Effects of gonadal steroid hormones on Hibernation in the Turkish hamster (*Mesocricetus brandti*). *J Comp Physiol B* 135: 107-114
- Heldmaier G, Steinlechner S (1981) Seasonal pattern and energetics of short daily torpor in the Djungarian hamster, *Phodopus sungorus*. *Oecologia* 48: 265-270
- Kerley GIH (1989) Diet of small mammals from the Karoo, South Africa. *S Afr J Wildl Res* 19: 67-72
- Kerley GIH, Knight MH, Erasmus T (1990) Resource partitioning of small mammals in the southern Kalahari. *S Afr J Wildl Res* 20: 123-126

- Körtner G, Geiser F (1998) Ecology of natural hibernation in the marsupial mountain pygmy-possum (*Burramys parvus*). *Oecologia* 113: 170-178.
- Körtner G, Song X, Geiser F (1998) Rhythmicity of torpor in a marsupial hibernator, the mountain pygmy-possum (*Burramys parvus*), under natural and laboratory conditions. *J Comp Physiol B* 168: 631-638
- Lee TM, Pelz K, Licht P, Zucker I (1990) Testosterone influences hibernation in golden mantled ground squirrels. *Am J Physiol* 259: R760-R767
- Lovegrove BG, Raman J (1998) Torpor patterns in the pouched mouse (*Saccostomus campestris*; Rodentia): a model animal for unpredictable environments. *J Comp Physiol B* 168: 303-312
- Lovegrove BG (2000) Daily heterothermy in mammals: coping with unpredictable environments. In: Heldmaier G, Klingenspor M (eds) *Life in the cold: 11th International hibernation symposium*. Berlin, Springer, pp 29-40
- Lyman CP, Willis JS, Malan A, Wang LCH (1982) *Hibernation and torpor in mammals and birds*. New York, Academic Press
- Meserve PL, Yunker JA, Gutierrez JR, Contreras LC, Milstead WB, Lang BK, Cramer KL, Herrera S, Lagos VO, Silva SL, Tabilo EL, Torrealba MA, Jaksic FM (1995). Heterogeneous responses of small mammals to an El Niño Southern Oscillation event in northcentral semiarid Chile and the importance of ecological scale. *J Mamm* 76: 580-595

- Nicol SC, Andersen NA (1996) Hibernation in the echidna: not an adaptation to cold? In: Geiser F, Hulbert AJ, Nicol SC (eds) Adaptations to the cold: Tenth International Hibernation Symposium. Armidale, University of New England Press, pp 7-12
- Nicol S, Anderson CG (2000) Patterns of Hibernation of Echidnas in Tasmania. In: Heldmaier G, Klingenspor M (eds) Life in the cold: 11th International Hibernation Symposium. Berlin, Springer, pp 21-28
- Ortmann S, Schmid J, Ganzhorn JU, Heldmaier G (1996) Body temperature and torpor in a Malagasy small primate, the mouse lemur. In: Geiser F, Hulbert AJ, Nicol SC (eds) Adaptations to the cold: Tenth International Hibernation Symposium. Armidale, University of New England Press, pp 55-61
- Philander SGH (1983) El Niño southern oscillation phenomena. *Nature* 302: 295-301
- Skinner JD, Smithers RHN (1990) The mammals of the southern African subregion. Pretoria, University of Pretoria
- Stone RC, Hammer GL, Marcussen T (1996) Prediction of global rainfall probabilities using phases of the Southern Oscillation Index. *Nature* 384: 252-255
- Tyson PD (1986) Climatic change and variability in southern Africa. Cape Town, Oxford University Press

Webb PI, Skinner JD (1996) Summer torpor in African woodland dormice *Graphiurus murinus* (Myoxida: Graphiurinae). *J Comp Physiol B* 166: 325-330

White RM, Kennaway DJ, Seamark RF (1996) Reproductive seasonality of the bush rat (*Rattus fuscipes greyi*) in South Australia. *Wildlife Research* 23: 317-336

White RM, Kerley GIH, Bernard RTF (1997) Pattern and controls of reproduction of the southern African rodent *Gerbillurus paebe* in the semi-arid Karoo, South Africa. *J Arid Environ* 37: 529-549

Chapter Two

Sex differences in patterns of summer torpor and metabolic rates of pouched mice,

Saccostomus campestris

Introduction

Many small endotherms utilize daily heterothermy and hibernation in response to energetically stressful conditions (Lyman et al. 1982; Geiser 1994; Geiser and Ruf 1995). The employment of these responses is usually associated with cold temperate zones where there is low food availability during winter (Lyman et al. 1982). However, many species from tropical and subtropical areas also enter induced and spontaneous torpor under winter and summer conditions (Geiser and Baudinette 1985; Geiser and Baudinette 1987; Ellison and Skinner 1992; Audet and Thomas 1996; Coburn and Geiser 1998; Lovegrove and Raman 1998). For example, the subtropical blossom-bat, *Syconycteris australis*, enters torpor more frequently in summer than in winter (Coburn and Geiser 1998). These authors attribute this pattern to unpredictable nectar availability, suggesting that torpor may be used for energy conservation by species experiencing periodic food shortages even in warm climates. In fact, in the majority of studies, summer torpor has been linked to unpredictable spatial and temporal food availability.

The Afrotropical pouched mouse (*Saccostomus campestris*) has been identified as a suitable model animal for investigating physiological and behavioural adaptations to unpredictable environments (Lovegrove and Raman 1998). *Saccostomus campestris* has a widespread distribution throughout southern Africa (Skinner and Smithers 1990), inhabiting both mesic and semi-arid habitats. Under laboratory conditions, both males and females are capable of breeding throughout the year and reproductive activity is not influenced by photoperiod (Bernard and Hall 1995). This species is also known to employ daily heterothermy in response to

low ambient temperatures and food deprivation (Ellison and Skinner 1992; Lovegrove and Raman 1998). However, Lovegrove and Raman (1998) have reported a differential propensity for daily torpor between the sexes, with males entering torpor less frequently than females.

This difference in use of torpor between the sexes raises the question of whether the reluctance of males to enter torpor is associated with their year-round maintenance of reproductive activity. It is well documented that reproductive activity restricts the use of daily torpor by male rodents (Hall and Goldman 1980; Goldman et al. 1986; Lee et al. 1990). The aseasonal breeding capacity of pouched mice is thought to represent an adaptation to unpredictability of the southern African region by enabling opportunistic breeding whenever it is nutritionally and energetically possible, regardless of season (Berry and Bronson 1992; Bernard and Hall 1995). However, it is possible that male *S. campestris* face a consequent conflict between the use of daily torpor to conserve energy and the maintenance of gonadal activity that presumably optimizes mating success and thus fitness.

In this study, I tested the hypothesis that male *S. campestris* may compensate for their limited heterothermic capacity with lower normothermic body temperatures (T_b 's) as observed by Lovegrove and Raman (1998) by maintaining lower normothermic metabolic rates relative to females (See Lovegrove and Raman 1998). The approach used was to compare body temperature and metabolic responses of female and male pouched mice in response to food *ad libitum* and food restricted treatments.

Materials and methods

Study animals and general maintenance

Animals that had attained an age of 3- 4 months and full reproductive status were used. Females were perforate and the males had clearly visible, well-developed testes that were descended in

the scrotal sac. The mean \pm SD body mass of females at the start of measurements was 66.9 ± 3.6 g, ($n = 8$), significantly lower than that of males, 79.9 ± 9.4 g, ($n = 8$) (ANOVA; $F_{1,14} = 13.12$, $P < 0.05$). Throughout the experimental period the animals were housed in a constant environment room ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) with a photoperiod of 14L:10D, in conventional rodent cages with steel mesh lids. They were fed a daily diet of commercial rodent pellets, apple, lettuce and a restricted quantity of sunflower seeds. Being rich in linoleic acid, sunflower seeds were provided as a source of polyunsaturated fatty acids that optimize membrane function during torpor and hibernation (Geiser and Kenagy 1987). Water was available *ad libitum*.

Experimental procedures

Measurement of T_b

Body temperatures were measured with temperature-sensitive telemeters (Model XM, Minimeter Co., Sunriver, Oregon, accuracy 0.1°C) calibrated with a standard mercury thermometer (0.05°C) in a water bath at temperatures from $10 - 40^{\circ}\text{C}$. Linear regressions were calculated from log-transformed transmitter pulse frequency as a function of temperature and were used as calibration curves for each transmitter. The telemeters (*ca.* 1.6g) never exceeded 1.7% of the animals' body mass. They were implanted surgically into the intra-peritoneal cavity under inhalation anaesthesia (Isoflorane in oxygen; induction and maintenance, 2%; flow rate, *ca.* $0.5 \text{ l}\cdot\text{min}^{-1}$). Telemeter signals were detected using antennae attached to Perspex sleeves surrounding each respirometer and were converted to TTL waves using a monostable multi-vibrator and then converted to voltages using a frequency-to-voltage converter. Ambient temperatures (T_a) in the cabinet and respirometers were measured with thermistor probes, calibrated in the same way.

Metabolic measurements

Metabolic rates were measured indirectly as oxygen consumption (VO_2). All metabolic measurements were made in 2.3 l, dome-shaped, perspex respirometers (length = 18cm, breadth = 12 cm, height = 12cm). The respirometers were placed in a 1m^3 sound-proof constant environment cabinet. The light:dark cycle in the cabinet was similar to that in the constant environment rooms (14L : 10D). Measurements were made using an open flow-through system interfaced with a PC.

Atmospheric air, acting as the control gas, was pumped from outside the building into the cabinet at approximately $5\text{ l}\cdot\text{min}^{-1}$. Silica gel was used as a water vapour scrubber to partially dry the air (relative humidity $\leq 60\%$). This air was drawn through individual respirometers at flow rates chosen to maintain $< 1\%$ oxygen depletion between the incurrent and excurrent air. The flow rates were measured with a Brooks Thermal Mass Flow Meter (Model 5810) factory calibrated to STP at sea level. The use of solenoid relay valves and a pump for each respirometer allowed five respirometers, as well as a control channel, to be used simultaneously. The excurrent air from each respirometer was passed through CO_2 – proof tubing, a water condenser and a CO_2 scrubber to remove water and carbon dioxide, respectively. After passing through the pumps, relay valves, filters and the mass flow meter, the excurrent air was subsampled with an Applied Electrochemistry Oxygen Analyser (Ametek S-3A/1) and an Oxygen Sensor (Ametek N-22M) to determine the fractional concentration of oxygen in the dry air.

Analogue signals from the thermistor probes, mini-mitters, mass flow meter and oxygen analyzer were digitized with an A/D converter and recorded on a multi-purpose channel, WINDOW-based recording program, with a sample interval of six minutes for each respirometer. Metabolic rates were calculated using the equation $V_{\text{O}_2} = V_E(F_{\text{I}\text{O}_2} - F_{\text{E}\text{O}_2})/(1 - F_{\text{I}\text{O}_2})$

where V_{O_2} = metabolic rate ($\text{ml O}_2 \cdot \text{h}^{-1}$), V_E = flow rate ($\text{ml} \cdot \text{min}^{-1}$), F_{IO_2} = incurrent fractional O_2 concentration and F_{EO_2} = excurrent fractional O_2 concentration (Withers 1977).

Experimental protocol

The animals were subjected to two food treatments, a food *ad libitum* (*ad lib*) and a food restricted treatment, as described in Lovegrove and Raman (1998).

During the food *ad lib* treatment, measurements of T_b and metabolic rate were made at T_a 's ranging from 5 – 35°C for the determination of BMR. Two hours prior to the commencement of measurements, all food was removed from the cages. Measurements were made between 08h00 and 16h00. The time of the experiments coincided with the known rest phase and removal of food ensured that the animals were post-absorptive during measurements. At each T_a , T_b and VO_2 were measured for four hours.

Throughout the food restriction treatment animals were maintained on *ca.* 70% of the *ad lib* diet. Measurements were made at T_a 's ranging from 5 - 25°C. On each measurement day, food was removed at 08h00. Since the animals were expected to enter torpor during the late scotophase or early photophase, measurements commenced at 17h00. If the animals did not enter torpor, the experiments were terminated at 16h00 the following day. However, if torpid animals were observed (T_b was continuously observed on computer monitor), the measurements were terminated after the animals had fully aroused from torpor.

Data and statistical analyses

The sample size was $n = 16$ (8 males; 8 females) during the food *ad lib* treatment. Due to failed telemeters this was reduced to $n = 12$ in the food restricted treatment, with six animals for each sex.

The minimum T_b below which animals were deemed to be torpid was chosen as 32°C, following the objective criteria used by Lovegrove and Raman (1998). The minimum T_b and resting metabolic rates (RMR's) were calculated as the mean of the three lowest consecutive data points, that is, a total time interval of 18 minutes, measured at each T_a .

Torpor frequency was calculated for each sex, as the proportion of torpid animals at each T_a , and these frequency data were arcsine-transformed before statistical analysis to allow for parametric tests.

A balanced repeated measures ANOVA (RM-ANOVA, Statistica, Statsoft, Inc) was used to test for sex and treatment effects, on metabolic rate, minimum T_b and torpor frequency at T_a 's of 5 - 25°C. To quantify the influence of the body mass covariate on RMR, a Multiple Analysis of Covariance (MANCOVA) was used to investigate two between factors (temperature and sex) and a within factor (RMR) at T_a 's ranging from 5-25°C. The MANCOVA was performed on log-transformed body mass and resting metabolic rates and showed no significant linear regressions between body mass and RMR at all T_a 's ($0.00 < r^2 < 0.235$; $P > 0.05$). Hence, resting metabolic rates were not influenced by body mass and thus differences between sexes and treatment were analyzed using a repeated measures ANOVA.

Torpor bout lengths were determined as the total time during which T_b was maintained below 32°C. Since all animals did not enter torpor at all temperatures during food restriction, a repeated measures ANOVA on bout lengths could not be employed.

The mean \pm SD bout length was calculated and a one way ANOVA was used to test for differences in bout length between sexes.

A slopes test was used to test for differences between the slopes of regression lines of metabolic rate as a function of ambient temperature.

Results

Body mass

At the commencement of the food restriction treatment the mean body masses were $71.1 \pm 8.6\text{g}$ ($n = 8$) and $85.0 \pm 7.3\text{g}$ ($n = 8$), for females and males, respectively. At the end of the food restriction treatment body masses had decreased to $54.9 \pm 4.5\text{g}$ and $64.3 \pm 7.1\text{g}$, representing a mass loss of $22.3 \pm 6.5\%$ for females and $24.0 \pm 6.8\%$ for males, respectively (Fig 1).

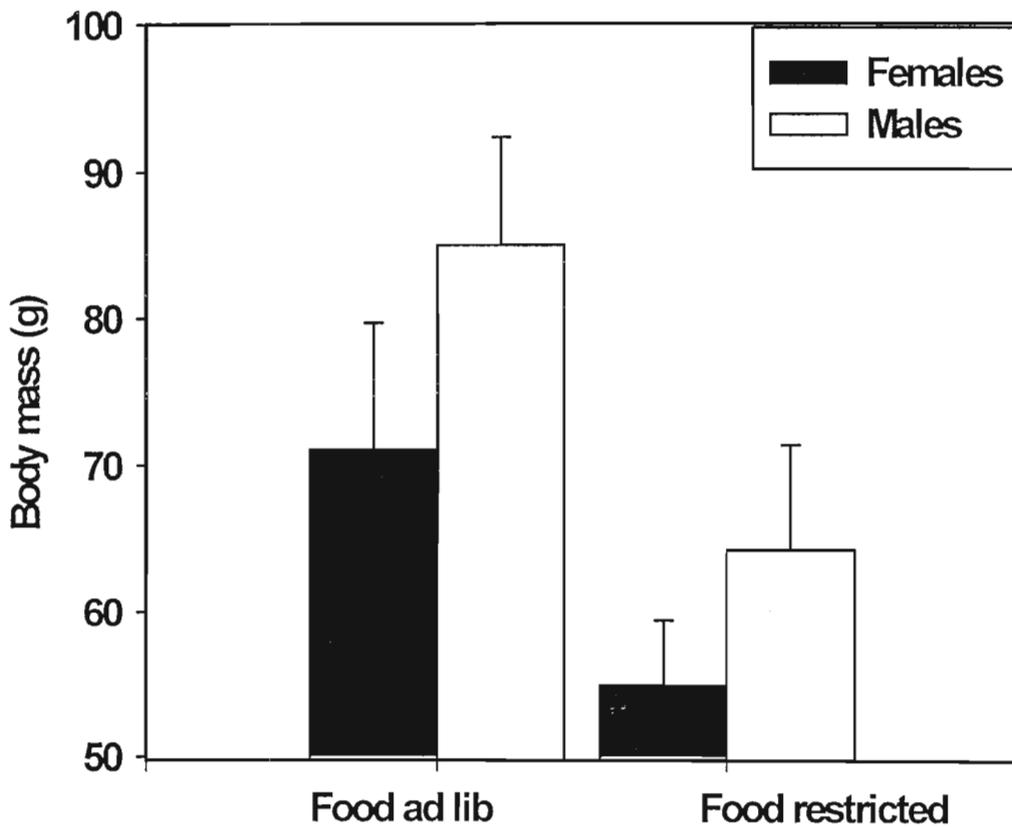


Figure 1. The mean \pm SD body mass of female ($n = 8$) and male ($n = 8$) *S. campestris* at the end of each food treatment.

Torpor frequency

All incidences of torpor occurred during the photophase, which is also the rest-phase of this species. The proportion of torpid animals was significantly affected by treatment (ANOVA; $F_{1,4} = 48.11$; $P < 0.05$). Only three incidences of shallow torpor ($30^{\circ}\text{C} < T_b < 32^{\circ}\text{C}$) were observed in *ad lib* animals (Fig 2). Under food restriction, however, both males and females exhibited daily torpor (Fig 3), but significantly fewer males entered torpor than females (RM-ANOVA; $F_{1,4} = 14.34$; $P < 0.05$). Treatment \times sex interactions were also significant (RM-ANOVA; $F_{1,4} = 11.56$; $P < 0.05$), confirming that significantly more females entered torpor than males.

Minimum body temperature

Minimum T_b was not significantly affected by sex alone (RM-ANOVA; $F_{1,24} = 3.89$; $P > 0.05$). However, minimum T_b was significantly affected by treatment, with food restricted animals exhibiting lower body temperatures than *ad lib* fed animals ($F_{1,24} = 43.37$; $P < 0.001$). On average, the minimum T_b decreased by $1.2 - 2.4^{\circ}\text{C}$ in males and $1.6 - 6.5^{\circ}\text{C}$ in females between treatments, depending on T_a . Moreover, there were significant sex \times treatment effects (RM-ANOVA; $F_{1,24} = 4.47$; $P < 0.05$) as well as sex \times temperature effects ($F_{4,96} = 4.27$; $P < 0.01$). Females exhibited deeper torpor bouts relative to males, with the lowest T_b values recorded for females at each T_a under food restriction. A Tukey *a posteriori* test revealed that, when food restricted, the lowest T_b values were observed at 15°C and 20°C for both sexes.

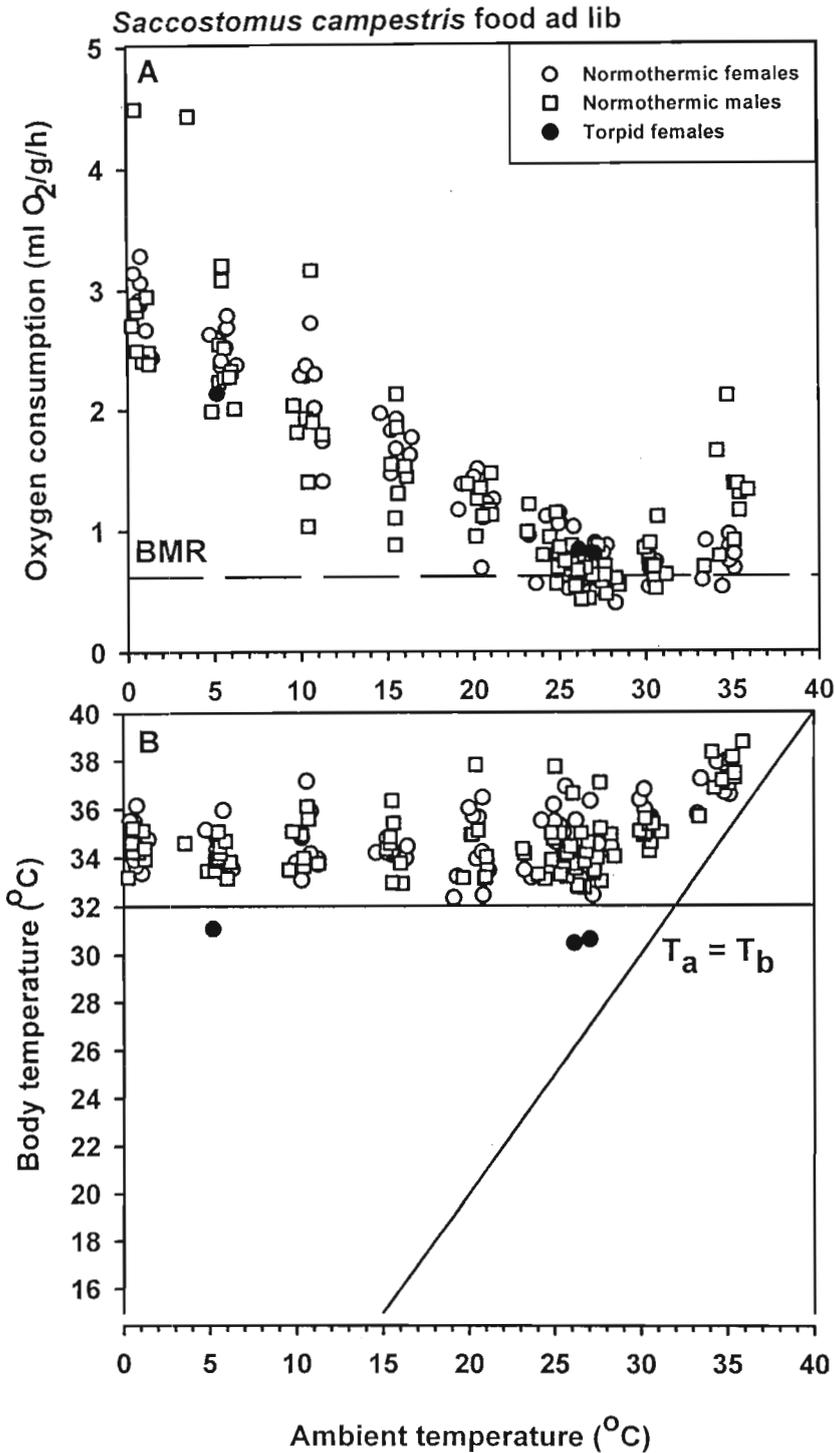


Figure 2. (A) The resting metabolic rates of female and male *S. campestris* at T_a 's ranging from 5-35°C under *ad lib* treatment. The hatched line represents basal metabolic rate. (B). Body temperatures of female and male *S. campestris* under *ad lib* treatment. Only three incidences of torpor were observed, below the minimum of normothermy, $T_b = 32^\circ\text{C}$, represented by the solid line.

Metabolic rates

In *ad lib* fed animals the mass-specific oxygen consumption increased linearly with decreasing ambient temperature (Fig 2). The basal metabolic rate (BMR) was calculated from the lowest RMR value at T_a 's $> 32^\circ\text{C}$ for each animal. No significant differences were found in BMR's of males and females (ANOVA; $F_{1,14} = 0.015$; $P > 0.05$). The BMR data were pooled for both sexes and the mean \pm SD BMR was calculated as $0.618 \pm 0.096 \text{ mlO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$. There were significant differences in metabolic rates between treatments ($F_{1,24} = 31.03$; $P < 0.01$), with reduced metabolic rates in all food restricted animals. Overall, there were no significant sex effects on metabolic rates ($F_{1,24} = 0.31$; $P > 0.05$).

However, when food restricted animals were considered separately, there were significant interactive effects of sex and temperature ($F_{4,40} = 5.22$; $P < 0.01$). Under food restriction, the animals exhibited the lowest metabolic rates at T_a 's of *ca.* 20°C and 25°C , a decrease corresponding to increased torpor frequency at these T_a 's. At T_a 's around 20°C and 25°C the metabolic rates decreased below basal levels in torpid animals (Fig 3). However, below 20°C the metabolic rates of torpid animals increased above mean BMR although the values were still lower than equivalent *ad lib* values. I suggest that this thermogenic response was associated with the defense of a torpor body temperature set-point of *ca.* 25°C (Fig 3). Metabolic rate reductions ranged from 14– 40 % of normothermic values in males and 10 – 70 % in females between treatments, depending on T_a and whether the animals were torpid or not. Although there were metabolic reductions in normothermic males the % metabolic rate reduction was not compared between treatments for non-torpid males because the sample size was reduced by the number of males that became torpid at the different T_a s.

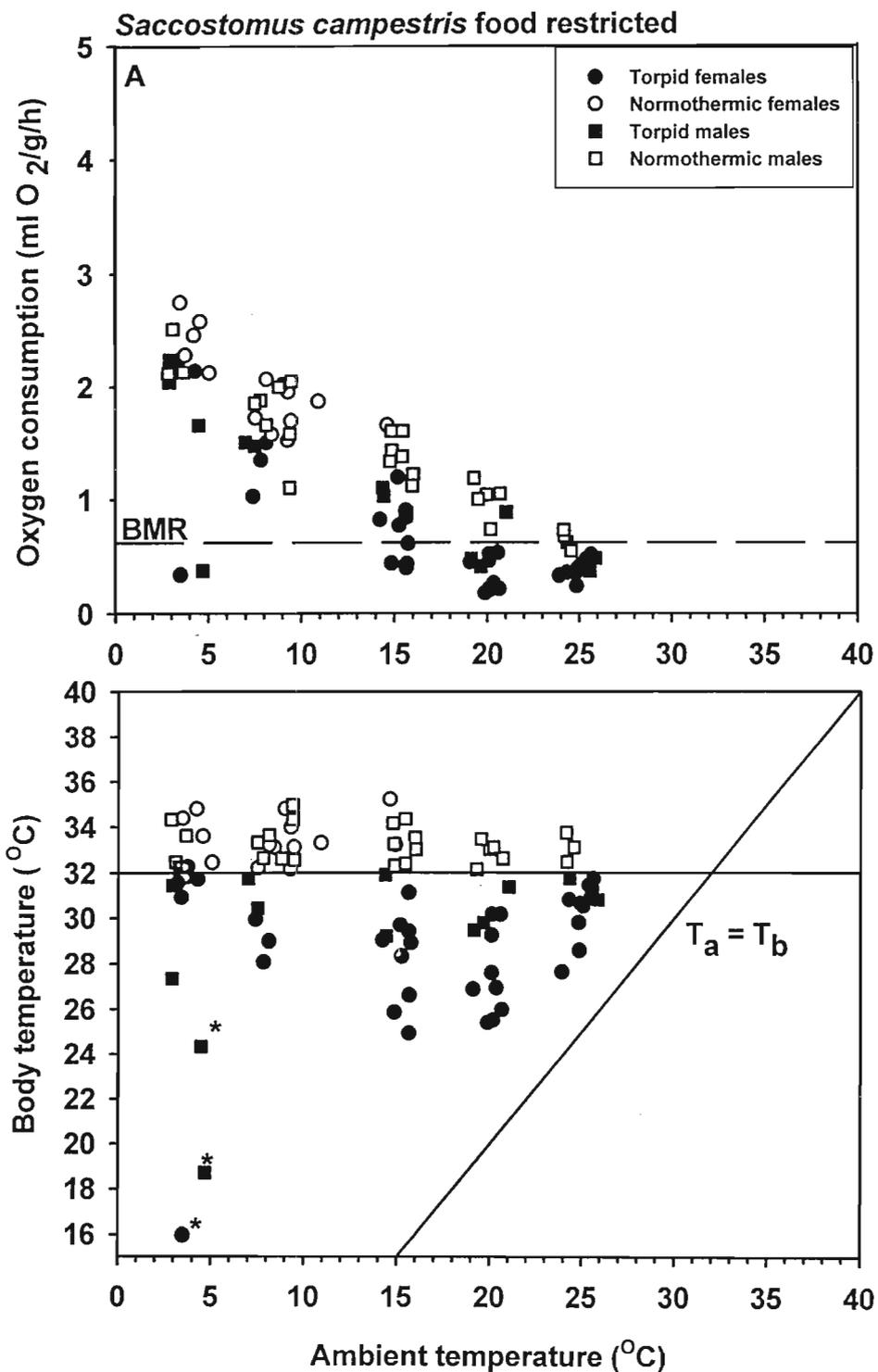


Figure 3. (A) Metabolic rates of female and male *S. campestris*, under food restriction at T_a 's ranging from 5-25°C. (B) Body temperatures of animals under food restriction. Data points marked with asterisks indicate pathologically hypothermic animals, i.e. animals that could not arouse through endogenous heat production. Note the markedly high RMR of some torpid males and females at the low ambient

Since there were no significant effects of sex the metabolic rates data were pooled in each food treatment and regression lines were fitted to these data. The slopes of these regression lines were $-0.087 \text{ ml O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1} \cdot ^\circ\text{C}^{-1}$ and $-0.078 \text{ ml O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1} \cdot ^\circ\text{C}^{-1}$ for *ad lib* and food restricted animals, respectively. These slopes were not significantly different ($t_{2,202} = 1.972$; $P > 0.05$), suggesting that conductance remained constant between treatments. The y-intercepts were 4.8 and 3.7 times greater than the basal metabolic rate ($0.618 \text{ ml O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$), for the food *ad lib* and food restricted treatments, respectively.

Torpor bout length

Since only three shallow torpor bouts were observed in *ad lib* fed animals, analyses of bout lengths were restricted to comparisons between sexes under food restriction, that is, comparisons between treatments were ignored. The mean bout lengths were 176 ± 122 min ($N = 6$; $n = 71$) and 152 ± 119 min ($N = 6$; $n = 17$) for females and males, respectively. These values were not significantly different ($F_{1,86} = 0.52$; $P > 0.05$). The combined mean bout length for females and males was 172 ± 122 min ($n = 88$).

Torpor usually commenced shortly after the onset of the photophase and was spontaneously terminated before the onset of the scotophase. Animals of both sexes entered torpor at T_a 's of $15^\circ - 25^\circ\text{C}$ and tended to avoid torpor at the lower temperatures (5°C and 10°C). At a T_a of *ca.* 5°C , several food-restricted animals, a female and three males, became pathologically hypothermic. Three of these animals were successfully reheated to normothermia with an artificial heat source. The body temperature and VO_2 traces of a female that showed the longest (6.8 hours) and deepest torpor bout ($T_b = 25.4^\circ\text{C}$ at $T_a = 20.0^\circ\text{C}$) is shown in Fig 4.

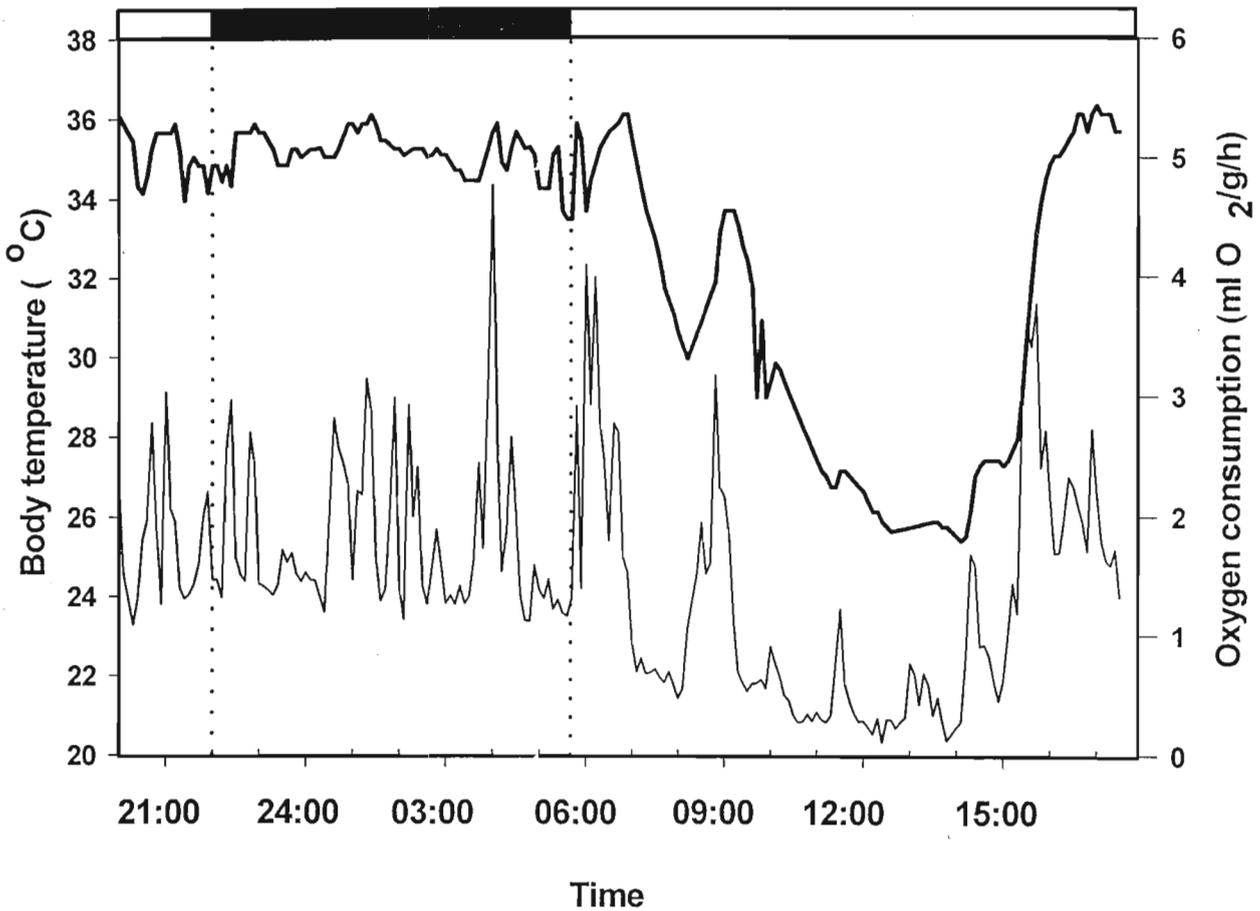


Figure 4. Body temperature (solid line) and VO_2 (thin line) profiles of a female *S. campestris* (#FB34) during a torpor bout at $T_a = 20^\circ\text{C}$. This was the longest (6.8h) and deepest ($T_b = 25.4^\circ\text{C}$) torpor bout measured in this study. The dark horizontal bar on top represents the scotophase.

Discussion

No T_b differences were observed between sexes in normothermic animals under *ad lib* food treatment. However, pronounced differences were observed in minimum T_b under food restriction. The females consistently exhibited lower T_b 's compared to the males under food restriction reflecting an increased incidence of torpor. The reluctance of males to enter torpor confirms Lovegrove and Raman's (1998) observations. The latter study found that males only displayed torpor at the lowest T_a 's and only after severe food restriction, whereas females readily entered torpor. However, even when the males did not display torpor they showed reduced normothermic T_b 's under food restriction. (Geiser 1988) also reported significant sex differences in patterns of torpor in two *Antechinus* species. In both species, females entered torpor more frequently than males, patterns attributed to differences in body mass.

The majority of animals in the present study entered torpor at T_a 's of 15- 25°C. The estimated "summer" torpor body temperature set-point was 24.9°C for females and 29.2°C for males. The majority of males commenced the metabolic defence of T_b around 20°C, with metabolic rates elevated above BMR levels, whereas torpid females showed increased metabolic heat production at *ca.* 15°C. This may explain why several animals in this study became pathologically hypothermic at T_a 's below 15°C. Presumably, their body temperatures decreased below the set-point and they could not arouse from torpor because their capacity for endogenous heat production was impaired.

Summer-acclimated *S. campestris* reportedly have a lower non-shivering thermogenesis (NST) capacity relative to winter acclimated animals (Haim et al. 1991). Thus, although the animals display seasonal adjustments in NST, summer acclimated animals seem unable to initiate NST if T_b falls below *ca.* 25°C at low ambient temperatures. It is well documented that

the capacity for NST is increased greatly under short photoperiod and further enhanced by exposure to cold (Heldmaier et al. 1981; Steinlechner and Heldmaier 1982). It is thus possible that the summer conditions under which our animals were acclimated may be insufficient for adequate physiological preparation for heterothermy, and explain the general avoidance of torpor at the lowest T_a 's. Presumably, an efficient summer NST capacity has never evolved in *S. campestris* because the ambient temperatures seldom fall below 10°C during summer.

There is increasing evidence that small mammals that employ summer torpor may not rely exclusively on endogenous heat production for arousal from torpor. For example, daily cycles of ambient temperature may assist some small mammals with the arousal from summer torpor (Ortmann et al. 1996). Lovegrove et al. (1999) have shown that the experimental cost of arousal in *Sminthopsis macroura* is significantly reduced when the animals are exposed to moderate ambient temperature cycles. However, it remains unknown whether free-ranging small mammals in unpredictable environments do indeed couple torpor to ambient temperature cycles to minimize arousal costs when in energy shortfall.

Lovegrove and Raman (1998) suggested that the limited heterothermic capacity of male *S. campestris* might be caused by a lack of seasonal testes regression and hence high testosterone blood titres. Testes regression is a requirement for male rodents to enter hibernation (Goldman et al. 1986; Darrow et al. 1987). Preliminary results in this study suggest that it is indeed high testosterone levels that inhibit torpor in male *S. campestris*. Castrated animals readily enter torpor relative to castrated animals implanted with silastic testosterone implants (Chapter 3).

Saccostomus campestris is a highly opportunistic breeder maintaining year-round reproductive activity (Bernard and Hall 1995). The oestrous cycle and spermatogenic cycle are not influenced by photoperiod, a reproductive pattern presumed to optimize fitness in unpredictable environments. In rodents several weeks are required following full testes

regression for males to become reproductively active (Bronson 1989). These time intervals may be too long in the southern African region where rainfall, resources and perhaps concomitantly the cues for female oestrous are unpredictable.

Based on Lovegrove and Raman's (1998) observation that males displayed lower normothermic T_b 's than females, males were expected to display lower metabolic rates. In this respect a lower BMR would also decrease the daily energy expenditure of males relative to females thus reducing the need for torpor to conserve energy. The males did not display decreased metabolic rates relative to the females under *ad lib* treatment. However, normothermic males showed decreases in metabolic rates at all T_a 's when food was restricted.

Normothermic subtropical blossom bats (*Syconycteris australis*) have been shown to reduce energy expenditure by lowering T_b 's and metabolic rates at low T_a 's (Coburn and Geiser 1996). Similarly, normothermic male *Sminthopsis macroura* reduced resting metabolic rates when food restricted (Song and Geiser 1997). In this latter study the males entered torpor at low T_a (18°C) accompanied by a decrease in average daily energy expenditure, whereas at moderate temperatures (28°C), the males did not enter torpor. The males instead showed reductions in resting metabolic rates and consequently had lower T_b 's when normothermic. Male *S. macroura* seem to adjust their energy expenditure by reducing metabolic rates in response to variable food availability at higher temperatures (Song and Geiser 1997). *Mus musculus* also reduces its metabolic rate to 50% of normothermic metabolic levels in response to food restriction, with only slight decreases in body temperatures, seldom below 32°C (Hudson and Scott 1979). The observation that *S. campestris* tended to avoid torpor at the lowest ambient temperatures suggests that daily torpor may be used in response to resource unpredictability than low T_a 's. It has been suggested that daily heterothermy in the Afrotropical, Australasian and Indomalayan regions is used more in response to variability in resource availability than to cold as observed in

the Nearctic and Palaearctic zones (Lovegrove 2000). This interpretation would be consistent with the observation that the highest number of mammalian orders exhibiting daily torpor occur in the Afrotropical zone and Australasian zones (Lovegrove 2000), regions that are rendered unpredictable by the ENSO-related rainfall anomalies.

Summary

There were no sex differences in body temperatures, resting and basal metabolic rates under food *ad lib* conditions. However, when confronted with energy stress, the females readily employed torpor to save energy, defending a lower torpor setpoint-body temperature than males. Males entered torpor less frequently and maintained a higher setpoint-body temperature. This may be a consequence of the maintenance of year-round gonadal activity. The males may at least partially compensate for their limited heterothermic capacity by lowering normothermic body temperatures and reducing resting metabolic rates during energetically stressful episodes.

Literature cited

Audet D, Thomas DW (1996) Evaluation of the accuracy of body temperature measurement using external radio transmitters. *Can J Zool* 74: 1778-1781

Bernard RTF, Hall J (1995) Failure of the estrous cycle and spermatogenesis to respond to day length in a subtropical African rodent, the pouched mouse (*Saccostomus campestris*). *Biol Reprod* 52: 1291-1295

Berry R, Bronson FH (1992) Life history and bioeconomy of the house mouse. *Biol Rev* 67: 519-550

- Bronson FH (1989) *Mammalian Reproductive Biology*. London, The University of Chicago Press
- Coburn DK, Geiser F (1996) Daily torpor and energy savings in a subtropical blossom-bat, *Syconycteris australis* (Megachiroptera). In: Geiser F, Hulbert AJ, Nicol SC (eds) *Adaptations to the Cold: Tenth International Hibernation Symposium*. Armidale, University of New England Press, pp 39-45
- Coburn DK, Geiser F (1998) Seasonal changes in energetics and torpor patterns in the subtropical blossom-bat *Syconycteris australis* (Megachiroptera). *Oecologia* 113: 467-473
- Darrow JM, Yogev L, Goldman BD (1987) Patterns of reproductive hormone secretion in hibernating Turkish hamster. *Am J Physiol* 253: R329-R336
- Ellison GTH, Skinner JD (1992) The influence of ambient temperature on spontaneous daily torpor in pouched mice (*Saccostomus campestris*: Rodentia-Cricetidae) from South Africa. *J therm Biol* 17: 25-31
- Geiser F, Baudinette RV (1985) The influence of temperature and photophase on daily torpor in *Sminthopsis macroura* (Dasyuridae: Marsupialia). *J Comp Physiol B* 156: 129-134
- Geiser F, Baudinette RV (1987) Seasonality of torpor and thermoregulation in three dasyurid marsupials. *J Comp Physiol* 157B: 335-344

- Geiser F, Kenagy GJ (1987) Polyunsaturated lipid diet lengthens torpor and reduces temperature in a hibernator. *Am J Physiol* 252: R897-R901
- Geiser F (1988) Daily torpor and thermoregulation in *Antechinus* (Marsupialia): influence of body mass, season, development, reproduction, and sex. *Oecologia* 77: 395-399
- Geiser F (1994) Hibernation and daily torpor in marsupials: a review. *Aust J Zool* 42: 1-16
- Geiser F, Ruf T (1995) Hibernation versus daily torpor in mammals and birds: Physiological variables and classification of torpor patterns. *Physiol Zool* 68: 935-966
- Goldman BD, Darrow JM, Duncan MJ, Yogev L (1986) Photoperiod, reproductive hormones, and winter torpor in three hamster species. In: Heller HC, Musacchia XJ, Wang LCH (eds) *Living in the cold: physiological and biochemical adaptations*. New York, Elsevier, pp 331-340
- Haim A, Racey PA, Speakman JR, Ellison GTH, Skinner JD (1991) Seasonal acclimation and thermoregulation in the pouched mouse *Saccostomus campestris*. *J therm Biol* 16: 13-17
- Hall V, Goldman BD (1980) Effects of gonadal steroid hormones on Hibernation in the Turkish hamster (*Mesocricetus brandti*). *J Comp Physiol B* 135: 107-114
- Heldmaier G, Steinlechner S, Rafael J, Vsiansky P (1981) Photoperiodic control and effects of melatonin on nonshivering thermogenesis and brown adipose tissue. *Sci* 212: 917-919

- Hudson JW, Scott IM (1979) Daily torpor in the laboratory mouse, *Mus musculus* var. Albino. *Physiol Zool* 52: 205-218
- Lee TM, Pelz K, Licht P, Zucker I (1990) Testosterone influences hibernation in golden mantled ground squirrels. *Am J Physiol* 259: R760-R767
- Lovegrove BG, Raman J (1998) Torpor patterns in the pouched mouse (*Saccostomus campestris*; Rodentia): a model animal for unpredictable environments. *J Comp Physiol B* 168: 303-312
- Lovegrove BG, Körtner G, Geiser F (1999) The energetic cost of arousal from torpor in the marsupial *Sminthopsis macroura*: benefits of summer ambient temperature cycles. *J Comp Physiol B* 169: 11-18
- Lovegrove BG (2000) Daily heterothermy in mammals: coping with unpredictable environments. In: Heldmaier G, Klingenspor M (eds) *Life in the cold: 11th International hibernation symposium*. Berlin, Springer, pp 29-40.
- Lyman CP, Willis JS, Malan A, Wang LCH (1982) *Hibernation and torpor in mammals and birds*. New York, Academic Press
- Ortmann S, Schmid J, Ganzhorn JU, Heldmaier G (1996) Body temperature and torpor in a Malagasy small primate, the mouse lemur. In: Geiser F, Hulbert AJ, Nicol SC (eds) *Adaptations to the cold: Tenth International Hibernation Symposium*. Armidale, University of New England Press, pp 55-61

Skinner JD, Smithers RHN (1990) The mammals of the southern African subregion. Pretoria, University of Pretoria

Song X, Geiser F (1997) Daily torpor and energy expenditure in *Sminthopsis macroura*: Interactions between food and water availability and temperature. *Physiol Zool* 70: 331-337

Steinlechner S, Heldmaier G (1982) The role of photoperiod and melatonin in seasonal acclimatization of the Djungarian hamster, *Phodopus sungorus*. *Int J Biometeor* 26: 329-337

Withers PC (1977) Measurement of VO_2 , VCO_2 , and evaporative water loss with a flow-through mask. *J appl Physiol* 42: 120-123

Chapter Three

The influence of testosterone on torpor in male pouched mice,

Saccostomus campestris

Introduction

Tropical and subtropical mammals enter induced and spontaneous torpor in response to unpredictable spatial and temporal food availability (Geiser and Baudinette 1985; Bozinovic and Rosenmann 1988; Lovegrove and Raman 1998). Resource unpredictability is enhanced in these regions mostly by summer rainfall anomalies related to the El Niño Southern Oscillations (ENSO) (Philander 1983; Stone et al. 1996). Lovegrove and Raman (1998) have suggested that in the Afrotropical context, the ideal model animal with which to investigate the influences of unpredictable resource availability on the evolution of physiological traits is the pouched mouse, *Saccostomus campestris*.

Saccostomus campestris is widespread throughout southern Africa and has a wide habitat tolerance (Skinner and Smithers 1990). Under laboratory conditions pouched mice are capable of year-round breeding (Bernard and Hall 1995). Spermatogenesis and the mass and histology of the testes are not influenced by photoperiodic changes. Similarly, the oestrous cycle is not photoresponsive. This lack of photoperiodic response is attributed to an opportunistic breeding capacity that enables aseasonal breeding presumed to be an adaptation to environmental unpredictability (Bernard and Hall 1995; White and Bernard 1999).

Saccostomus campestris also employs daily torpor in response to variable food availability (Lovegrove and Raman 1998). However, sexual disparity exists in the use of daily torpor in this species. Whereas females readily displayed torpor upon food restriction, males

were more reluctant to enter torpor (Lovegrove and Raman 1998). Moreover, males displayed significantly lower thermoneutral body temperatures than females (Lovegrove and Raman 1998).

I have subsequently tested the hypothesis that the lower body temperature of males may be indicative of lower normothermic metabolic rates (Chapter 2). A lower basal metabolic rate (BMR), for example, could alleviate the requirement for daily torpor in males because their daily energy requirements would be reduced. However, I found no sex differences in these parameters at normothermia (Chapter 2), although there were pronounced sex differences particularly in the use of daily torpor under food restriction. I concluded that although the males seemed to lack compensation for their limited heterothermic capacity under food *ad lib* conditions, they reduced normothermic metabolic rates when food restricted. I further suggested that the limitation to enter torpor in males may be associated with aseasonal maintenance of functional gonads.

High levels of circulating male reproductive hormones are known to influence daily and seasonal heterothermy (Hall and Goldman 1980; Goldman et al. 1986; Darrow et al. 1987; Barnes 1996). Hibernating Turkish hamsters, *Mesocricetus brandti*, exhibited low serum testosterone levels when sampled during torpor whereas non-hibernating animals had higher levels (Darrow et al. 1987). Furthermore, Goldman et al. (1987) showed that testicular regression and hence decreased testosterone levels were necessary for initiation of hibernation as administration of testosterone inhibited torpor in castrated male hamsters. A similar response was observed in castrated ground squirrels (Lee et al. 1990).

In this study I investigated the association between gonadal activity (testosterone) and the propensity for daily torpor in male pouched mice. I predicted that the administration of testosterone would inhibit daily torpor in castrated pouched mice. I compared torpor frequency in castrated mice implanted with silastic capsules containing testosterone (experimental group) and saline (control group) in response to food *ad libitum* and food restriction treatments.

Materials and Methods

Study animals and general maintenance

Male pouched mice that had attained an age of 7-8 months and full reproductive status were used in this study. They had well-developed testes, descended into the scrotal sac. Experimental animals were implanted with testosterone-filled silastic capsules whereas control animals received saline-filled capsules. The mean \pm SD body mass of experimental animals was 76.9 ± 10.5 g, ($n = 8$), not significantly different to that of control animals, 83.8 ± 7.4 g, ($n = 8$) at the start of measurements (ANOVA; $F_{1,14} = 2.17$; $P > 0.05$).

The animals were housed in conventional rodent cages with steel mesh lids at an ambient temperature (T_a) = 25 ± 1 °C, and a 10L: 14D photoperiod, for two months prior to data measurement. They were acclimated to a short photoperiod to simulate winter conditions and thus maximize physiological readiness and propensity for torpor (Heldmaier et al. 1982; Heldmaier et al. 1985). Animals were fed an *ad libitum* diet of commercial rodent pellets, apple, lettuce and a controlled quantity of sunflower seeds. Being rich in linoleic acid, sunflower seeds were provided as a source of polyunsaturated fatty acids that optimizes membrane function during torpor and hibernation (Geiser and Kenagy 1987). Water was available *ad libitum*.

Experimental procedures

Gonadectomy, as well as telemeter and silastic capsule implantation were performed simultaneously. All surgery was performed under inhalation anesthesia (Isoflorane on oxygen; 2% induction and maintenance; flow rate *ca.* 0.5 l min^{-1}).

Gonadectomy

The scrotal sacs of anaesthetized mice were prepared with 70% alcohol and betadine solution. A small incision (*ca.* 1 cm) was made through the skin at the tip of the scrotum between the bulges of the testicles. The subcutaneous connective tissue was cleared and each testis gently squeezed out of the scrotum. A small, 5mm incision was then made into the tip of the testicle sac and the cauda epididymis, the testis, the caput epididymis, the vas deferens and the spermatic blood vessels were removed. A single ligature of silk was inserted around the vas deferens and the spermatic blood vessels, which were then severed distal to the ligature. The testis and the epididymis were then removed, the scrotum sutured and the wound treated with an antiseptic.

Preparation and implantation of silastic capsules

Silastic tubing (2mm diameter) was prepared in 10mm lengths. Testosterone-filled capsules of this length have previously been shown to inhibit torpor in similar-sized Turkish hamsters (Hall and Goldman 1980). One end of the tube was sealed with a clear silicone sealant and was allowed to dry overnight. The tubing was then filled with crystalline testosterone and sealed. Control capsules were filled with saline. The capsules were placed in saline for 24 hours before implantation. Capsules were implanted subcutaneously in the abdominal region.

Blood samples and hormone analysis

Blood samples were obtained from anaesthetized animals by cardiac puncture. Approximately 0.3 – 0.5 ml of blood was obtained and allowed to clot for a period of 24 hours under refrigeration at 4°C. Thereafter, blood was centrifuged and serum was extracted and frozen until hormone analysis. All analyses were performed at the Department of Reproduction, Faculty of

Veterinary Science, University of Pretoria, South Africa. The “Coat-A-Count” procedure (Coat-A-Count total testosterone, Diagnostic Products Company) was used. This method is a solid-phase ^{125}I radioimmunoassay, designed for the quantitative measurement of total testosterone in serum. It is based on testosterone-specific antibody immobilized to the wall of a propylene tube. ^{125}I -labelled testosterone competes for a fixed time with testosterone in the serum sample for antibody sites. The tube is then decanted, to separate bound from free testosterone, and radioactivity counted on a gamma counter. The amount of testosterone in the sample is then determined from a standard calibration curve. Typically, parallelism between the sample and the standard, (% Observed/Expected) performed at different dilutions is $86\% < \%O/E < 108\%$. The intra-assay coefficient of variation, based on 17-21 degrees of freedom per reading over a range of 0-1600ng Testosterone/dL, never exceeds 20%. The interassay coefficient of variation never exceeds 12%. The sensitivity of the assay, defined as the concentration twice the standard deviation of the buffered blank, is approximately 4ng/dL. A volume of 50 μL of sera in duplicate was used to assess the testosterone concentration.

Measurement of body temperatures

Body temperatures were measured with temperature-sensitive telemeters (Model XM. Minimitter Co., Sunriver, Oregon, accuracy 0.1°C) calibrated with a standard mercury thermometer (0.05 °C) in a water bath at temperatures from 10 - 40°C. Linear regressions were calculated from natural log-transformed transmitter pulse frequency as a function of temperature and were used as calibration curves for each transmitter. The telemeters were implanted surgically into the intra-peritoneal cavity. Radio signals from the telemeters were detected using antennae mounted on sleeves surrounding each cage connected to AM radio receivers. The signals were then

converted to square TTL waves using a monostable multi-vibrator and then converted to voltages using a frequency to voltage converter. The radio receivers were interfaced with a PC connected to an uninterrupted power supply and the data recorded every three minutes for all animals for 52 consecutive days.

Experimental protocol

After the surgical procedures animals were maintained at $T_a = 20^\circ\text{C}$ in constant environment rooms (relative humidity $\leq 60\%$) for a one-week acclimation period. Thereafter, animals were maintained at $T_a = 15^\circ\text{C}$ for the entire duration of data measurement. After one week at $T_a = 15^\circ\text{C}$ data measurement commenced. The animals were maintained on an *ad lib* diet and T_b measurements were recorded for two weeks. During this period, the daily food intake was determined as described in Lovegrove and Raman (1998). Thereafter, the food was restricted to 70% of the original diet for a further two weeks in order to induce torpor. Following the food restriction phase the diet was again restored to *ad libitum* for a further 26 days.

Blood samples were obtained on four occasions. The first sample was obtained during gonadectomy and capsule implantation and the remaining samples were taken at three-week intervals thereafter. Blood sampling was not exactly coincident with the treatment cycles because at the end of the food restriction the animals had lost a considerable amount of weight and I was concerned that cardiac puncture might be relatively stressful. Hence there was a weekly delay between the end of each treatment and blood sampling.

Data and statistical analysis

Sample sizes were reduced to $n = 7$ in both the experimental and control group by failed telemeters. Furthermore, one control animal died and one experimental animal removed its

sutures and dislodged the capsule. Data for these animals were discarded, thus restricting data analysis to 12 animals, six in each treatment.

Minimum daily T_b (T_b min) was determined for both torpid and non-torpid animals as the mean of three lowest consecutive data points (i.e. representing a period of 9 minutes) obtained during the known rest phase for this species. The minimum T_b below which animals were deemed to be torpid was 32°C, following the objective criteria used by Lovegrove and Raman (1998). Torpor bout length was determined as the total time during which T_b remained consistently below 32°C. Torpor frequency was calculated for individual animals as the proportion of the experimental days during which the animals displayed torpor.

For statistical comparisons of T_b min and torpor frequency between the two treatments, data from the first 26 days of data measurement were used, i.e. 13 days on the food *ad lib* diet and 13 days restricted diet. Data from a subsequent 13 days on the restored food *ad lib* diet were also used for the descriptive frequency distributions of T_b min.

A balanced three-way repeated measures ANOVA was used to test for effects of diet, treatment and time on T_b min. Bout lengths were not compared between experimental and control animals because there were too few observations of torpor in the former group. Repeated measures ANOVA requires balanced time-series designs, with no missing observations. Thus, because torpor was not observed on a daily basis in all animals, this test could not be employed to determine diet effects on torpor bout length. Data for torpor bout lengths were therefore pooled for both diets and the mean \pm SD was calculated. A two-way repeated measures ANOVA was used to test for treatment and diet effects on arcsine-transformed proportions of torpor frequency.

A repeated measures ANOVA incorporating a time effect was used to compare blood testosterone levels between treatments. All values are expressed as mean \pm 1SD.

Results

Body mass

At the end of food restriction the body mass of all animals had decreased by 8.6% in controls and 6.1% in experimental animals (Fig 1), but these reductions were not significant (ANOVA; $F_{1,14} = 2.17$; $P > 0.05$). By the end of data measurement (52 days), following restoration of the *ad lib* diet, all animals had regained their original body mass.

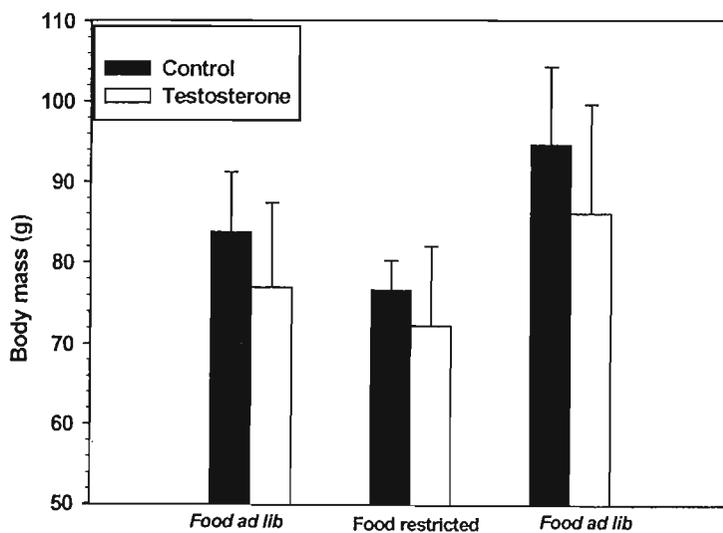


Figure 1. The mean \pm SD body mass of control ($n = 7$) and testosterone-implanted *S. campestris* at the end of each food treatment.

Torpor frequency

There were significant diet effects on torpor frequency ($F_{1,10} = 9.87$; $P < 0.05$). When food was available *ad lib* the incidence of torpor was low but increased under food restriction. On the *ad lib* diet only three control animals displayed torpor whereas no experimental animals were observed in torpor. Double circadian plots of $T_{b \min}$ of a testosterone-implanted animal (# TE13) under both diet treatments illustrates the total lack of torpor (Fig 2A).

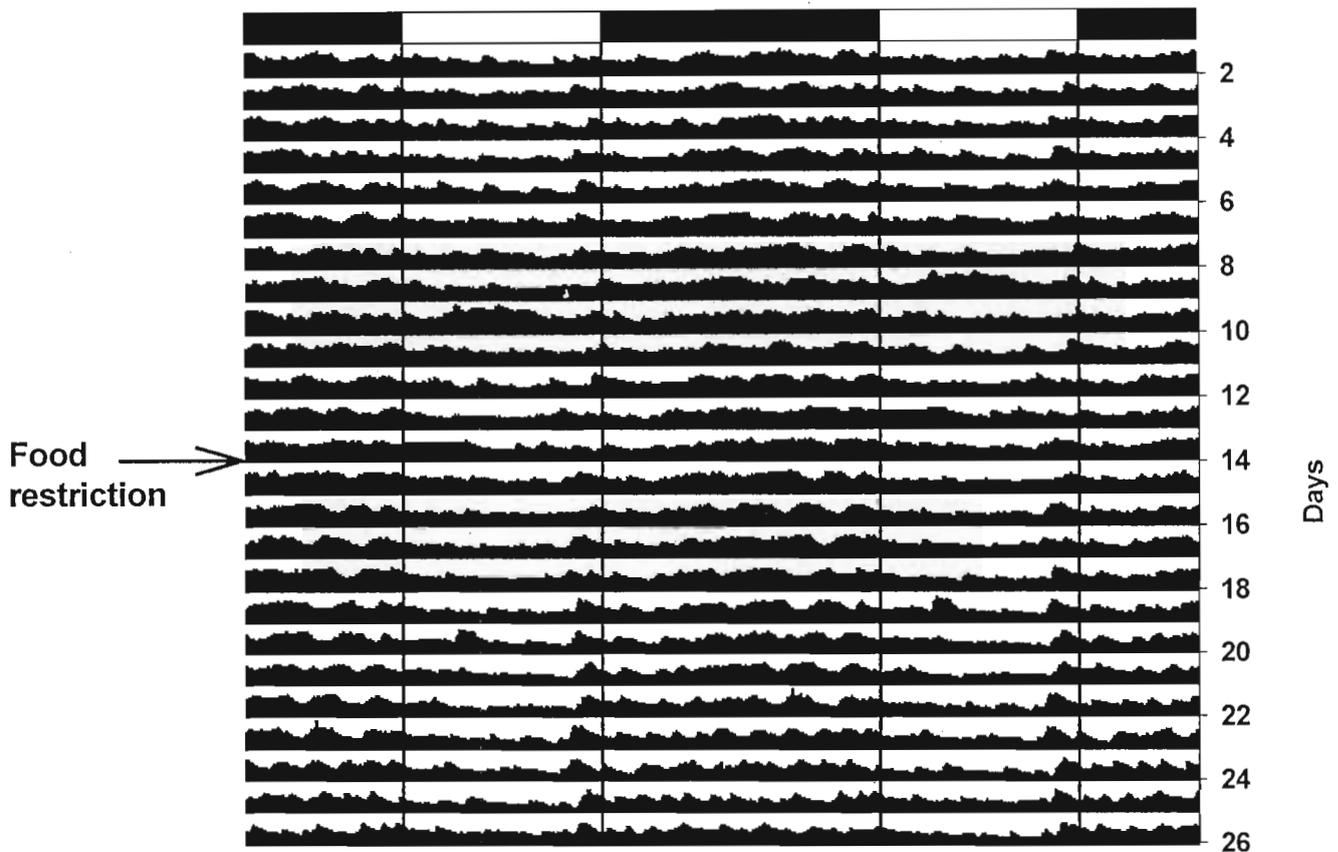


Figure 2A. Double circadian plots of body temperature (T_b) of a testosterone implanted-animal (#TE13). The dark horizontal bars on top indicate hours of scotophase. The T_b scale ranges from 32–38°C in each plot of each day.

On the other hand one control animal (#CO10), entered torpor on all thirteen days of food restriction and displayed shallower and shorter spontaneous torpor bouts on ten days of the 13-day *ad lib* treatment (Fig 2B).

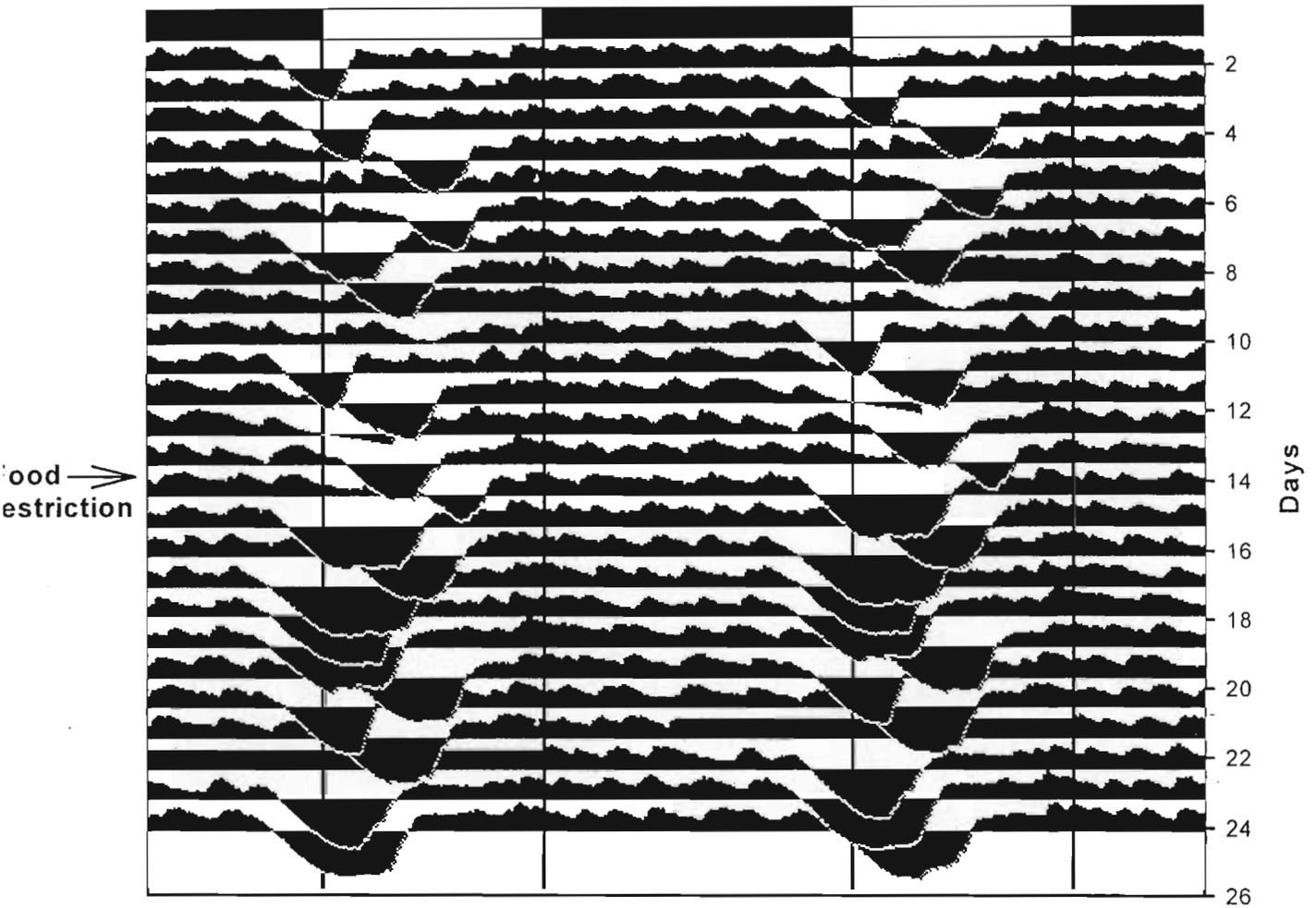


Figure 2B. Double circadian plots of body temperature (T_b) of a control animal (#CO10). The dark horizontal bars on top indicate hours of scotophase. The T_b scale ranges from 32-38°C in each plot of each day. Torpor bouts are indicated as T_b less than 32°C.

Testosterone had a significant effect on the frequency of torpor ($F_{1,10} = 6.28$ $P < 0.05$). The majority of torpid animals were observed in the control group, under both treatments (Fig 3A). Only one testosterone-implanted animal displayed torpor, exhibiting two bouts (Fig 3A). This animal became pathologically hypothermic on the second bout and had to be re-warmed with an artificial heat source.

There were also significant interactive effects of diet and treatment ($F_{1,10} = 8.38$; $P < 0.05$). In essence, more control animals became torpid under food restriction (Figs 3A and B). After the *ad lib* diet was restored, control animals continued to display daily torpor whereas only three torpor bouts were observed in experimental animals (Fig 3A).

Blood testosterone and exhibition of torpor

The testosterone level in intact males was 1.76 ± 2.14 ng/ml ($n = 10$). There were significant differences in the blood testosterone levels between the two groups following castration and capsule implantation (ANOVA; $F_{1,6} = 14.53$; $P < 0.05$). Blood testosterone decreased rapidly in the control group following castration and was virtually undetectable in the last two blood samples (Fig 3B). This decrease in testosterone concentrations corresponded with an increase in the incidence of torpor in the control animals (Figs 3A and B). Between the third and fourth sampling sessions, the *ad lib* diet had been restored but this did not terminate exhibition of daily torpor as the majority of control animals continued to employ daily torpor.

The testosterone titres of the experimental animals, however, increased to *ca.* 2-3 times those measured at gonadectomy. After attaining a peak at the onset of food restriction blood levels decreased to *ca.* 1.8 times that at gonadectomy.

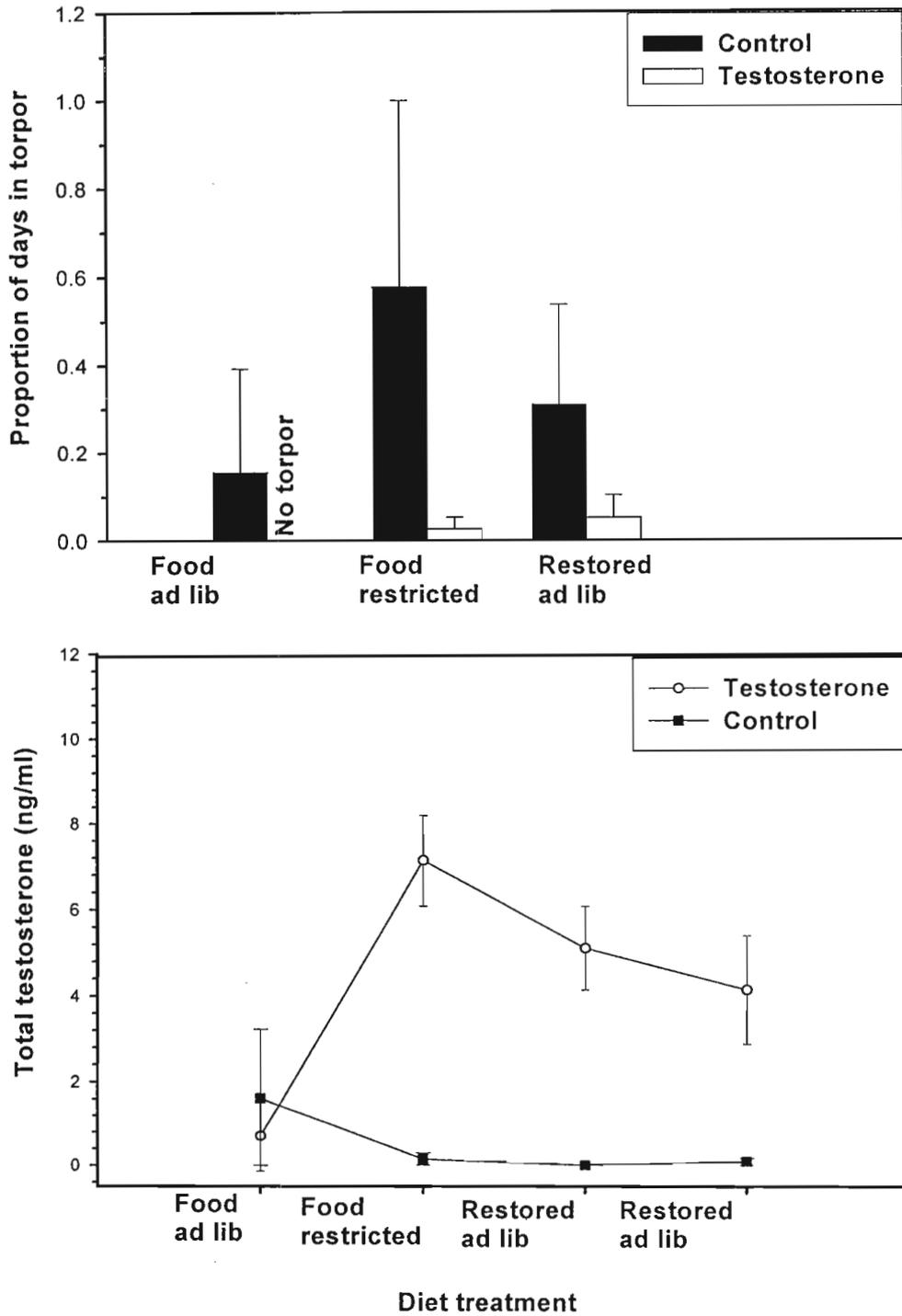


Figure 3. (A) The mean \pm SD proportion of days in which torpor bouts were recorded in control and experimental animals under the different food treatments. (B). Mean \pm SD blood testosterone concentrations during the different food treatments.

Daily minimum T_b

Diet significantly affected the daily minimum T_b attained by the animals ($F_{1,20} = 5.54$; $P < 0.05$).

Under food *ad lib* the lowest T_b recorded for a control animal was 25.9°C and 32.3°C for an experimental animal (Fig 4A). The normothermic T_b min (i.e. $T_b > 32^\circ\text{C}$) was $33.48 \pm 0.58^\circ\text{C}$ and $34.0 \pm 0.52^\circ\text{C}$ for control ($N = 6$, $n = 65$) and experimental animals ($N = 6$, $n = 78$), respectively.

During food restriction, the lowest T_b min recorded was 23.6°C for a control animal and 26.2°C for a testosterone animal, a value obtained from the single observation in which the animal could arouse by endogenous heat production. This was the only torpor bout observed for all experimental animals under food restriction (Fig 4B). The T_b min under food restriction for all control animals was $30.01 \pm 3.57^\circ\text{C}$ ($n = 78$). When normothermic animals were excluded (i.e. $T_b > 32^\circ\text{C}$) the minimum torpor T_b for the controls was $27.68 \pm 2.93^\circ\text{C}$ ($n = 45$). The mean \pm SD T_b min was $33.29 \pm 1.10^\circ\text{C}$ ($N = 6$; $n = 77$) for experimental animals.

These treatment differences in T_b min were significant ($F_{1,20} = 9.26$; $P < 0.05$). Control animals consistently displayed lower body temperatures compared to testosterone-implanted animals. Except for the one animal, the T_b of testosterone animals never decreased below 32°C, even under food restriction (Fig 4B). For the control group, the mean temperature gradient (ΔT_b) between torpor T_b min and T_a was 12.89°C.

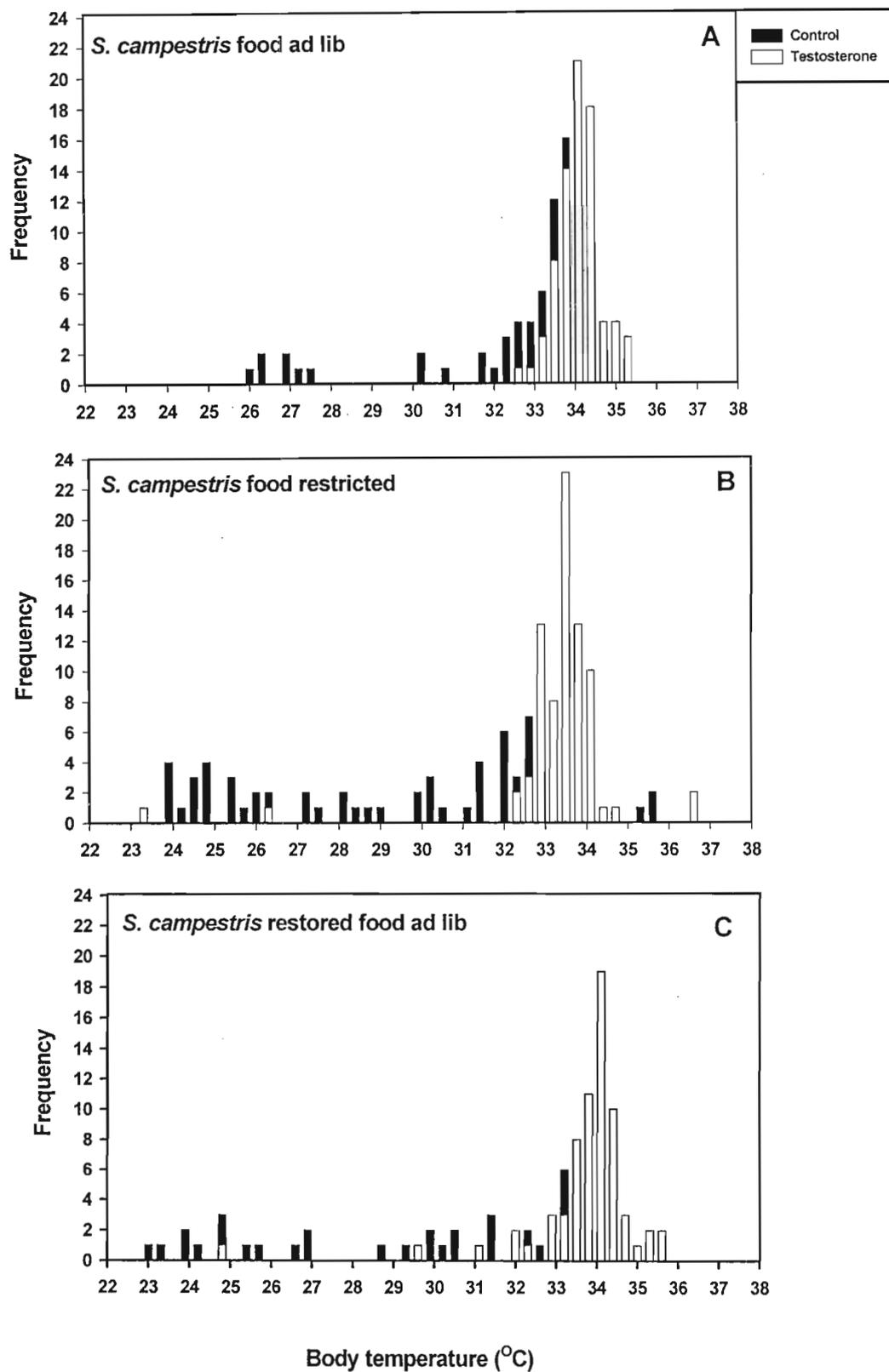


Figure 4. Frequency distributions of minimum daily body temperature of control and testosterone-implanted animals under the different diets.

Torpor bout duration

Because only two torpor bouts were observed in testosterone-implanted animals under food restriction, no statistical analyses could be performed to determine treatment and diet effects on torpor bout length. These two bouts were thus excluded from calculations of the mean \pm SD bout length. Torpor bout duration ranged from 9 – 426 minutes (Fig 5). The mean bout length excluding the restored *ad lib* period was 219 ± 107 minutes, (N = 6; n = 64, median = 241 mins). The majority of bouts lasting between 9 and 100 minutes represented shallow “test drops” because T_b seldom decreased below 30°C.

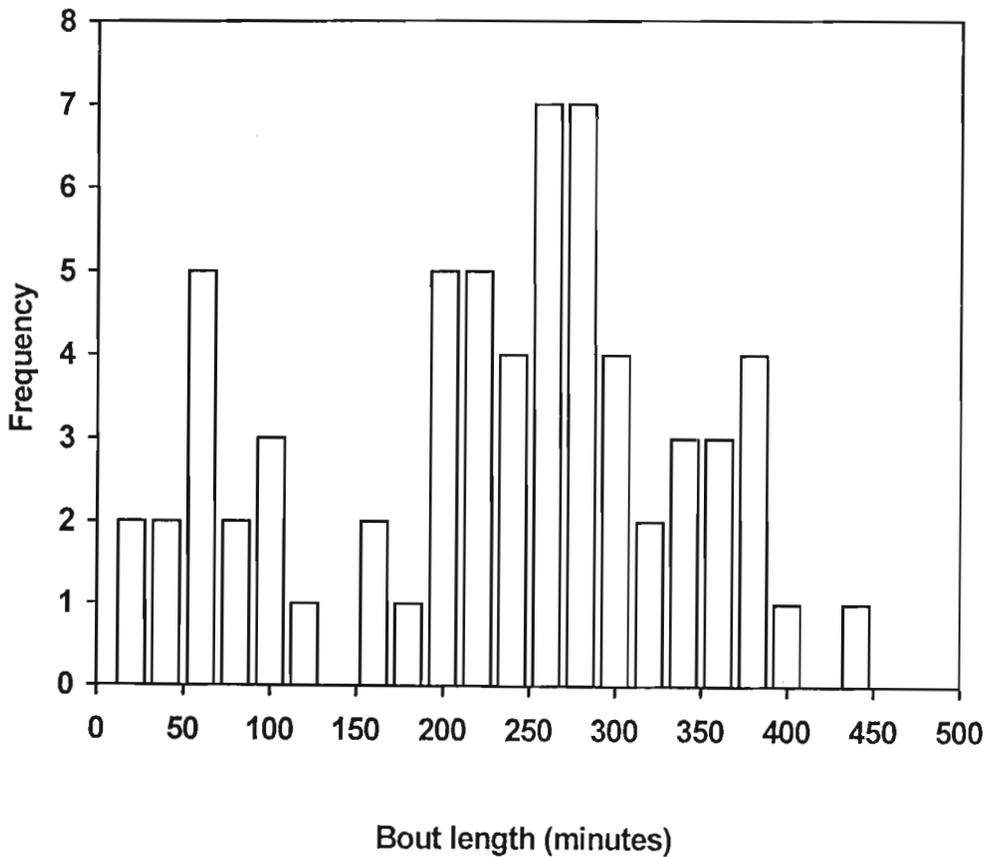


Figure 5. The frequency distribution of torpor bout duration in all control animals observed during the food *ad libitum* and food restriction diets.

Discussion

Administration of testosterone markedly affected the capacity for daily heterothermy in *S. campestris*. The control animals readily entered torpor under food restriction, whereas experimental animals very rarely displayed torpor. Moreover, torpor was more frequent and deeper in the control animals. These results are in agreement with our prediction that testosterone inhibits torpor in pouched mice.

Testosterone has previously been shown to inhibit torpor in a number of mammalian species (Goldman et al. 1986; Darrow et al. 1987; Fiest et al. 1988; Lee et al. 1990). Castrated golden mantled squirrels (*Spermophilus lateralis*), implanted with testosterone capsules do not hibernate, or have shorter torpor bouts compared to normally cycling individuals (Lee et al. 1990). In non-castrated Djungarian hamsters, *Phodopus sungorus*, decreased testosterone levels that permit hibernation are achieved by gonadal regression (Ouarour et al. 1991). Gonadal regression in temperate zones usually occurs as a response to decreasing photoperiod and low ambient temperatures, normally associated with cold, predictable winters (Bronson 1989). Reproduction in these zones occurs during predictable, long day, warm summers. It is therefore advantageous for small mammals to regress testes and hibernate, thereby coping with the energetically stressful winter.

Tropical and subtropical small mammals also breed during summer months but may utilize summer torpor in response to unpredictability of energy resources (Bozinovic and Rosenmann 1988; Coburn and Geiser 1996; Lovegrove and Raman 1998). However, for males, entry into torpor should require gonadal atrophy. Although no studies have investigated the effects of summer torpor on spermatogenesis (Grinevitch et al. 1995), summer breeding and the use of daily heterothermy during summer seem highly incompatible in males. Moreover, summer is associated with long photoperiod and warm ambient temperatures, environmental

conditions not known to trigger gonadal atrophy. Animals used in this study were acclimated to short photoperiod ($L < D$) and moderate ambient temperature (15°C) conditions to enhance physiological readiness for torpor. Hence, the torpor observed in this study may not necessarily be characterized as summer torpor. Nevertheless, this is of little consequence because Bernard and Hall (1995) reported that *S. campestris* displayed an aseasonal, non-photoresponsive and opportunistic, breeding pattern. Lack of seasonality in reproduction presumably permits breeding whenever there is sufficient calorific intake to support a breeding effort, irrespective of season (Berry and Bronson 1992). This opportunistic breeding pattern has been observed in a number of other short-lived southern African small mammals, where winters are relatively mild and rainfall unpredictable (Bronson 1989; Bernard and Hall 1995; White et al. 1997).

Bronson (1989) defines pure opportunism as the lack of environmental cues for initiation of the breeding season. He argues that it becomes more advantageous (i) as the life span and reproductive cycles shorten, (ii) as latitude of residence decreases and (iii) in areas such as desert and semi-arid environments where availability of resources is unpredictable. In the Karoo, South Africa, *Gerbillurus paeba* retains the physiological capacity for breeding throughout the year even though successful breeding is confined to the summer months (White et al. 1997).

Similarly, in South Australia a proportion of male and female bush rats, *Rattus fuscipes greyi*, retain the ability to mate throughout the year, with reproduction observed during mild winters in the wild in some individuals (White et al. 1996). Although the marsupial *Sminthopsis crassicaudata*, employs torpor under both summer and winter conditions, reproductive activity ceases under short photoperiod (Holloway and Geiser 1996). These authors suggest that reproduction and daily torpor may be controlled by different environmental factors.

Although reproduction and torpor have been suggested to be mutually exclusive, reproductively active animals may undergo torpor (Geiser and Masters 1994). Geiser and

Masters (1994) attribute the ability to enter torpor during the reproductive season to the low cost of gestation in marsupials and monotremes. They argue that neonate litters of marsupials usually weigh less than 0.3% of the mother's weight and that energetic costs during lactation in marsupials tend to be very low. In bats, fetal development is slow and energy expenditure during gestation may be much lower than in small rodents that usually have large neonate litters and a relatively short developmental period after birth, following an energetically expensive gestation and lactation period. Geiser and Masters (1994) therefore argue that mammals that spread their reproductive effort and its associated metabolic costs over a long time may display torpor during the reproductive period. In contrast, mammals with short reproductive periods adhere to strict homeothermy during reproduction to ensure fast production of offspring during times of plenty food supplies (Geiser 1996).

In a number of ground squirrel species as well as the marsupial pygmy-possum, males emerge from hibernation earlier than females (Michener 1992). This early emergence is explained as a preparation for the breeding season as it allows for sufficient re-development of testes as well as migration into female ranges (Körtner and Geiser 1998). Since the males emerge before the end of the hibernation season they usually utilize food caches as energy reserves (Barnes et al. 1986). *Saccostomus campestris* has been described as predominantly herbivorous, but has some omnivorous habits as well as granivorous tendencies (Kerley 1989; Skinner and Smithers 1990). Individuals have also been observed to store seeds in their cheek pouches. Kerley (1989) notes that although cheek pouch contents are relatively poor indicators of diet, animals possessing cheek pouches are adapted for transporting food for later consumption, or hoarding. No studies have specifically investigated the use of food caches by *S. campestris*. Nevertheless, it is possible that male *S. campestris* may cope with periods of unpredictable resource availability by using food caches.

Although control animals exhibited daily torpor, the mean torpor bout length of 3.7 hours observed for the control animals was considerably lower than that obtained by Lovegrove and Raman (1998) for female *S. campestris* (5.5 hours). It was also lower than the 11.2 hours mean for all daily heterotherms (Geiser and Ruf 1995). Furthermore, the animals in our study never decreased their T_b 's below 23°C, an observation consistent with the study of Lovegrove and Raman (1998), where minimum T_b never fell below 21°C at an ambient temperature of 15°C. Since the energetic savings accrued from torpor depend partly on the bout length (Ruf and Heldmaier 1992), it seems that *S. campestris* may not always maximize the potential benefits associated with long torpor bouts. Lovegrove and Raman (1998) argued that in summer, elevated ambient temperature cycles minimize the gradient between body and ambient temperature and thus shorten the potential bout length. They also attributed short torpor bouts to the fact that the coolest periods of the day, during which torpor would be most cost-effective, are associated with the active phase of nocturnal mammals.

Summary

This study demonstrated that the limited ability of male *S. campestris* to display torpor is a consequence of reproductive activity. The control males successfully entered torpor even though the bouts were shorter than those observed in females. On the other hand, elevated testosterone levels inhibit daily torpor and therefore a reduction circulating gonadal hormones is required before torpor can be expressed. The opportunistic, year-round maintenance of reproductive activity observed in *S. campestris* conflicts with the use of daily torpor for energy conservation by males. The difference in the response of males and females of the same species to the same energetic stresses appears to be a consequence of differences in their reproductive physiology.

In males, a period of up to six weeks is required before spermatogenesis and re-development of functional gonads can be completed after gonadal atrophy, whereas females only require a few days for follicular development and breeding readiness (Bronson 1989). Male *S. campestris* may cope with energetically stressful periods by lowering their normothermic body temperatures and metabolic rates and possibly by utilizing food caches.

Literature cited

Barnes BM (1996) Relationship between hibernation and reproduction in male ground squirrels.

In: Geiser F, Hulbert AJ, Nicol SC (eds) Adaptation to the Cold: Tenth International Hibernation Symposium. Armidale, University of New England Press, pp 71-79

Barnes BM, Kretzmann M, Licht P, Zucker I (1986) The influence of hibernation on testis

growth and spermatogenesis in the golden mantled ground squirrel, *Spermophilus lateralis*. Biol Reprod 35: 1289-1297

Bernard RTF, Hall J (1995) Failure of the estrous cycle and spermatogenesis to respond to

daylength in a subtropical African rodent, the pouched mouse, *Saccostomus campestris*.

Biol Reprod 52: 1291-1295

Berry R, Bronson FH (1992) Life history and bioeconomy of the house mouse. Biol Rev 67:

519-550

Bozinovic F, Rosenmann M (1988) Daily torpor in *Calomys musculus*, a South American rodent. *J Mamm* 69: 150-152

Bronson FH (1989) *Mammalian Reproductive Biology*. London, The University of Chicago Press

Coburn DK, Geiser F (1996) Daily torpor and energy savings in a subtropical blossom-bat, *Syconycteris australis* (Megachiroptera). In: Geiser F, Hulbert AJ, Nicol SC (eds) *Adaptations to the Cold: Tenth International Hibernation Symposium*. Armidale, University of New England Press, pp 39-45

Darrow JM, Yogev L, Goldman BD (1987) Patterns of reproductive hormone secretion in hibernating Turkish hamster. *Am J Physiol* 253: R329-R336

Fiest D, Fiest C, Lynch G (1988) The effects of castration and testosterone on thermogenesis and pelage condition in white-footed mice (*Peromyscus leucopus*) at different photoperiods and temperatures. *Physiol Zool* 61: 26-33

Geiser F, Baudinette RV (1985) The influence of temperature and photophase on daily torpor in *Sminthopsis macroura* (Dasyuridae: Marsupialia). *J Comp Physiol B* 156: 129-134

Geiser F, Kenagy GJ (1987) Polyunsaturated lipid diet lengthens torpor and reduces temperature in a hibernator. *Am J Physiol* 252: R897-R901

- Geiser F, Masters P (1994) Torpor in relation to reproduction in the mulgara, *Dasyercus cristicauda* (Dasyuridae: Marsupialia). *J therm Biol* 19: 33-40
- Geiser F, Ruf T (1995) Hibernation versus daily torpor in mammals and birds: Physiological variables and classification of torpor patterns. *Physiol Zool* 68: 935-966
- Geiser F (1996) Torpor in reproductive endotherms. In: Geiser F, Hulbert AJ, Nicol SC (eds) *Adaptations to the cold: Tenth International Hibernation Symposium*. Armidale, University of New England Press, pp 81-86
- Goldman BD, Darrow JM (1987) Effects of photoperiod on hibernation in castrated Turkish hamsters. *Am J Physiol* 253: R337-R343
- Goldman BD, Darrow JM, Duncan MJ, Yogev L (1986) Photoperiod, reproductive hormones, and winter torpor in three hamster species. In: Heller HC, Musacchia XJ, Wang LCH (eds) *Living in the cold: physiological and biochemical adaptations*. New York, Elsevier, pp 331-340
- Grinevitch L, Holroyd SL, Barclay RMR (1995) Sex differences in the use of daily torpor and foraging time by big brown bats (*Eptesicus fuscus*) during the reproductive season. *J Zool Lond* 235: 301-309
- Hall V, Goldman BD (1980) Effects of gonadal steroid hormones on hibernation in the Turkish hamster (*Mesocricetus brandti*). *J Comp Physiol B* 135: 107-114

- Heldmaier G, Böckler H, Buchberger A, Lynch GR, Puchalski W, Steinlechner S, Wiesinger H (1985) Seasonal acclimation and thermogenesis. In: Gilles R (ed) Circulation, Respiration and Metabolism. Berlin, Springer-Verlag, pp 490-501
- Heldmaier G, Steinlechner S, Rafael J (1982) Nonshivering thermogenesis and cold resistance during seasonal acclimitization in the Djungarian Hamster. *J Comp Physiol B* 149: 1-9
- Holloway JC, Geiser F (1996) Reproductive status and torpor of the marsupial *Sminthopsis crassicaudata*: Effect of photoperiod. *J therm Biol* 21: 373-380
- Kerley GIH (1989) Diet of small mammals from the Karoo, South Africa. *S Afr J Wildl Res* 19: 67-72
- Körtner G, Geiser, F (1998) Ecology of natural hibernation in the marsupial mountain pygmy-possum (*Burramys parvus*). *Oecologia* 13: 170-178.
- Lee TM, Pelz K, Licht P, Zucker I (1990) Testosterone influences hibernation in golden mantled ground squirrels. *Am J Physiol* 259: R760-R767
- Lovegrove BG, Raman J (1998) Torpor patterns in the pouched mouse (*Saccostomus campestris*; Rodentia): a model animal for unpredictable environments. *J Comp Physiol B* 168: 303-312

- Michener GR (1992) Sexual differences in over-winter torpor patterns of Richardson's ground squirrels in natural hibernacula. *Oecologia* 89: 397-406
- Ouarour A, Kirsch R, Pevét P (1991) Effects of temperature, steroids and castration on daily torpor in the Djungarian hamster *Phodopus sungorus*. *J Comp Physiol A* 168: 477-481
- Philander SGH (1983) El Niño southern oscillation phenomena. *Nature* 302: 295-301
- Ruf T, Heldmaier G (1992) The impact of daily torpor on energy requirements in the Djungarian Hamster, *Phodopus sungorus*. *Physiol Zool* 65: 994-1010
- Skinner JD, Smithers RHN (1990) The mammals of the southern African subregion. Pretoria, University of Pretoria
- Stone RC, Hammer GL, Marcussen T (1996) Prediction of global rainfall probabilities using phases of the Southern Oscillation Index. *Nature* 384: 252-255
- White RM, Bernard RTF (1999) Lack of a response of the subtropical rodent (*Saccostomus campestris*) to a secondary plant compound, 6-methoxybenzoxazolinone-consequences for a reproductive strategy. *S Afr J Zool* 34: 108-113
- White RM, Kennaway DJ, Seamark RF (1996) Reproductive seasonality of the bush rat (*Rattus fuscipes greyi*) in South Australia. *Wildlife Research* 23: 317-336

White RM, Kerley GIH, Bernard RTF (1997) Pattern and controls of reproduction of the southern African rodent *Gerbillurus paeba* in the semi-arid Karoo, South Africa. *J Arid Environ* 37: 529-549

Chapter Four

General conclusions

This study showed that *ad libitum* fed *Saccostomus campestris* display no significant sex differences in normothermic body temperatures and resting metabolic rates. However, under food restriction, there were pronounced differences. Females employed torpor more frequently and maintained lower torpor set-point body temperatures than males.

Although the animals were capable of summer torpor, it was confined to moderate ambient temperatures and was not employed at low temperatures where several animals became pathologically hypothermic. This suggests that summer torpor in this species may be used in response to resource unpredictability rather than low ambient temperatures. Furthermore summer conditions (long photoperiod) to which the animal were acclimated may inhibit sufficient physiological preparedness for torpor, such as the capacity for non-shivering thermogenesis and optimal membrane function. It remains unknown whether free ranging *S. campestris* may use diel cycles of ambient temperature to minimize the cost of arousal from torpor.

Testosterone administration inhibited torpor display in male *S. campestris*. Low testosterone blood levels were required before males could express torpor. These results demonstrate that the limited capacity for daily torpor in male *S. campestris* is a consequence of reproductive activity. It was therefore concluded that reproductive activity and use of torpor were highly incompatible in male *S. campestris*. Furthermore, the opportunistic breeding pattern observed in this species conflicts with torpor use in males.

The difference in response to the same energy stress between sexes was attributed to the differences in the reproductive physiology of the sexes. Whereas males may require a period of

several weeks for gonadal recrudescence, females only require a few days to attain breeding readiness.

This study therefore demonstrated that the ability of males to maintain functional gonads throughout the year limits their ability to use daily torpor. Whereas females derive energetic benefits from use of torpor, normothermic males may partially compensate for their limited heterothermic capacity by lowering their resting metabolic rates, accompanied by moderate reductions in body temperature when in energy shortfall. Because the animals possess cheek pouches it is also possible that males may cope with energetically stressful conditions by utilizing food caches.