1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

**Comparative Water Metabolism of Barrow Island Macropodid Marsupials: Hormonal
versus Behavioural-Dependent Mechanisms of Body Water Conservation.**

J. M. King and S.D. Bradshaw*

School of Animal Biology M092 and Centre for Native Animal Research, University of
Western Australia, Perth, WA 6009

*Corresponding author

Don.Bradshaw@uwa.edu.au

Tel: +618 9381 5010 Fax: +618 6488 1029

Running Head: Water metabolism of desert marsupials

Abstract

Seasonal variations in rates of water turnover were measured over a 7-year period in four species of macropodid marsupials, (*Lagorchestes conspicillatus*, *Bettongia lesueur*, *Petrogale lateralis* and *Macropus robustus isabellinus*), on Barrow Island off the arid Pilbara coast of Western Australia. These ranged from over 100 mL.kg^{-0.82}.d⁻¹ in wet seasons to as low as 28.2 mL.kg^{-0.82}.d⁻¹ in dry seasons in the Spectacled Hare wallaby, *Lagorchestes conspicillatus*. This marsupial species has the lowest recorded rate of water turnover of any desert mammal. Although total body water content did not vary significantly between species, plasma osmolality increased significantly in both Barrow Island euros (*Macropus robustus isabellinus*) and Spectacled hare wallabies in November 1994, in the driest year yet recorded on the Island. In contrast, there was no change in plasma osmolality of the other two species (Black-footed rock wallaby, *Petrogale lateralis* and Lesueur's burrowing bettong, *Bettongia lesueur*) that exploit cool and humid thermal refugia such as caves and underground warrens to avoid diurnal temperature extremes. Plasma levels of the marsupial antidiuretic hormone, lysine vasopressin (LVP), were for the most part below the detectable limit of the assay of 0.41 pg.mL⁻¹ in rock wallabies and bettongs, but reached high levels of 16.7 ± 4.6 pg.mL⁻¹ and 20.25 ± 5.1 pg.mL⁻¹ in euros and hare wallabies respectively in dry seasons. LVP levels were positively correlated with plasma osmolality in both euros and hare wallabies, and negatively correlated with total body water content in euros, supporting its rôle as an antidiuretic hormone in these two species. The study highlights the importance of environmental features, such as caves and underground warrens, which are critical for the long-term survival of endangered species such as the Black-footed rock wallaby and the Lesueur's bettong. These species appear to lack hormonally-controlled renal systems for the conservation of body water and are thus dependent on behavioural strategies for the maintenance of fluid homeostasis in arid environments.

Key Words: marsupial, water turnover, desert, behaviour, ADH, lysine vasopressin

1 Introduction

2 In arid environments the major environmental factor limiting the survival and
3 persistence of species is the availability of water. Animals living in these environments have
4 adapted behaviourally, physiologically and in some cases anatomically, in order to survive
5 with little or no free water for long periods of time. Reducing the amount of water lost from
6 the body enables animals to conserve water and thus reduce the potential for dehydration.
7 For an animal to remain in water balance it is necessary to control the amount of water
8 leaving the body via excretion, cutaneous evaporation and from the respiratory tract in
9 order to balance that entering the body via food, drinking and metabolic water production.
10 Many desert species are able to restrict water loss to a greater extent than temperate
11 species through explicit physiological adaptations. Some desert species, for example, are
12 able to tolerate a high loss of body water e.g., up to 30% of body mass in camels
13 (Macfarlane, Morris and Howard, 1963) and 35% of initial body mass in black Bedouin goats
14 (Shkohnik, Borut and Chosniak, 1972). This is primarily because water is lost from the gut and
15 intracellular spaces whilst the water content of the blood is maintained (Wilson, 1989).
16 (Denny and Dawson, 1975) showed that 90% of the water lost from the body during
17 dehydration in the kangaroo *Macropus rufus* (then *Megaleia rufa*) and 74% in *Macropus*
18 *robustus* was from the gut and intracellular space, whereas blood volume only fell by 7-8%.
19 Species that maintain a relatively constant blood volume attenuate the detrimental
20 cardiovascular effects associated with increased blood turgor, viscosity and osmolality and
21 are therefore more likely to survive dehydration (Borut, Horowitz and Castel, 1972; Denny and
22 Dawson, 1975; Hewitt, 1981; Siebert and Macfarlane, 1971; Wilson, 1989). A similar protection
23 of the vascular and extracellular volumes at the expense of the intracellular compartment
24 was also described in desert lizards exposed to chronic dehydration (Bradshaw, 1986, 1997a),
25 suggesting that this is a common adaptation in desert vertebrates.

26

27 Behavioural modifications may be equally effective in economising vital body water
28 reserves. By seeking refuge from the heat during the day, and adopting a nocturnal habit,

many animals are able to avoid temperature extremes in hot, arid environments, maintain a constant body temperature and thus reduce evaporative water loss. Many arid-zone mammals are crepuscular or nocturnal and rest in the shade during the hottest part of the day, feeding mainly in the early mornings and late evenings when temperatures are low and food plants have taken up moisture from the humid night air, (Ealey, 1967; Nagy, 1987, 1994; Nagy and Gruchacz, 1994; Nagy and Knight, 1994). Many small arid-adapted mammals make use of burrows which have a relatively cool, humid environment and are thus able to reduce further evaporative water loss (Bradshaw, Cheniti and Lachiver, 1976; Lachiver, Cheniti, Bradshaw, Berthier and Petter, 1978; Nagy and Gruchacz, 1994).

Water turnover, as measured by the dilution of injected tritiated water (TOH), has been shown to increase with body mass and the relationship between water influx rate and body mass is highly significant (Peters, 1983). The slope of the regression of $\log(\text{body mass})$ against $\log(\text{water flux, mL}\cdot\text{d}^{-1})$ found for 5 macropodids was 0.8 (Denny and Dawson, 1975), and 0.82 when data from 41 species of eutherians and marsupials were combined (Nicol, 1978). (Nagy and Peterson, 1988) derived an exponent of 0.71 for 18 marsupial species and (Green, 1989, 1997) reported exponents of 0.87 and 0.82 for 15 species of macropodids measured in wet and dry seasons respectively. Slightly lower exponents of 0.64 and 0.69 were reported for 20 non-arid and 9 arid marsupials by (Nagy and Bradshaw, 2000). Few of these estimates of the allometric exponent relating water turnover rate to body mass vary significantly, however, due to the relatively small sample sizes, and 0.82 has been used as a convenient average reference point in the present study that facilitates comparison with published data on eutherians mammals.

Reduction of water loss from the body through physiological means is mediated by the release of antidiuretic hormone (ADH) from the pituitary gland. Elevation in blood osmolality as a result of water loss in mammals is detected by osmoreceptors in the hypothalamus which activate the release of ADH from the neurohypophysis of the pituitary

1 into the blood (Robertson, Mahr, Athar and Sinha, 1973). ADH stimulates the re-absorption of
 2 water in the kidney which limits the amount of water that is lost in the urine thus enabling
 3 animals to conserve water and reduce the potential for dehydration.

4
 5 Lysine, rather than arginine vasopressin (AVP), has been found to be the primary
 6 neuropeptide stored in the *pars nervosa* of macropodid marsupials (Chauvet, Colne, Hurpet,
 7 Chauvet and Acher, 1983b; Chauvet, Colne, Hurpet, Chauvet and Acher, 1983a; Hurpet,
 8 Chauvet, Chauvet and Acher, 1980), as is the case with members of the Suina (Ferguson,
 9 1969; Ferguson and Pickering, 1969) and the Peru strain of mouse (Stewart, 1971). LVP, along
 10 with AVP, has also been isolated from the pituitary of a number of north and south American
 11 marsupials and both LVP and AVP occur in the pituitary of the northern bandicoot *Isodon*
 12 *macrourus* (Rouille, Chauvet and Acher, 1988). Circulating concentrations of LVP have
 13 been measured in tammar joeys (Wilkes and Jannsens, 1986) and correlate well with the
 14 development of concentrating capacities in the kidney of this species. The effect of
 15 available surface water on circulating levels of LVP in the quokka was documented by
 16 (Jones, Bradshaw, Fergusson and Watts, 1990), who concluded from this study that LVP is the
 17 physiological antidiuretic hormone in this species. Phenypressin is also found in the
 18 neurohypophysis of macropodid marsupials (Chauvet, Hurpet, Chauvet and Acher, 1980)
 19 but the study of (Bradshaw, Morris and Bradshaw, 2001) on kidney function of two species of
 20 desert wallabies indicates that LVP, rather than phenypressin, is the physiological ADH of
 21 macropodid marsupials.

22
 23 Barrow Island, off the arid northwest coast of Western Australia, harbours many
 24 marsupial species that are now extinct on the mainland and still offers a rare glimpse of the
 25 ecological relationships that existed prior to the major habitat modifications and destruction
 26 wrought by the European colonisation of Australia. Their persistence in a habitat devoid of all
 27 sources of free water suggests that their study *in situ* will provide insights into the means by
 28 which they are able to maintain body fluid homeostasis (Bradshaw, 1997b).

1

2 The aims of this study were thus twofold:

3 1. To document changes in body water content and water influx of four species
4 of macropodid marsupials occurring on Barrow Island that have differing access to low-
5 temperature and high humidity environmental refugia.6 2. To compare plasma levels of LVP in an effort to establish the significance in
7 these species of physiological homeostatic responses acting at the level of the kidney to
8 conserve body water.

9

10 *Materials and Methods*11 *Barrow Island*

12 Barrow Island (Lat 20°49'33" Long 115°26'42"E) has an area of 20,000 ha and is
13 situated 1,400 km north of Perth, 60 km off the north-west Pilbara coast of Western Australia.
14 It is a landbridge island that was separated from the mainland by rising sea levels between 8
15 and 10 thousand years ago (Dortch and Morse, 1984). Barrow Island has an arid climate:
16 average annual rainfall over the period 1967-1994 is 306.9 mm but varies markedly from year
17 to year - from 750 mm in 1974 to only 54 mm in 1994. Average maximum and minimum shade
18 temperatures in summer are approximately 35°C and 25°C respectively, and in winter are
19 25°C and 15°C. Most of the annual rainfall is associated with cyclones that occur between
20 November and April, with some rain also falling in May-June. Surface water is only present
21 when creeks and clay pans flood after rain and the island has no other sources of free water
22 available to animals. Mean annual rainfall is shown in Figure 1. Barrow Island is unique within
23 Australia in having 14 species of terrestrial mammals, 8 of which are marsupials, with 4 of
24 these being either extinct, or virtually so, on the mainland of Australia.

25

26 *Study Species*

27 Four species of macropodids (superfamily: *Macropodoidea*) were studied on Barrow
28 Island: Barrow Island euro (*Macropus robustus isabellinus*); Black-footed rock-wallaby

1 (*Petrogale lateralis*); Lesueur's bettong (*Bettongia lesueur*) and Spectacled hare-wallaby
 2 (*Lagorchestes conspicillatus*). The Barrow Island euro is a sub-species of the euro or common
 3 wallaroo (*Macropus robustus*) found in north-western Australia and eastern Australia. This
 4 sub-species is endemic to Barrow Island and is approximately half the size of the mainland
 5 form, *M. robustus erubescens*. The average mass of male and female Barrow Island euros
 6 sampled during this study was 18.1 ± 0.57 kg ($n = 29$) and 8.6 ± 0.3 kg ($n = 13$) respectively.
 7 The population estimate on Barrow Island is 1,800 individuals (Short and Turner, 1991).
 8 Animals are found over the whole island, however they are most abundant on the west of
 9 the island where there are dry creek beds, rocky outcrops and floodout flats. Individuals
 10 seek shade during the day under rocky overhangs and next to man-made structures within
 11 the oil field.

12 The Black-footed rock wallaby, *Petrogale lateralis*, occupies less than 5% of its former
 13 range on the mainland (Johnson, Burbidge and McKenzie, 1989) and is listed as threatened.
 14 Only scattered populations are now found in Western Australia, with population estimates of
 15 some of these isolated colonies as low as a few individuals. The population estimate on
 16 Barrow Island is 112-154 individuals (Hall, Onus and Kinnear, 1993), which is thought to be one
 17 of the largest remaining populations. Rock-wallabies live in rocky habitats, preferring steep
 18 rocky slopes, cliffs, and gorges where caves and overhangs are present. On Barrow Island
 19 they are restricted to the western side of the island on coastal cliffs and rocky outcrops. They
 20 use caves and overhangs as thermal refugia during the day, thereby reducing the ambient
 21 temperatures to which they are exposed and providing shelter from the wind. Average
 22 body mass of male and female *P. lateralis* sampled during this study were 3.6 ± 0.13 kg ($n = 9$)
 23 and 2.9 ± 0.74 kg ($n = 25$) respectively.

24 Lesueur's bettong or "boodie", *Bettongia lesueur*, once occurred over a large area of
 25 the arid, Australian mainland, however, it was last recorded there in the early 1960s. It is now
 26 found on four islands off the West Australian coast and is extinct on the mainland other than
 27 a recently re-introduced population in Shark Bay, W.A. This species occupies less than 0.01%
 28 of its former range, and has rare and endangered status (Short, 1994). The largest population

is found on Barrow Island, with an estimated 3,400 individuals (Short, 1994; Short and Turner, 1993). Average body mass of bettongs studied was 0.78 ± 0.02 kg ($n = 29$) and 0.73 ± 0.02 kg ($n = 20$) for males and females respectively. *Bettongia lesueur* is the only macropodid species known to construct and live in warrens. Warrens occur in natural holes in limestone cap-rock, and are also excavated in firm soil. The number of individuals inhabiting a warren may vary considerably but may be as large as 70-100. Animals seek refuge in the cool, moist warrens during the day and emerge to forage after dusk.

Spectacled hare-wallabies, *Lagorchestes conspicillatus*, were once widespread on the Australian mainland, however their distribution has been reduced in the last 50 years primarily due to clearing of land for agriculture, and introduction of exotic predators and competitors. *Lagorchestes conspicillatus* is the most abundant macropodid on Barrow Island, with an estimated population of 10,000 individuals (Short and Turner, 1991). The species commonly inhabits hummock grasslands, where large spinifex tussocks (*Triodia angusta*) provide their only refuge from high diurnal environmental temperatures.

Capture and Sampling methods

The study involved 10 field trips, each of 4-5 weeks duration, to Barrow Island from November 1990 to November 1996. Animals were captured using several different techniques including trapping, hand-netting and darting. *Petrogale lateralis* were caught using Bromilow traps (Kinnear, Bromilow, Onus and Sokolowski, 1988), which minimise injury to the animal and have been designed specifically for trapping rock-wallabies. *Bettongia lesueur* were trapped using wire box traps (Tomahawk®) placed around burrow entrances. Traps were baited with apple and universal bait (peanut butter, oats and honey). *Lagorchestes conspicillatus* were caught at night with the use of spotlights and long-handled hand-nets. Wallabies used for water turnover studies were transported immediately to an environmental laboratory on the island and held in metabolism cages for up to 12 hours in a dark, quiet room at a constant temperature of 25°C.

1 *Macropus robustus* were anaesthetised using tranquiliser darts. Two darting methods
 2 were used: a Pneu-Dart model 171C cartridge-fired rifle, equipped with 1 mL syringe darts,
 3 and 2.5 mL syringes, charged with air and fired using a 2 m blowpipe. The darts were primed
 4 with an anaesthetic mixture of 53 mg.mL⁻¹ Xylazine hydrochloride (Rompun dry; Bayer)
 5 dissolved in 100 mg.mL⁻¹ Ketamine hydrochloride (Ketamine injection; Parnell) at a dose rate
 6 of 0.1 mL.kg⁻¹. Rapid recovery was ensured by intravenous injection of Reversine (Yohimbine
 7 hydrochloride; Parnell) at a dose rate of 0.1 mL.kg⁻¹. Blood samples were collected from the
 8 tail vein of the animal within minutes of capture using ammonium Heparin or EDTA(K₃)
 9 (ethylenediamine tetraacetate, Becton Dickinson) as anticoagulant. A small number of euros
 10 (6 out of 74) was injured when being captured using explosive darts and subsequent
 11 measurement revealed unusually high levels of LVP in their plasma (126.1 ± 79.6 pg.mL⁻¹)
 12 which were excluded from any further analyses.

13 Water turnover studies were conducted on the Barrow Island populations of
 14 macropodids in November 1990, April and December 1991, December 1993, November
 15 1994, and in March, June and September 1995, and in September and November 1996.
 16 March and June 1995 were classified as "wet" trips, based on a very high rainfall of 715.4 mm
 17 that year, and all others were "dry" trips, based on prior rainfall data.

18 Bettongs, rock-wallabies and hare-wallabies were taken to the field-laboratory once
 19 captured and, after taking weight and skeletal measurements, a single injection of tritiated
 20 water (TOH) of measured radioactivity (from 310-420 µCi.mL⁻¹, 11.47-15.54 MBq.mL⁻¹) was
 21 injected intramuscularly. Rock-wallabies and hare-wallabies were injected with 1 mL of TOH,
 22 and bettongs were injected with 0.25 mL. Animals were then held in metabolism cages for 12
 23 hr to collect urine samples. A 1-2 mL blood sample was collected from the caudal vein after
 24 this time, and designated the equilibration sample (ES). The blood was centrifuged and the
 25 plasma stored at -20°C. The animals were then released at their site of capture and
 26 attempts were made to re-capture the same individuals 5-10 days later. On re-capture, the
 27 animal was again weighed and a second recapture blood sample (RS) taken, and the time
 28 between ES and RS was calculated.

Water turnover studies were carried out on *M. robustus isabellinus* in December 1991 and 1993. Animals were captured using anaesthetic darts, weighed, and injected with TOH (3 mL for males and 1.5 mL for females). The animals were kept lightly sedated for approximately 6 hr using pentothal® (thiopentone sodium - Abbott laboratories), after which time the equilibration sample was collected from the caudal vein. Euros were re-captured from 5-10 days after they had been released, re-weighed, and a second blood sample collected on re-capture.

Analysis of tritium Samples and turnover calculations

Radioactivity in plasma samples was counted to 1% error in a Packard 300CD liquid scintillation analyser. 100 µL of plasma was counted in 5 mL of scintillant (Packard Emulsifier Safe®) with appropriate correction for quenching by external standardisation. The specific activity of all plasma samples was corrected for protein content prior to calculation of total body water content by isotopic dilution. Total body water and water turnover rates were calculated using the equations of (Nagy and Costa, 1980) and the marsupial correction of (Bakker and Main, 1980) applied, where total body water = $0.92x - 0.097$ with x being the total body water estimated by isotopic dilution. Linear change in body water volume over time was also assumed (Speakman, 1997).

Radioimmunoassay of Lysine Vasopressin (LVP)

Lys⁸-vasopressin (LVP) was assayed by RIA using the method originally developed for arginine vasotocin by (Rice, 1982) and modified for the measurement of LVP in marsupial plasma by (Jones, *et al.*, 1990). Synthetic [Lys⁸]-vasopressin (Sigma V-6879) was iodinated with iodine-125 using the chloramine-T oxidation method of (Hunter and Greenwood, 1962) and a high specific activity fraction isolated on a Sephadex G25 column. The antibody used in this assay (R153) was raised in a rabbit against [Arg⁸]-vasopressin (AVP), and was kindly provided by D. Casley in the Department of Medicine, University of Melbourne. It showed high crossreactivity with LVP, AVP and phenylpressin (Phe³-Arg⁸-vasopressin). LVP in 1 mL plasma

1 samples was extracted by absorption of plasma proteins on octadecasilyl silica followed by
 2 subsequent elution with acetonitrile (Bennett, Hudson, McMartin and Purdon, 1977) using C-
 3 18 Sep-Paks® (Waters No 51910). Free and protein-bound LVP were separated by
 4 precipitation with anti-rabbit globulin (IDS AA-PPT1) and all samples were counted to less
 5 than 1% error in a Packard Prias® autogamma scintillation counter. Intra and inter-assay
 6 variations were 9.8% and 6.4% respectively. The sensitivity of the assay, defined as the
 7 minimal detection limit using the method of (Frankel, Cook, Graber and Nalbandov, 1967),
 8 was 0.41 pg.mL⁻¹.

10 *Urine Collection*

11 12-hour voided urine samples were collected under Ondina® oil (Shell) in metabolism
 12 cages and stored at -20°C until analysed. Osmolality of plasma and (1:2 diluted) urine
 13 samples was measured in 8 µL aliquots with a Wescor Inc 5100B Vapour Pressure osmometer
 14

15 *Temperature and Humidity Data*

16 Temperature and humidity data were recorded over a 3-week period in the driest
 17 part of the year (November/December) from inside a rock cave using a THIESS®
 18 hygrothermograph, and both inside a warren and within a spinifex tussock (*Triodia angusta*)
 19 using a Dataflow® 691 data logger with temperature and humidity sensors. Rainfall records
 20 for Barrow Island were obtained from the Australian Bureau of Meteorology.

22 *Statistical Analyses*

23 The distribution of all data was assessed for normality by constructing probability plots
 24 (Gnanadesikan, 1977) and, where appropriate, variables were logarithmically transformed
 25 prior to statistical analyses. Patterns of variation in turnover and hormonal data between
 26 species were investigated through analysis of variance (ANOVA) coupled with either a
 27 Student-Newman Keuls Test (SNK), Bonferroni test or Tukey HSD *post hoc* multiple
 28 comparisons. The significance of differences between selected group means was also

assessed, where appropriate, by Student's t-test. In analysing plasma LVP concentrations, non-detectable levels in the assay (ND) were assigned a notional value of 0.1 pg.mL⁻¹.

Results

Environmental Conditions of Temperature and Humidity

Figure 2 shows typical temperature and humidity records over an 8-day period in November 1993, comparing those within a bettong warren with data taken from the centre of a spinifex bush (*Triodia angusta*), and shade ambient measurements (air). The spinifex bush provides little protection from air temperatures that exceed 45°C on a number of days and contrast with the microclimate in the bettong warren where temperatures did not exceed 34°C and relative humidity averaged 70%. Figure 3 shows records taken over another 7-day period in November 1993 from a cave that was used as a diurnal refuge by rock wallabies. In this case the cave temperature remained virtually constant at 24-25°C and the relative humidity ranged from 80-95%. Mean data from these and other traces are summarised in Table 1.

Plasma and Urinary Osmolality

Table 2 summarise changes in plasma osmolality for the four species from trips grouped as either "wet" or "dry" depending on the prior and prevailing climatic conditions. Plasma osmolality at 266.6 ± 4.1 mOsm.kg⁻¹ of rock wallabies in the wet season was significantly lower than that of both hare wallabies and bettongs ($F_{3,28} = 5.844$, $P = 0.0031$) and, in the dry season, plasma osmolality of hare wallabies was significantly greater than that of the euros ($F_{3,157} = 6.856$, $P = 0.0002$). There were no significant differences between overall means for wet and dry seasons for any of the 4 species. Both euros and hare wallabies, however, had significantly elevated plasma osmolalities in November 1994, when compared with the overall means for dry trips (see Table 2). This suggests that, in this driest year ever measured on Barrow Island, rock wallabies and bettongs were better able to maintain electrolyte homeostasis than euros and hare wallabies. Table 3 lists changes in urine

osmolality for hare wallabies, rock wallabies and bettongs, again grouped by season. Urine samples were not collected from euros, which were only held for a minimum of time before being released. The only significant difference between wet and dry season data is for the hare wallaby with $t_{36} = 2.925$ and $P = 0.0059$. Urine osmolality did not vary seasonally in the case of rock wallabies and bettongs but, in both cases, the urine they voided was significantly less concentrated than that of hare wallabies in both wet and dry seasons.

Total Body Water and Rates of Turnover

Variation in total body water content (TBW) in the 4 species, by season, is shown in Table 4. In the wet period the TBW of bettongs at $59.8 \pm 1.4\%$ was significantly lower than that of the other 3 species with $F_{3,81} = 15.71$ and $P = 0.0001$. TBW increased significantly from $68.0 \pm 2.2\%$ to $78.0 \pm 2.2\%$ in euros in the dry season ($t_{14} = 3.214$, $P = 0.0062$), probably as a result of a loss of body condition, with the other 3 species remaining unchanged. Rates of water turnover of the 4 species are shown in Figure 4, grouped by wet and dry season. Hare wallabies had high rates of water turnover in the wet seasons that were significantly greater than rates measured in both rock wallabies and bettongs ($F_{3,19} = 5.725$, $P = 0.0058$). In the dry season, water turnover rates of euros and rock wallabies were similar but those of hare wallabies at $28.2 \pm 6.1 \text{ mL.kg}^{-0.82}.\text{d}^{-1}$ were much lower than all the other species ($F_{3,31} = 14.519$, $P < 0.0001$). Bettongs also had significantly higher rates than either euros or rock wallabies under these conditions.

Plasma LVP Levels

The analysis of variation in LVP levels in the 4 species was rendered difficult by the fact that, in many cases, levels were below the detection limit of the assay, which was 0.41 pg.mL^{-1} or approximately $0.4 \text{ fmol LVP per tube}$. This was particularly the case with both rock wallabies and bettongs where overall means were $0.55 \pm 0.49 \text{ pg.mL}^{-1}$ ($n = 20$) and $0.48 \pm 0.31 \text{ pg.mL}^{-1}$ ($n = 26$) respectively. The overall mean LVP level in euros (see Figure 5) was not significantly different from those of rock wallabies and bettongs ($4.2 \pm 1.3 \text{ pg.mL}^{-1}$, $n = 62$, $P >$

0.05), but there was significant variation in the data set, ranging from a peak of 16.7 ± 4.6 pg.mL⁻¹ (n = 11) in November 1994, the driest year of the study, to a low of 2.1 ± 0.6 pg.mL⁻¹ (n = 6) in June 1995, which was the wettest year of the study with a total of 715.4 mm of rain recorded ($F_{6,55} = 3.84$, $P = 0.009$). Plasma samples in hare wallabies were from 3 dry trips and levels were all high, reaching 20.25 ± 5.1 pg.mL⁻¹ in April 1991, and with an overall mean of 14.7 ± 3.2 pg.mL⁻¹ (n = 28) and no significant variation ($F_{2,25} = 0.06$, $P = 0.94$). Variation in plasma LVP levels with plasma osmolality in hare wallabies and euros is shown in Figure 6 with a positive correlation in both cases (hare wallaby LVP in pg.mL⁻¹ = $10^{0.035x}$ $r^2 = 0.583$ and euro LVP = $10^{0.016x}$ $r^2 = 0.172$). Variation in total body water content in euros was also negatively correlated with plasma LVP levels, as shown in Figure 7, where LVP in pg.mL⁻¹ = $2.881e+037x^{-20}$ with $r^2 = 0.988$.

Discussion

Australia's record as a country in terms of conservation of native fauna species ranks with the poorest in the world, with some 200 species becoming extinct within as many years following European colonisation of the continent. The importance of Barrow Island in this context is that it has been an important A Class Reserve since 1910 and still harbours a number of marsupial species that are either extinct on the mainland continent, or virtually so. Despite the fact that the island has also supported an operating oilfield since 1965, this has had little impact on the indigenous fauna of the island, with many rare species being found in large numbers (Bradshaw, 1992; Bradshaw, Morris, Dickman, Withers and Murphy, 1994; Short and Turner, 1991, 1993). This situation thus provides an unique opportunity to study a number of rare species of marsupials that have become extinct on the mainland as a result of habitat destruction and deterioration, but which still survive close to their original state on Barrow Island (Bradshaw, 1997b).

The present study extended over a period of 7 years and included, in 1994, the driest year ever recorded on Barrow Island, with only 54 mm (17.6%) of rainfall recorded, compared

1 with a long-term average of 306.9 mm. Such extreme conditions provide a rare opportunity
 2 to assess a species' ability to maintain fluid homeostasis under drought conditions in its
 3 natural environment. Critical parameters are total body water content, plasma and urinary
 4 osmolality and circulating levels of antidiuretic hormone (ADH), in this case lysine vasopressin
 5 (LVP). When data from the 2 wet trips (March and June 1995) were compared with
 6 combined data for all the dry trips, plasma osmolality did not vary significantly between any
 7 of the 4 species. If the data from the November 1994 trip were compared with the grouped
 8 data for dry trips, however, significant increases in plasma osmolality were observed in the
 9 case of both the euro ($P = 0.0014$) and the hare wallaby ($P = 0.0329$) but not for either rock
 10 wallabies or bettongs. This suggests that the two species that utilise thermal refugia (caves
 11 and deep warrens respectively) were better able to maintain fluid homeostasis during this
 12 exceptional drought in 1994. This conclusion is reinforced by the LVP data available for that
 13 trip. Levels in the euro at $16.7 \pm 4.6 \text{ pg.mL}^{-1}$ were very high and compare with plasma samples
 14 from 5 rock wallabies that were all below the detectable limit of the LVP assay of 0.41 pg.mL^{-1} .
 15 The urine of both rock wallabies and bettongs was always relatively dilute (i.e. osmolality 2-
 16 3.5 times that of the plasma) and did not change between wet and dry seasons. That of
 17 hare wallabies was always highly concentrated, with U/P_{osm} ratios averaging 7.9 in the dry
 18 seasons.

19

20 (Bradshaw, *et al.*, 2001) compared the maintenance of water and electrolyte
 21 homeostasis and kidney function in two species of wallabies in the arid Pilbara region of
 22 Western Australia, *Lagorchestes conspicillatus* from Barrow Island and the rock wallaby
 23 *Petrogale rothschildi* from Enderby Island. They found that hare wallabies evidenced a
 24 typical mammalian pattern of hormonal control of kidney function and water excretion, with
 25 plasma LVP levels correlating highly with the U/P_{osm} ratio of the urine. The rock wallaby, on
 26 the other hand, had low and constant plasma levels of LVP, regardless of the dryness of the
 27 season, and responded to water deprivation by voiding less of a similarly-concentrated urine
 28 to that elaborated in wet seasons. Similar work on the kidney function of the Barrow Island

1 Black-footed rock wallaby, *Petrogale lateralis*, has yet to be carried out but the exceedingly
 2 low plasma LVP levels recorded in this study, and the lack of any seasonal change in plasma
 3 and urinary osmolalities, suggests that it too may lack an hormonally-based control of kidney
 4 function.

5
 6 Plasma ADH levels vary from 3-6 pg.mL⁻¹ in hydrated laboratory Wistar rats (Windle,
 7 Forsling, Smith and Balement, 1993) but extraordinarily high levels of arginine vasopressin
 8 (AVP) of 479 ± 59 pg.mL⁻¹ were reported in two species of jeboas (*Jaculus orientalis* and *J.*
 9 *deserti*) maintained on a dry diet in the laboratory (Baddouri, Butlen, Imbert-Teboul, Le
 10 Bouffant, Marchetti, Chabardes and Morel, 1984; Baddouri, Marchetti, Hilali and Menard,
 11 1981). These were reported in a later study to fall to 130 ± 30 pg.mL⁻¹ when water-loaded by
 12 gavage (Baddouri, El Hilali, Marchetti and Menard, 1987) but have yet to be confirmed by
 13 other workers. Much lower levels of AVP of 6.0 ± 0.7 pg.mL⁻¹ were reported in another desert
 14 rodent (*Dipodomys spectabilis*) fed a normal grain diet (Stallone and Braun, 1988), and these
 15 increased progressively to a maximum of 68.8 ± 4.4 pg.mL⁻¹ after 192 hours dehydration
 16 during which the rodents lost approximately 20% of their initial body mass. Plasma ADH levels
 17 were first reported in the field in the marsupial tammar wallaby, *Macropus eugenii*, by
 18 (Bakker and Bradshaw, 1978; Bakker, Bradshaw and Main, 1982) using a toad bioassay and
 19 later assayed as LVP by (Wilkes and Jannsens, 1986). Water metabolism and changes in LVP
 20 levels were also studied in another small wallaby, the quokka *Setonix brachyurus*, on Rottnest
 21 Island by (Jones, *et al.*, 1990). They found that sub-populations of this species living in areas
 22 lacking all free water had significantly elevated levels of LVP, averaging 89.2 ± 19.5 pg.mL⁻¹,
 23 compared with a level of 35.6 ± 15.8 pg.mL⁻¹ in quokkas that had access to brackish drinking
 24 water on the margins of hyper-saline salt lakes.

25
 26 There is thus good evidence for LVP acting as a physiological ADH in a number of
 27 marsupials but the study of (Bradshaw, *et al.*, 2001) on desert wallabies suggests that some
 28 species, such as the rock wallaby, may lack hormonal control of renal antidiuretic systems.

Plasma LVP levels in the rock wallaby *Petrogale rothschildi* were always low (3.4 – 3.7 pg.mL⁻¹) and did not vary between wet and dry seasons on Enderby Island off the Pilbara coast of Western Australia and showed no correlation with the U/P_{osm} ratio of the urine. Renal clearance data suggest that an effective antidiuresis is achieved in this species by reduction in renal plasma flow and glomerular filtration rate (GFR), rather than by enhanced tubular reabsorption of filtrate (Bradshaw, *et al.*, 2001).

The rates of water turnover in the marsupial species studied here are all low by eutherians standards (Nagy and Peterson, 1988) and Spectacled hare wallabies averaged 28.2 mL.kg^{-0.82}.d⁻¹ over the dry trips in this study. (Bakker and Bradshaw, 1989) first reported a turnover figure of 43.5 mL.kg^{-0.82}.d⁻¹ for this species on Barrow Island with a significantly lower figure of 27.5 ± 2.0 mL.kg^{-0.82}.d⁻¹ later being recorded by (Bradshaw, *et al.*, 2001). This represents a turnover rate of less than 3.5% of the animal's total body water volume per day and reflects an extraordinary level of water economy for a mammal, sharing with the Namib desert rodent, *Petromyscus collinus*, the distinction of having the lowest rate yet measured for any species worldwide (Nagy and Peterson, 1988; Withers, Louw and Henschel, 1980). Scaling of energy and water fluxes in 6 species of Barrow Island marsupials was reported by (Nagy and Bradshaw, 2000), 4 of which overlapped with those in this study. Rates of water influx of the hare wallaby were, again, exceptionally low averaging 3.3% of the total body water content exchanged per day at 29.4 mL.kg^{-0.82}.d⁻¹. Water influx rates of euros at 67.1 mL.kg^{-0.82}.d⁻¹ were also comparable with those reported for dry trips in the present study (59.1 ± 3.8 mL.kg^{-0.82}.d⁻¹) and this was also the case for both rock wallabies and bettongs (70.6 mL.kg^{-0.82}.d⁻¹ and 85.7 mL.kg^{-0.82}.d⁻¹ respectively). Regressing water influx values on body mass for 9 species of arid-living marsupials produced a significantly lower relationship than that for 23 species of non-arid species with a reduction of approximately 35% in daily water utilisation rates. This is similar to the difference found between desert and non-desert eutherians mammals (Nagy and Peterson, 1988) and provides a convincing example of convergent evolution.

Perhaps the most significant result to emerge from this study is the extent to which behavioural avoidance mechanisms exhibited by the rock wallabies and bettongs, and the importance of their thermal refugia, underscore their ability to withstand the aridity of the Barrow Island habitat. Mean rates of water turnover of bettongs were significantly higher than those of the other 3 species in the dry trips, averaging 25.7% of the total body water content exchanged per day, compared with only 3-4% in the case of the hare wallabies and 5.5% for the euro. This situation is reminiscent of that reported during mid-summer in desert rodents in the Sahara, Israel, and in North America where water influxes typically range from 13-20% per day (Bradshaw, 2003), indicating that these desert species are not water deprived and benefit substantially from their burrowing and nocturnal habit (Ben Chaouacha-Chekir, Lachiver and Cheniti, 1983; Bradshaw, *et al.*, 1976; Degen, 1997; Degen, Hazan, Kam and Nagy, 1991; Degen, Pinshow and Ilan, 1990; Lachiver, *et al.*, 1978; Nagy and Gruchacz, 1994; Petter, Lachiver and Chekir, 1984). The impressive hormonal control of water loss from the kidney seen in the hare wallaby, and to a lesser extent in the euro, is a necessary concomitant of their lack of an adequate diurnal thermal refuge which forcibly exposes them to the full onslaught of the arid situation and prescribes the need for physiological adaptations of a high order if they are to survive.

Acknowledgements

We gratefully acknowledge the substantial assistance provided by the Western Australia Petroleum Commission (WAPET) to JMK over the course of this study. Permits to collect and study endangered species were provided by the Department of Conservation and Land Management (CALM, now DEC) and all animal procedures were approved by the Animal Ethics Committee of the University of Western Australia. Financial support was provided by the Australian Research Council to SDB and by the Department of Zoology of the University of WA. We gratefully acknowledge the assistance of Felicity Bradshaw with hormone assays.

References

- Baddouri, K., Butlen, D., Imbert-Teboul, M., Le Bouffant, F., Marchetti, J., Chabardes, D. and Morel, F. (1984) Plasma antidiuretic hormone levels and kidney responsiveness to vasopressin in the jerboa *Jaculus orientalis*. *Gen. Comp. Endocrinol.*, **54**: 203-215.
- Baddouri, K., El Hilali, M., Marchetti, J. and Menard, J. (1987) Renal excretion capacity in hydrated desert rodents (*Jaculus orientalis* and *Jaculus deserti*). *J. Comp. Physiol. B.*, **157**: 237-240.
- Baddouri, K., Marchetti, J., Hilali, M. and Menard, J. (1981) Mesure de l'hormone antidiurétique et de l'activité rénine plasmatique chez les Rongeurs désertiques (*Jaculus orientalis* et *Jaculus deserti*). *C.R. Acad. Sci. (Paris)*. **292**: 1143-1146.
- Bakker, H. R. and Bradshaw, S. D. (1978) Plasma antidiuretic hormone levels in a marsupial as measured with a toad bioassay. *J. Endocrinol.*, **76**: 167-168.
- Bakker, H. R. and Bradshaw, S. D. (1989) Water turnover and electrolyte balance of the Spectacled Hare Wallaby (*Lagorchestes conspicillatus*) on Barrow Island. *Comp. Biochem. Physiol.*, **92A**: 521-529.
- Bakker, H. R., Bradshaw, S. D. and Main, A. R. (1982) Water and electrolyte metabolism of the tammar wallaby (*Macropus eugenii*). *Physiol. Zool.*, **55**: 209-219.
- Bakker, H. R. and Main, A. R. (1980) Condition, body composition and total body water estimation in the quokka, *Setonix brachyurus* (Macropodidae). *Aust. J. Zool.*, **28**: 395-406.
- Ben Chaouacha-Chekir, R., Lachiver, F. and Cheniti, T. (1983) Données préliminaires sur le taux de renouvellement d'eau chez un Gerbillidé désertique, *Psammomys obesus*, étudié dans son environnement naturel en Tunisie. *Mammalia*. **47**: 543-547.
- Bennett, H. P. J., Hudson, A. M., McMartin, C. and Purdon, G. E. (1977) Use of octa decylsilyl-silica for the extraction and purification of peptides in biological samples. *Biochem. J.*, **168**: 9-13.
- Borut, A., Horowitz, M. and Castel, A. (1972) Blood volume regulation in the spiny mouse: capillary changes due to dehydration. *Symp. Zool. Soc. Lond.*, **31**: 175-189.
- Bradshaw, S. D. (1986) *Ecophysiology of Desert Reptiles*. Academic Press, Sydney. 324 pp.
- Bradshaw, S. D. (1992) L'Ecophysologie d'une île désertique en Australie: études de bilans énergétiques et homéostasie de vertébrés terrestres dans un milieu aride. *Bull. Soc. Ecophysiol. (Paris)*. **17**: 83-92.
- Bradshaw, S. D. (1997a) *Homeostasis in Desert Reptiles*. Springer, Berlin and Heidelberg. 213 pp.
- Bradshaw, S. D. (1997b) Water metabolism of endangered Marsupial species. IN *Advances in Comparative Endocrinology*. (Ed. Kawashima, S. and Kikuyama, S.), 1701-1705. Monduzzi Editore. Bologna, Italy.
- Bradshaw, S. D. (2003) *Vertebrate Ecophysiology: An Introduction to its Principles and Applications*. Cambridge University Press, Cambridge. 287 pp.
- Bradshaw, S. D., Cheniti, T. and Lachiver, F. (1976) Echanges hydriques chez deux rongeurs désertiques, *Meriones shawii* et *Meriones libycus* étudiés dans leur environnement naturel en Tunisie. *Bull. Soc. Ecophysiol. (Paris)*. **1**: 30-31.

- 1 Bradshaw, S. D., Morris, K. D. and Bradshaw, F. J. (2001) Water and electrolyte homeostasis and
2 kidney function of desert-dwelling marsupial wallabies in Western Australia. *J. Comp.*
3 *Physiol. B.*, **171**: 23-32.
- 4
- 5 Bradshaw, S. D., Morris, K. D., Dickman, C. R., Withers, P. C. and Murphy, D. (1994) Field
6 metabolism and turnover in the Golden Bandicoot (*Isodon auratus*) and other small
7 mammals from Barrow Island, Western Australia. *Aust. J. Zool.*, **42**: 29-41.
- 8
- 9 Chauvet, M. T., Colne, T., Hurpet, D., Chauvet, J. and Acher, R. (1983a) A multigene family of
10 vasopressin-like hormones? Identification of mesotocin, lysipressin and phenypressin in
11 Australian macropods. *Biochem. Biophys. Res. Comm.*, **116**: 258-263.
- 12
- 13 Chauvet, M. T., Colne, T., Hurpet, D., Chauvet, J. and Acher, R. (1983b) Marsupial
14 neurohypophysial hormones: identification of mesotocin, LVP and phenypressin in the quokka
15 wallaby. *Gen. Comp. Endocrinol.*, **52**: 309-315.
- 16
- 17 Chauvet, M. T., Hurpet, D., Chauvet, J. and Acher, R. (1980) Phenypressin (Phe²-Arg⁸-
18 vasopressin), a new neurohypophysial peptide found in marsupials. *Nature (Lond.)*. **287**:
19 640-642.
- 20
- 21 Degen, A. A. (1997) *Ecophysiology of Small Desert Mammals*. Springer, Berlin; New York. xii, 296
22 pp.
- 23
- 24 Degen, A. A., Hazan, A., Kam, M. and Nagy, K. A. (1991) Seasonal water influx and energy
25 expenditure of free-living sand rats. *J. Mammal.*, **72**: 652-657.
- 26
- 27 Degen, A. A., Pinshow, B. and Ilan, M. (1990) Seasonal water flux, urine and plasma osmotic
28 concentrations in free-living sand rats feeding solely on saltbush. *J. Arid Envs.*, **18**: 59-66.
- 29
- 30 Denny, M. J. S. and Dawson, T. J. (1975) Comparative metabolism of tritiated water by macropodid
31 marsupials. *Amer. J. Physiol.*, **228**: 1794-1799.
- 32
- 33 Dortch, C. E. and Morse, K. (1984) Prehistoric stone artifacts on some offshore islands in Western
34 Australia. *Aust. Archeology*. **19**: 31-47.
- 35
- 36 Ealey, E. H. M. (1967) Ecology of the euro, *Macropus robustus* (Gould) in north-western Australia.
37 CSIRO Wild. Res., **12**: 67-80.
- 38
- 39 Ferguson, D. R. (1969) The genetic distribution of vasopressins in the peccary (*Tayassu angulatus*)
40 and warthog (*Phacochoerus aethiopicus*). *Gen. Comp. Endocrinol.*, **12**: 609-613.
- 41
- 42 Ferguson, D. R. and Pickering, B. T. (1969) Argine and lysine vasopressin in the Hippopotamus
43 neurohypophysis. *Gen. Comp. Endocrinol.*, **13**: 425-429.
- 44
- 45 Frankel, A. I., Cook, B., Graber, J. and Nalbandov, A. V. (1967) Determination of corticosterone in
46 plasma by fluorimetric techniques. *Endocrinology*. **80**: 181-194.
- 47
- 48 Gnanadesikan, R. (1977) *Methods for Statistical Data Analysis of Multivariate Observations*. John
49 Wiley & Sons Inc., New York. pp.
- 50
- 51 Green, B. (1989) Water and energy turnover in free-living macropods. IN *Kangaroos, Wallabies,*
52 *Rat-kangaroos*. (Ed. Grigg, G., Jaman, P. and Harmann, D.), 222-229. Surrey Beatty &
53 Sons LTD. Sydney.
- 54
- 55 Green, B. (1997) Field energetics and water fluxes in marsupials. IN *Marsupial Biology: Recent*
56 *Research, New Perspectives*. (Ed. Saunders, N. and Hinds, L.), 143-162. University of New
57 South Wales Press. Sydney.
- 58

- 1 Hall, G., Onus, M. L. and Kinnear, J. E. (1993) *A survey of Barrow Island for the Black-flanked rock*
2 *wallaby (Petrogale lateralis lateralis)*. Department of Conservation and land Management
3 (CALM), Perth, WA. pp
4
- 5 Hewitt, S. (1981) Plasticity of renal function in the Australian desert rodent, *Notomys alexis*. *Comp.*
6 *Biochem. Physiol.*, **69A**: 297-304.
7
- 8 Hunter, W. M. and Greenwood, F. C. (1962) Preparation of iodine-131 labelled human growth
9 hormone of high specific activity. *Nature (Lond.)*. **194**: 495-496.
10
- 11 Hurpet, D., Chauvet, M. T., Chauvet, J. and Acher, R. (1980) Identification of lysine vasopressin in
12 two Australian marsupials, the Red kangaroo and the tammar. *Biochem. Biophys. Res.*
13 *Comm.*, **95**: 1585-1590.
14
- 15 Johnson, K. A., Burbidge, A. A. and McKenzie, N. L. (1989) Australian Macropodoidea: Status,
16 causes of decline and future research and management. IN *Kangaroos, Wallabies and Rat*
17 *Kangaroos*. (Ed. Grigg, G., Jarman, P. and Hume, I.), 641-657. Surrey Beatty & Sons Pty.
18 Ltd. Sydney.
19
- 20 Jones, M. E. E., Bradshaw, S. D., Fergusson, B. and Watts, R. (1990) The effect of available
21 surface water on levels of anti-diuretic hormone (lysine vasopressin) and water and
22 electrolyte metabolism of the Rottnest Island quokka (*Setonix brachyurus*). *Gen. Comp.*
23 *Endocrinol.*, **77**: 75-87.
24
- 25 Kinnear, J. E., Bromilow, R. M., Onus, M. L. and Sokolowski, R. E. S. (1988) The Bromilow trap:
26 a new risk-free soft trap suitable for small to medium-sized macropodids. *Aust. Wildl. Res.*,
27 **15**: 235-237.
28
- 29 Lachiver, F., Cheniti, T., Bradshaw, S. D., Berthier, J. L. and Petter, F. (1978) Field studies in
30 southern Tunisia on water turnover and thyroid activity in two species of *Meriones*. IN
31 *Environmental Endocrinology*. (Ed. Assenmacher, I. and Farner, D. S.), 81-84. Springer-
32 Verlag. Berlin.
33
- 34 Macfarlane, W. V., Morris, R. J. H. and Howard, B. (1963) Turnover and distribution of water in
35 desert camels, sheep, cattle and kangaroos. *Nature (Lond.)*. **197**: 270-271.
36
- 37 Nagy, K. A. (1987) How do desert animals get enough water? IN *Progress in Desert Research*.
38 (Ed. Berkofsky, L. and Wurtele, M. G.), 89-98. Rowman & Littlefield. Totowa, New Jersey.
39
- 40 Nagy, K. A. (1994) Seasonal water, energy and food use by free-living, arid-habitat mammals.
41 *Aust. J. Zool.*, **42**: 55-63.
42
- 43 Nagy, K. A. and Bradshaw, S. D. (2000) Scaling of energy and water fluxes in free-living arid-zone
44 Australian marsupials. *J. Mammal.*, **81**: 962-970.
45
- 46 Nagy, K. A. and Costa, D. P. (1980) Water flux in animals: Analysis of potential errors in the tritiated
47 water method. *Amer. J. Physiol.*, **238**: R454-R465.
48
- 49 Nagy, K. A. and Gruchacz, M. J. (1994) Seasonal water and energy metabolism of the desert-
50 dwelling kangaroo rat (*Dipodomys merriami*). *Physiological Zoology*. **67**: 1461-1478.
51
- 52 Nagy, K. A. and Knight, M. H. (1994) Energy, water and food use by springbok antelope (*Antidorcas*
53 *marsupialis*) in the Kalahari desert. *J. Mammal.*, **75**: 860-872.
54
- 55 Nagy, K. A. and Peterson, C. C. (1988) Scaling of water flux rate in animals. *Univ. Calif. Publ.*
56 *Zool.*, **120**: 1-172.
57
- 58 Nicol, S. C. (1978) Rates of water turnover in marsupials and eutherians: a comparative review with
59 new data on the Tasmanian devil. *Aust. J. Zool.*, **26**: 465-473.
60

- Peters, R. H. (1983) *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge. 329 pp.
- Petter, F., Lachiver, F. and Chekir, R. (1984) Les adaptations des rongeurs Gerbilidés à la vie dans les régions arides. Bull. Soc. bot. Fr., **131**: 365-373.
- Rice, G. E. (1982) Plasma arginine vasotocin concentrations in the lizard *Varanus gouldii* (Gray) following water loading, salt loading and dehydration. Gen. Comp. Endocrinol., **47**: 1-6.
- Robertson, G. L., Mahr, E. A., Athar, S. and Sinha, T. (1973) Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. J. clin. Invest., **52**: 2340-2352.
- Rouille, Y., Chauvet, M.-T. and Acher, R. (1988) Dual duplication of neurohypophysial hormones in an Australian marsupial: mesotocin, lysine vasopressin and arginine vasopressin in a single gland of the Northern bandicoot (*Isodon macrourus*). Biochem. Biophys. Res. Comm., **154**: 346-350.
- Shkolnik, A., Borut, A. and Chosniak, J. (1972) Water economy of the Bedouin goat. Symp. Zool. Soc. Lond., **31**: 1-13.
- Short, J. (1994) Burrowing bettong. Aust. Nat. Hist., **24(9)**: 22-23.
- Short, J. and Turner, B. (1991) Distribution and abundance of Spectacled hare-wallabies and euros on Barrow Island. Wild. Res., **18**: 421-429.
- Short, J. and Turner, B. (1993) The distribution and abundance of the Burrowing bettong (Marsupialia: Macropodoidea). Wild. Res., **20**: 525-534.
- Siebert, B. D. and Macfarlane, W. V. (1971) Water turnover and renal function of dromedaries in the desert. Physiological Zoology, **44**: 225-240.
- Speakman, J. R. (1997) *Doubly Labelled Water: Theory and Practice*. Chapman & Hall, London. 399 pp.
- Stallone, J. N. and Braun, E. J. (1988) Regulation of plasma antidiuretic hormone in the dehydrated kangaroo rat (*Dipodomys spectabilis* M). Gen. Comp. Endocrinol., **69**: 119-127.
- Stewart, A. D. (1971) Genetic variation in the neurohypophysial hormones of the mouse (*Mus musculus*). J. Endocrinol., **51**: 191-201.
- Wilkes, G. E. and Janssens, P. A. (1986) Development of urine concentrating ability in pouch of a young marsupial, the tammar wallaby (*Macropus eugenii*). Journal of Comparative Physiology, B, **156**: 573-582.
- Wilson, R. T. (1989) *Ecophysiology of the Camelidae and Desert Ruminants*. Springer-Verlag, Berlin; New York. x, 120 pp.
- Windle, R. J., Forsling, M. L., Smith, C. P. and Balement, R. J. (1993) Patterns of neurohypophysial release during dehydration in the rat. J. Endocrinol., **137**: 311-319.
- Withers, P. C., Louw, G. N. and Henschel, J. (1980) Energetics and water relations of Namib desert rodents. Sth. Afr. J. Zool., **15**: 131-145.

Figure Legends

Figure 1: Average monthly rainfall data for Barrow Island for the period 1967-1994 courtesy of the Australian Bureau of Meteorology

Figure 2: Temperature and relative humidity records over an 8-day period in November/December 1993 on Barrow Island. Continuous records were taken from within an occupied bettong (*Bettongia lesueur*) warren (Warren), from the centre of a large spinifex (*Triodia angusta*) bush (Bush) and shaded ambient temperature 1 m above ground (Air).

Figure 3: Temperature and relative humidity records from a cave on Barrow Island used as a daytime refuge by Black-footed rock wallabies (*Petrogale lateralis*) (Cave), compared with shaded ambient temperature 1m above ground (Air).

Figure 4: Comparison of mean rates of water influx in $\text{mL.kg}^{-0.82}.\text{d}^{-1}$ in four species of macropodid marsupials on Barrow Island in wet and dry seasons. The species are euro = *Macropus robustus isabellinus*, Hare wallaby = *Lagorchestes conspicillatus*, Rock wallaby = *Petrogale lateralis*, and Bettong = *Bettongia lesueur*. Data are presented as Mean $\pm$ SE.

Figure 5: Comparison of mean circulating levels of the antidiuretic hormone, lysine vasopressin (LVP) in four species of macropodid marsupials on Barrow Island. The species area as detailed in Figure 4 and the data are presented as Mean $\pm$ SE in pg.mL^{-1} .

Figure 6: Variation in plasma levels of lysine vasopressin (LVP) in pg.mL^{-1} in Barrow island euros, *Macropus robustus isabellinus* and hare wallabies, *Lagorchestes conspicillatus*, in relation to plasma osmolality.

Figure 7: Variation in plasma levels of lysine vasopressin (LVP) in pg.mL^{-1} in Barrow Island euros, *Macropus robustus isabellinus*, in relation to changes in total body water content (%TBW). Equation for the power curve is $y = 2.881 \text{e}+037x^{-20}$ with $r^2 = 0.988$ where y = plasma LVP level and x = TBW in mL.100g^{-1} .

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5:

Figure 6:

Figure 7:

Table 1: Microclimatic data from a Rock wallaby cave and a bettong warren on Barrow Island recorded in November/December 1993. Comparative ambient temperatures and relative humidity are also given under AIR

	CAVE		AIR	
	Temperature (°C)	Humidity (%)	Temperature (°C)	Humidity (%)
Mean ± SD	23.9 ± 0.96	89.6 ± 6.9	26.9 ± 3.8	68.9 ± 16.0
Maximum	25.5	97.0	37.0	92.0
Minimum	22.0	65.0	21.5	22.0

	WARREN		AIR	
	Temperature (°C)	Humidity (%)	Temperature (°C)	Humidity (%)
Mean ± SD	29.1 ± 1.59	68.6 ± 8.61	30.5 ± 6.62	50.8 ± 18.85
Maximum	33.8	83.8	46.7	94.8
Minimum	25.2	45.3	20.5	13.1

Table 2: Seasonal variation in plasma osmolality (mOsm.kg⁻¹) of four species of macropodid marsupials on Barrow Island. Data expressed as Mean $\pm$ SE (n). * P = 0.0329, *** P = 0.0014 data for November 1994 compared with overall means for dry trips for each species. NS = not significant with P > 0.05.

Species	Plasma Osmolality (mOsM.kg ⁻¹)			Significance Wet vs Dry
	Wet	Dry	Nov 1994	
Euro	274.2 ± 4.5 (5)	268.9 ± 2.1 (55)	NS	285.9 ± 4.4 (11)***
Hare wallaby	287.0 ± 1.7 (8)	283.2 ± 1.6 (70)	NS	288.7 ± 1.7 (36)*
Rock wallaby	266.6 ± 4.1 (13)	271.8 ± 9.6 (16)	NS	288.1 ± 5.3 (10)
Bettong	281.7 ± 4.2 (10)	277.2 ± 4.8 (24)	NS	282.0 ± 5.7 (3)

Table 3: Osmolality of voided urine in mOsm.kg⁻¹ of three species of macropodid marsupials on Barrow Island during the dry season of the year, Data expressed as Mean $\pm$ SE (n). NS = not significant with P > 0.05.

Species	Urine Osmolality (mOsm.kg ⁻¹)		Significance
	Wet	Dry	
Hare wallaby	1239 $\pm$ 21 (2)	2256 $\pm$ 81 (36)	P = 0.0059
Rock wallaby	840 $\pm$ 81 (5)	1018 $\pm$ 292 (2)	NS
Bettong	741 $\pm$ 6.5 (5)	870 $\pm$ 134 (5)	NS

Table 4: Variation in total body water content as mL.100g⁻¹ (%) of four species of macropodid marsupials on Barrow Island – wet versus dry season comparison. Data expressed as Mean $\pm$ SE (n)

Season	Euro	Hare Wallaby	Rock Wallaby	Bettong
Wet	68.0 $\pm$ 2.2 (8)	66.6 $\pm$ 1.0 (25)	70.7 $\pm$ 1.0 (27)	59.8 $\pm$ 1.4 (22)
Dry	78.0 $\pm$ 2.2 (8)	66.0 $\pm$ 1.1 (20)	70.7 $\pm$ 1.1 (17)	60.1 $\pm$ 1.8 (16)