Ecophysiology of Australian Arid-Zone Marsupials

S. Don Bradshaw

> *Short-sighted indeed are the Anglo-Australians, or they would long ere this have made laws for the preservation of their highly singular, and in many cases noble, indigenous animals: and doubly short-sighted are they for wishing to introduce into Australia the production of other climes…. Let me then urge them to bestir themselves, ere it be too late, to establish laws for the preservation of the large kangaroos, the Emu and other conspicuous indigenous animals: without such protection the remnant that is left will soon disappear, to be followed by unavailing regret for the apathy with which they have been previously regarded.* (Gould 1863)

Introduction

The suite of marsupials now found inhabiting the arid zone is vastly reduced compared with that which confronted the first European settlers in the late eighteenth and early nineteenth centuries (Woinarski et al. 2015; Burbidge 2009). Early explorers of the arid zone described how they needed to erect barriers around their tents to prevent marauding 'rat kangaroos' from raiding their stores of biscuits and other provisions. The situation is vastly different today. In the Little Sandy Desert in Western Australia, for example, of the 43 mammal species present at the time of European settlement, 19 are still present, but a further 19 marsupial species and 6 rodents once collected are now regionally extinct (Start et al. 2013; Burbidge et al. 2007).

The loss of species has not been uniform but focuses on marsupials in what has become known as the 'critical weight range' (CWR) of 35 g–5.5 kg (Burbidge and McKenzie 1989). Why species in this particular weight range are more vulnerable than others remains a subject of continued interest and debate, with some arguing the correlation reflects simply the fact that the majority of recent Australian mam-

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mals fall within this size range (Cardillo and Bromham 2001), with another phylogenetic analysis failing to find any effect of body mass on the pattern of decline among marsupials (Fisher et al. 2003).

A recent careful analysis has shown that the form of the CWR relationship differs for ground-dwelling versus tree-dwelling species and for species from low- versus high-rainfall areas (Johnson and Isaac 2009). Australian mainland marsupials in the CWR were found to be most extinction prone, and the effect was most pronounced in ground-dwelling species from areas with low annual rainfall (i.e. the arid zone). This conclusion has also been reinforced by a broad analysis of extinction factors showing that faunal losses have been greatest in arid regions and lowest in high-rainfall areas (McKenzie et al. 2007) and by a recent analysis arguing that the CWR is also relevant for species in tropical areas of Australia (Murphy and Davies 2014).

As was the case when considering arid-zone reptiles in Chap. 6 arid-zone marsupials must contend with the same environmental contingencies characteristic of arid environments, viz.:

(a) High day temperatures that must be tolerated, regulated or avoided
(b) A shortage of available free water that renders difficult
(c) The regulation of water and electrolyte balance
(d) Seasonal deficiencies of nitrogen (protein) and energy (carbohydrate and fat) essential for growth and reproduction

The accepted explanation for the vulnerability of CWR species in the arid zone is that they are prime targets for the two major marsupial predators, feral cats and foxes (Burbidge and McKenzie 1989). Some or all of the factors listed above, however, may also be involved, as failure to deal adequately with one of these may predispose an individual to enhanced levels of predation. It is thus important to be able to discern whether a given species is ever exposed to stressful environmental conditions (e.g. heat and drought) that reduce its physiological condition and make it an easy target for predators.

The Nature of Stress

Documenting the incidence and measuring the intensity of stress responses in free-ranging marsupials should be a conservation priority but is as yet a field in its infancy (Bradshaw 2007, 2010, 2017). One of the main difficulties is clarifying what is meant by stress after it was first defined, somewhat poetically, by Hans Selye as *a state of non-specific tension in living matter* (Selye 1946, 1952). An essential step is to distinguish ‘stressors’, emanating from the environment, from ‘stress’, which is the impact of these on the organism, thus avoiding meaningless contradictions such as ‘the stress of reproduction’.

The medical concept of ‘allostasis’ or ‘maintaining stability through change’ (Sterling and Eyer 1988) was adopted by McEwen (1998), who proposed that animals experience an ‘allostatic load’ divided into three levels: A = baseline cortico-

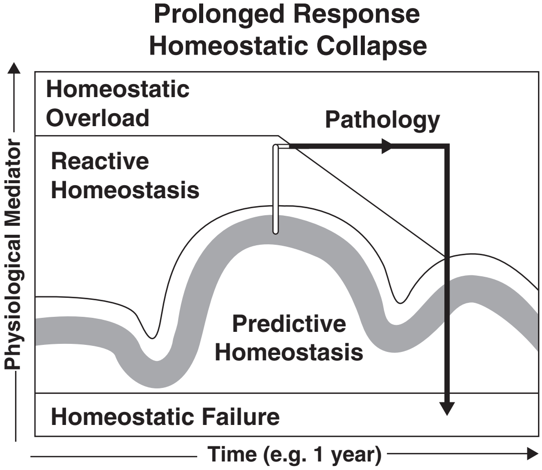

Fig. 1 The 'Reactive Scope Model' of stress. Diagrammatic representation of the impact of prolonged stressors on 'wear and tear' as depicted by a progressive decrease in the threshold between reactive homeostasis and homeostatic overload or stress. (From Romero et al. 2009 with permission)

steroid levels, B = daily and seasonal changes in levels and C = 'allostatic overload' leading to chronic stress if not alleviated (McEwen and Wingfield 2003). The use of the balance between energy expenditure and intake as a measure of allostatic load has been criticised by Walsberg (2003), and Michael Romero has countered with an innovative 'reactive scope model' of stress (Romero et al. 2009; Romero 2012). This is based on the concept that reacting to a stress (reactive homeostasis or level B in the allostatic system) involves 'wear and tear' and erodes the safety gap between B and C, leading with chronic exposure to allostatic overload (Fig. 1).

Whatever system one uses, it is obviously important to be able to discern when individuals in natural populations cross the threshold between homeostatic adaptive responses to environmental stressors and experience 'allostatic overload' or stress. A recent review of factors influencing the stress response in Australian marsupials, for example, documents studies that have measured either plasma or faecal levels of glucocorticoids (Hing et al. 2014). Elevated steroid levels alone, however, are not necessarily an index of stress (Johnstone et al. 2012) as they form part of the animal's normal adaptive response to the stressor (Angelier and Wingfield 2013). Opinion is also still divided on whether total levels or non-protein-bound 'free' levels of corticosteroids are what should be measured in studies of stress (Breuner et al. 2013; Schoech et al. 2013, Desantis et al. 2013).

An Operational Definition of Stress

An early simple operational definition proposed for stress, which avoids some of the difficulties referred to above, is 'the physiological resultant of demands that exceed an animal's homeostatic capacities' (Bradshaw 1997, 1986, 2003). This allows one to both discern the incidence of stress and measure its severity. Inherent in the definition is the understanding that the animal has maximally deployed whatever defences it has against the stressor and that, despite this, its internal state or ‘milieu intérieur’ has been significantly perturbed. By simultaneously monitoring the extent of the animal’s adaptive response to a stressor (e.g. levels of adrenocortical hormones, antidiuretic hormone) along with measures of its internal state (e.g. total body water content, plasma osmolality, body temperature), one can effectively detect individuals that are in stress. The primary aim of this review is to use ecophysiological data to identify, where possible, instances of arid-zone marsupials evincing stress response to environmental stressors.

High temperatures and lack of water characterise the arid zone, and when considering the relative impact of the four environmental constraints experienced by marsupials listed above, these two would appear to be the most relevant as loss of CWR species has been highest in the arid zone since European colonisation.

Temperature Regulation

‘Adaptive Heterothermy’

There have been few studies of thermoregulation of free-ranging marsupials in the Australian arid zone, but adaptive heterothermy, described for eutherian mammals, has been suggested as a response helping to ameliorate high diurnal temperatures (McCarron and Dawson 1989; Brown and Dawson 1977; McCarron et al. 2001). Pioneering research by Knut Schmidt-Nielsen with camels (Schmidt-Nielsen et al. 1956) and Richard Taylor on East African ungulates (Taylor 1970) established the paradigm of large arid-zone mammals using heterothermy to conserve water by storing heat in the body during the day, which it then lost by radiation at night (Bradshaw 2003). Added to this were studies suggesting that in those species possessing a carotid rete, such as the eland, selective brain cooling operates to protect cerebral function from high core body temperatures (Taylor and Lyman 1972). Recent studies, however, incorporating body temperature recording from free-ranging animals in desert conditions (bio-logging) have questioned this established view. Body temperatures of African antelopes, for example, are higher at night than during the day (Mitchell et al. 2002). Selective brain cooling as an adaptive response has also been questioned. It has never been observed in free-living mammals and may be a response restricted to captive animals exposed to heat or exercising on a treadmill (Fuller et al. 2014). In a relatively recent review, the authors comment that ‘With the possible exception of the camel, we doubt that arid-zone mammals use adaptive heterothermy at all.’ (Mitchell et al. 2002).

There have been a number of studies of changes in diurnal body temperatures of resting arid-zone kangaroos carried out by Terry Dawson and colleagues at the Fowlers Gap research station in northern New South Wales. These enable us to pose the question of whether these large macropods ever give evidence of stress resulting from high environmental temperatures. Using the operational definition of stress

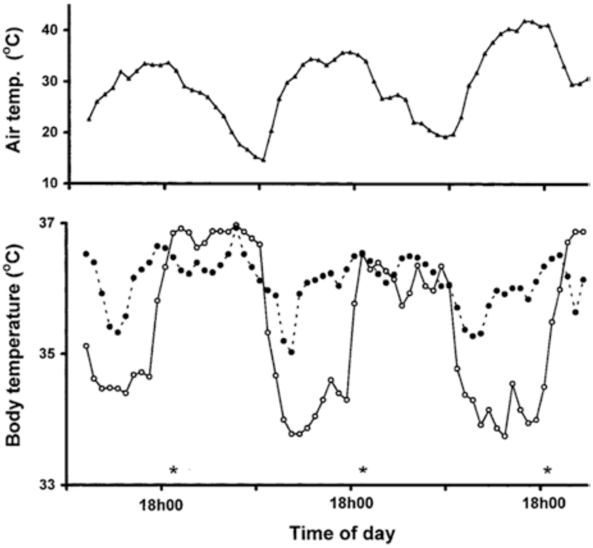

Fig. 2 Diel changes in air temperature and the comparison of the nychthermal cycle in deep body temperature under ad libitum conditions for a representative eastern grey kangaroo (open circles) and red kangaroo (solid circles). The asterisk indicates the time of day at which eastern grey kangaroos went to drink. (From McCarron et al. 2001 with permission)

above, this would mean finding evidence of a significant elevation of the body temperature above what is considered to be the normal range, despite the deployment by the kangaroo of its full suite of physiological and behavioural defences (e.g. shade seeking, sweating, saliva spreading). Thermoregulatory responses of mesic eastern grey (*Macropus giganteus*) and arid red kangaroos (*Macropus rufus*), held in small pens, to ambient temperatures ranging from –5 to 45 °C were compared by Dawson et al. (2000). Both species maintained their body temperature within the normal range of 36.5–37 °C; the only difference was the slightly lower rates of respiratory evaporative water loss of the red kangaroo at 45 °C. Somewhat different results were obtained when the same two species were compared while ‘free-ranging’ in an 8 ha enclosure with access to water (McCarron et al. 2001). Body temperatures of the eastern grey kangaroos were approximately 2 °C lower than those of red kangaroos during the middle of the day and 0.3 °C higher at night (Fig. 2). The daily body temperature range of the mesic grey kangaroos was thus higher than that of red kangaroos with access to water (2.76 °C vs 1.72 °C) and much higher when denied access to water (4.70 °C vs 2.29 °C). Even so, the mean body temperature of the eastern greys when denied access to water was still only 36.55 ± 0.40 °C, which would not appear to be above its ‘normal’ range and thus not supporting a case for thermal stress. The eastern grey, which is normally thought of as a ‘mesic’ species, has recently extended its range into the arid zone (Dawson 1995; Dawson et al. 2006), but the adequacy of its thermoregulatory response would appear to enable it to do this without experiencing stress responses or ‘allostatic overload’.

A major and obvious difference between eutherians and marsupials is that the latter, with one exception (the numbat), are essentially all nocturnal, or at most crepuscular. This means that they are not normally exposed to the full onslaught of the arid situation as they are typically active and forage at dawn and dusk and throughout the night. Although there are few data on the subject, it seems likely that adaptive heterothermy in the 'classical' context (i.e. as a response to high diurnal temperatures) is absent in arid-zone marsupials, but what is very clear is that heterothermy is very important in the group as a response to low, rather than high, temperatures. Hibernation and daily torpor (i.e. temporal heterothermy) have been reported in approximately 15% of all marsupials with data from 41 species included in a recent review (Riek and Geiser 2014). Deserts and arid zones are rather cold at night, because of the lack of cloud cover and the high levels of radiation to the sky, and temperature ranges between night and day may be as high as 60 °C (Monod 1973). Nocturnal animals, such as marsupials inhabiting the arid zone, must thus contend with potential heat losses during their prime activity period.

Torpor

Although it might be thought that mammals do not need to utilise torpor in a 'warm' country such as Australia, there is in fact a large body of literature on the incidence and intensity of torpor in Australian marsupials, and it is clear that arid-zone species, especially small insectivorous dasyurids, employ it routinely (Geiser 2004; Riek and Geiser 2014). Known heterothermic dasyurids range in body size from 6 g planigales (*Planigale* spp.) to the western quoll, *Dasyurus geoffroii*, weighing almost a kilogram (Geiser and Kortner 2010). Minimum body temperatures during torpor range from 11 to 15 °C in the smaller species (<100 g) and from 15 to 23 °C in larger species (>100 g) with the metabolic rate typically falling to 30–50% of the basal metabolic rate (Geiser 2004). Torpor also has a considerable impact on the water economy of arid-zone marsupials, with a reduction in absolute evaporative water loss (EWL) from 23.5 to 42.3% of normothermic values reported for the stripe-faced dunnart, *Sminthopsis macroura*, in a recent laboratory study (Fig. 3) (Cooper et al. 2005).

It is perhaps not surprising that arid-zone species using torpor are primarily small carnivores that depend on food sources such as grasshoppers, termites and alates that often fluctuate in availability. In captivity, torpor can be readily induced in dasyurids by reducing the ambient temperature but also by withholding food (Geiser 2003). Field data from *Pseudantechinus macdonnellensis*, *Planigale gilesi* and the mulgara, *Dasycercus blythi*, add weight to this. Long torpor bouts of 20 h were initiated in up to 100% of days during winter in these free-ranging desert dasyurids when food supplies were low (Geiser and Pavey 2007; Pavey and Geiser 2008; Kortner et al. 2008b; Warnecke and Geiser 2009). Torpor bouts in the field may vary from a little as 2 h to as long as 26 h in the stripe-faced dunnart (*Sminthopsis macroura*) (Kortner and Geiser 2011). Female brush-tailed mulgara (*Dasycercus*

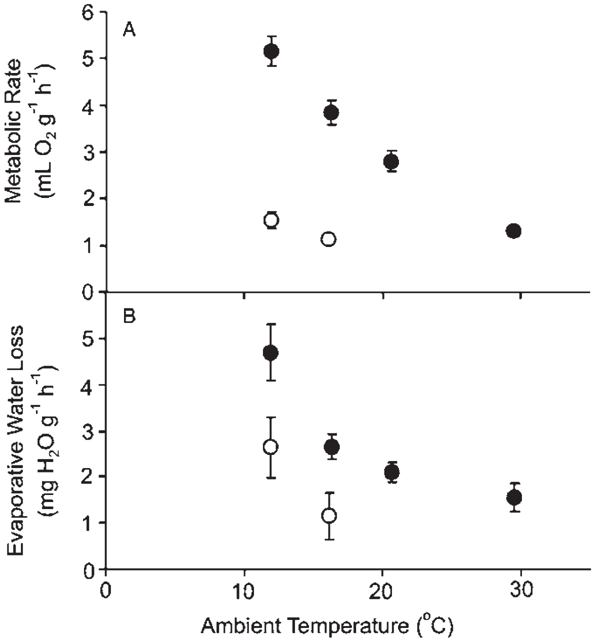

Fig. 3 Metabolic rate (**a**) and evaporative water loss EWL (**b**) of normothermic (dots) and torpid (circles) stripe-faced dunnarts (*Sminthopsis macroura*) at various ambient temperatures. Values are mean ± SE and *n* = number of observations. For normothermic dunnarts, *n* = 6 at T_a = 11–20 °C and *n* = 10 at T_a = 30 °C; for torpid dunnarts, *n* = 6 at T_a = 11 and *n* = 10 at T_a = 16 °C. (From Cooper et al 2005 with permission)

blythi) employ torpor throughout most of their pregnancy, but not during lactation (Kortner et al. 2008a). These studies on free-ranging individuals, equipped with temperature-sensitive radio-transmitters, have shown that daily torpor is a crucial survival strategy for small dasyurids in the arid zone during the cold months of the year and contributes to their long-term persistence in these habitats (Kortner and Geiser 2009; Masters and Dickman 2012; Dickman et al. 2001). A recent study on the 27 g kaluta (*Dasykaluta rosamondae*) in the Pilbara region of Western Australia has revealed that, during the colder winter months, animals were essentially diurnal and retreated into burrows overnight (Kortner et al. 2010). Torpor also differed between males and females, with females torpid at body temperatures of 21 °C throughout the night, whereas males entered multiple short and shallow bouts during the night (Fig. 4). The sandy inland mouse, *Pseudomys hermannsburgensis*, also displays an unusual response to low environmental temperatures, falling into torpor but apparently lacking the ability to arouse spontaneously (Tomlinson et al. 2007). As spontaneous arousal forms part of the accepted definition of ‘torpor’ (Lyman et al. 1982), this appears to be an instance of ‘adaptive hypothermia’ in an arid-zone marsupial, rather than torpor.

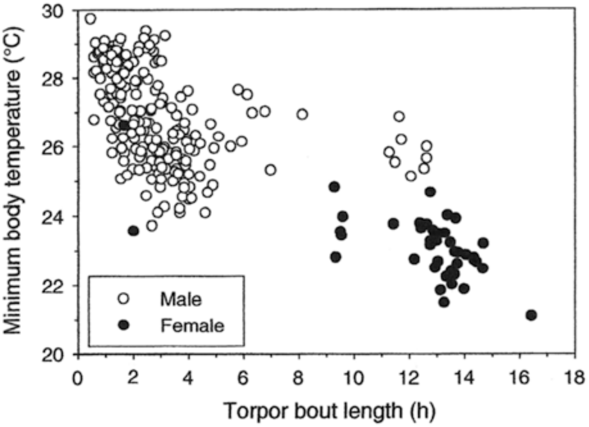

Fig. 4 Relationship between minimum T_b during torpor and torpor bout length in free-ranging male and female Kaluta (*Dasykaluta rosamondae*). (From Kortner et al. 2010 with permission)

Given the substantial change in the milieu intérieur of the torpid animal (i.e. the dramatic fall in body temperature and reduced metabolic rate), it is reasonable to ask whether torpor is a stress response. Our 'operational definition' of stress 'the physiological resultant of demands that exceed an animal's homeostatic capacities' mandates, however, that the animal is 'attempting' to maintain its normal level of homeostasis, despite the stressor. In the case of torpor, the animal clearly 'abandons' the normal physiological processes that maintain its body temperature within a narrow range, allowing it to fall close to ambient levels. Recent work on the endocrinological and molecular mechanisms underlying torpor in mammals has identified leptin as a key factor in initiating recovery from torpor bouts. Leptin, a peptide hormone, is produced by lipid tissue and is thought to relay nutritional status from peripheral fat stores to sites within the hypothalamus, especially the arcuate nucleus (Sahu 2003). The other hormone that has been implicated is ghrelin, secreted by the stomach which activates neuropeptide Y neurones (NPY) in the arcuate nucleus, leading to increased food intake and rate of metabolism (Swoap 2008). Thyroidectomy has also long been known to disrupt torpor in mammals by uncoupling mitochondrial heat production (Bechtold and Loudon 2007), and recent research has found that type 3 deiodinase reduces the availability of tri-iodothyronine (T_3, the active form of the hormone thyroxine) in the hypothalamus, initiating torpor in Siberian hamsters (Murphy et al. 2011). Recent studies implicate the melatonin-expressing cells in the pars tuberalis of the pituitary as the source of the information passed to hypothalamic tanycytes, where the deiodinase is expressed (Scherbarth and Steinlechner 2010). All this research has been undertaken in eutherian mammals, but data suggesting that leptin levels may also be involved in the initiation of torpor in marsupials is supported by a study with the stripe-faced dunnart, *Sminthopsis macroura*, in which leptin treatment halved the duration of torpor bouts in animals deprived of food for 24 h (Geiser et al. 1998).

Torpor in marsupials is thus not a stress response[1] but an apparently adaptive regulated response to an unfavourable energy balance, whereby normal homeostatic maintenance of body temperature by increased metabolism is temporarily uncoupled, allowing the body temperature to fall with significant savings in energy expenditure. Marsupials also lack brown fat, the metabolically highly active tissue in eutherian mammals that is used to restore body temperature from torpor bouts, and a number of studies have identified the importance of solar basking and huddling behaviour in rewarming of marsupials (Geiser and Pavey 2007; Pavey and Geiser 2008; Warnecke and Geiser 2009; Tomlinson et al. 2014). Basking during rewarming from torpor has been reported in at least four small dasyurids (*Planigale gilesi* (8 g), *Sminthopsis crassicaudata* (10 g), *Sminthopsis macroura* (15 g) and *Pseudantechinus macdonnellensis* (31 g)) with basking commencing approximately 3 h after sunrise when their body temperatures range from 13.8 to 19.3 °C (Warnecke and Geiser 2009, Geiser and Kortner 2010). The significant saving of energy expenditure by these small arid-zone marsupials through the reduction in metabolic rate and access to solar energy during passive rewarming from daily torpor has been estimated at 80% (Warnecke et al. 2008).

Water and Electrolyte Balance

Low availability of free water is the other primary factor characterising the arid zone, and animals living there must contend with this potential stressor. Rates of water turnover in free-ranging marsupials have been measured using tritiated water (^{3}HHO) in a wide range of species and vary allometrically with body mass (Nagy and Peterson 1988; Nagy 1994). The exponent of the relationship in macropodid marsupials varies from 0.82 to 0.87 (Green 1997), which is higher than 0.75, the exponent for metabolic rate of eutherian mammals, and also much higher than the exponent for the field metabolic rate (FMR) of marsupials at 0.69. This means that, as body mass increases, water turnover does not decrease on a mass-specific basis as rapidly as metabolic rate. This discrepancy between the two exponents, with respect to water, means that larger marsupials require relatively more water for a given energy intake than smaller animals and thus may have more difficulty obtaining their daily water requirements from their food. This question was explored in an early paper with data from two species of kangaroos, the red kangaroo and the euro, *Macropus robustus*, in the Pilbara region of Western Australia (Main and Bakker 1981). Males are twice the size of females in both species, and, with the onset of the dry season, males were found to lose body mass in contrast to females, which retained their initial mass. The authors interpreted this as evidence of differential dehydration in the larger males, which was supported by the first field study of euros by Tim Ealey (Ealey 1967; Ealey and Main 1967; Main 1970).

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[1] To date plasma corticosteroid levels during torpor have not been reported in any marsupial, but they would be of interest.

The Quokka

Evidence of stress due to lack of water is difficult to establish in these kangaroo species, because of the lack of information on regulatory hormonal responses, and a case study of a small macropodid wallaby, the quokka (*Setonix brachyurus)* (Fig. 5), is informative as an example, providing details of the nature of the data required. The quokka is originally a forest dweller, not an arid-zone species but a relictual population that survives on Rottnest Island 20 km off the coast of Perth in Western Australia, where there is no free water and the environment is severely degraded due to fire and overgrazing (Bradshaw 1983; Pen and Green 1983). There are a number of hypersaline lakes on the island, formed by intrusions from the ocean, and seeps into these lakes are all brackish. Quokkas living close to the lakes drink at these soaks, and a study was undertaken in late summer to determine whether this marginal intake of water would benefit lakeside dwellers compared with quokkas living on the dry West End of the island (Jones et al. 1990).

Table 1 compares rates of water and sodium turnover of quokkas living in the two habitats – both rates are substantially lower in the West End animals. Table 2 gives information on the body mass and condition of the two populations, along with plasma levels of antidiuretic hormone (ADH, lysine rather than arginine vasopressin in these marsupials), plasma osmolality and urine function. What is evident is that the West End animals are in poorer condition, have very high levels of ADH and have a significantly elevated plasma osmolality. They thus exemplify our operational definition of stress: a significant perturbation of the milieu intérieur (raised plasma osmolality), despite the maximal deployment of regulatory responses (the

Fig. 5 Adult female marsupial quokka wallaby (*Setonix brachyurus*), drinking at a brackish seep that flows into one of the hypersalline lakes, on Rottnest Island, 20 km off the coast of Perth in Western Australia

Table 1 Rates of turnover of water and sodium measured as influx in quokkas (*Setonix brachyurus*) from two locations in late summer on Rottnest Island, Western Australia

Turnover	Lake Baghdad	West End	Significance
Water influx (mL.Kg^{-1}.day^{-1})	133.5 ± 12.1	55.7 ± 15.5	$P < 0.005$
Sodium influx (mmol.Kg^{-1}.day^{-1})	34.5 ± 5.0	10.2 ± 2.9	$P < 0.001$

Modified from Jones et al. (1990)

Table 2 Comparison between body mass, condition and plasma levels of antidiuretic hormone (lysine vasopressin) and kidney function in quokkas (*Setonix brachyurus*) from two locations in late summer on Rottnest Island in Western Australia

Parameter	Lake Baghdad	West End	Significance
Body mass (kg)	2.66 ± 0.13	2.09 ± 0.15	$P < 0.01$
Condition index	6.45 ± 0.25	5.33 ± 0.26	$P < 0.01$
Lysine vasopressin pg.mL^{-1}	35.6 ± 15.8	89.2 ± 19.5	$P < 0.05$
Urine production (mL.Kg^{-1}.day^{-1})	58.18 ± 13.79	20.35 ± 2.75	$P < 0.02$
Urine osmolality (mOsm.Kg^{-1})	968.7 ± 101.3	1253.1 ± 44.7	$P < 0.02$
Plasma osmolality (mOsm.Kg^{-1})	279.8 ± 2.4	301.3 ± 6.0	$P < 0.005$
U/P$_{osm}$	3.46	4.16	$P < 0.02$

Modified from Jones et al. (1990)

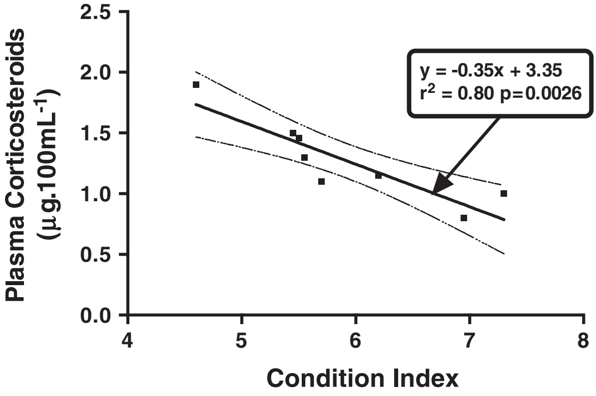

Fig. 6 Regression of plasma corticosteroid concentrations against body condition index in quokkas (*Setonix brachyurus*) in late summer on Rottnest Island in Western Australia. (Modified from Miller and Bradshaw 1979)

highest levels of ADH yet recorded in a marsupial). Measurement of plasma corticosteroid levels of quokkas in late summer also shows that the HPA axis is chronically stimulated, with a highly significant negative correlation with body condition (Fig. 6) (Miller and Bradshaw 1979).

Barrow Island Macropods

A long-term study of the ecophysiology of a suite of marsupials on Barrow Island, some 1400 km north of Perth in the Pilbara region of Western Australia, has provided valuable insights into the level of adaptation of arid-zone species (Bradshaw 1992). Barrow Island is Australia's foremost A-Class Reserve, harbouring 13 mammal species, 8 of them marsupial, of which 4 are either extinct on the mainland of Australia or virtually so (Moro and Lagdon 2013). Normally when rates of water turnover and FMR are measured in free-ranging animals, the study involves only single species, with perhaps a seasonal component. The study of Barrow Island, however, involved 12 vertebrate species (including a lizard and a bird) all measured simultaneously in different seasons over a 4-year period. Rates of metabolism in the field can then be used as 'metabolic currency' to compare the differential impact on available resources of the many vertebrates inhabiting this 234 km^2 arid island. Six marsupial species were included in the study, and variation in rates of water turnover was assessed using doubly-labelled water. An earlier study on the spectacled hare-wallaby, *Lagorchestes conspicillatus*, on Barrow Island had already established that this species has an extremely slow rate of water turnover in the driest part of the year, averaging 47.1 ± 4.1 $mL.kg^{-0.82}.day^{-1}$, which equates to only 5.1 ± 0.6% of its total body water content per day (Bakker and Bradshaw 1989).

An allometric comparison of rates of water turnover and FMR of Australian marsupials, using the doubly-labelled water method, shows that both parameters are substantially lower in arid-zone marsupials than in species inhabiting mesic habitats (Fig. 7) (Nagy and Bradshaw 2000). Rates of water turnover were compared in four species of macropodids on Barrow Island in the wet season (summer/autumn with cyclonic rains) and the dry winter season over a 5-year period in the 1990s (King and Bradshaw 2008). The species were the dwarf Barrow Island euro kangaroo, *Macropus robustus isabellinus*; the spectacled hare-wallaby; the black-footed rock wallaby, *Petrogale lateralis*; and Lesueur's bettong or 'boodie', *Bettongia lesueur*. Water influx rates were highest in bettongs during the dry season at 79.6 ± 6.1 $mL.kg^{-0.82}.day^{-1}$ and lowest in the hare wallaby, averaging 28.2 ± 6.1 $mL.kg^{-0.82}.day^{-1}$, with the other two species intermediate between these values (Fig. 8). The water turnover has also been expressed as the percentage of the animal's total body water content exchanged per day, and the high fraction for the bettongs (13.98 ± 0.6%) contrasts with that for the hare wallabies (3.51 ± 0.4%). The latter is an extraordinarily slow rate of water turnover for a desert mammal. Rates of water turnover in summer in desert rodents from southern Tunisia, for example, average between 15.3 and 21.8% of the total body water content per day (Bradshaw et al. 1976). There were no significant differences between plasma osmolality recorded in the dry and wet seasons for any of the macropods on Barrow Island; urine osmolality, however, was significantly elevated in hare wallabies in the dry season, averaging 2256 $mOsm.kg^{-1}$.

In evaluating the impact of the many vertebrate species that inhabit Barrow Island, and measuring the way in which they share scarce resources, the FMR can be used as 'metabolic currency' to compare the environmental cost of different lifestyles. If one takes the FMR of a single euro kangaroo (approximately 96 L of CO_2 per animal per day) as our unit of measurement, then multiplying this by the estimate number of euros on the island[2] gives total energy turnover by euros in

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[2] There are no accurate population estimates for Barrow Island marsupials, but available data from transects have been aggregated and then extrapolated, based on the area surveyed. As this assumes that animals are uniformly distributed across the island, the estimates generated are thus upper limits. SHORT, J. & TURNER, B. 1991. Distribution and abundance of Spectacled hare-wallabies and euros on Barrow Island. *Wildlife Research,* 18**,** 421–429, SHORT, J. & TURNER, B. 1993.

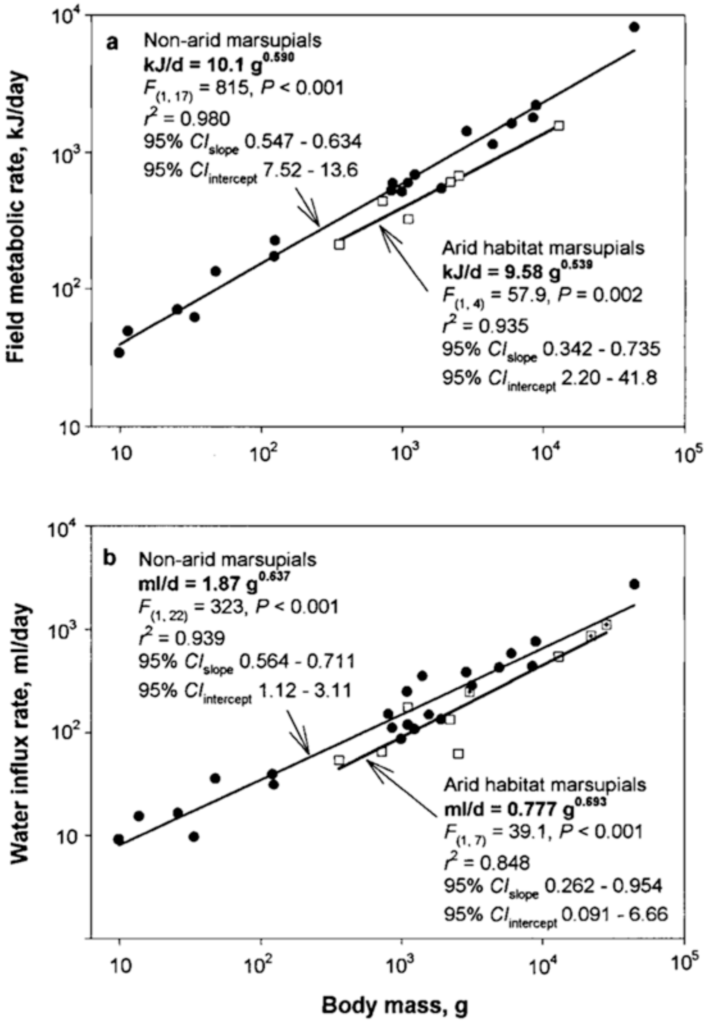

Fig. 7 Allometric regressions of (**a**) field metabolic rate (FMR) against body mass (g) for free-ranging marsupials from arid (open squares) and non-arid habitats (black circles) and (**b**) water influx rate against body mass for free-ranging marsupials from arid (open squares) and non-arid habitats (black circles). (From Nagy and Bradshaw 2000, with permission)

'Kangaroo Equivalents'. FMR data are available for a wide range of other Barrow Island vertebrates from which one can estimate their energy usage and express this

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The distribution and abundance of the Burrowing bettong (Marsupialia: Macropodoidea). *Wildlife Research,* 20**,** 525–534, LAGDON, R. 1988. Barrow Island Environmental Review: 1963–1988. Perth, Western Australia: West Australian Petroleum Pty. Limited.

A.A. Burbidge (pers. com.).

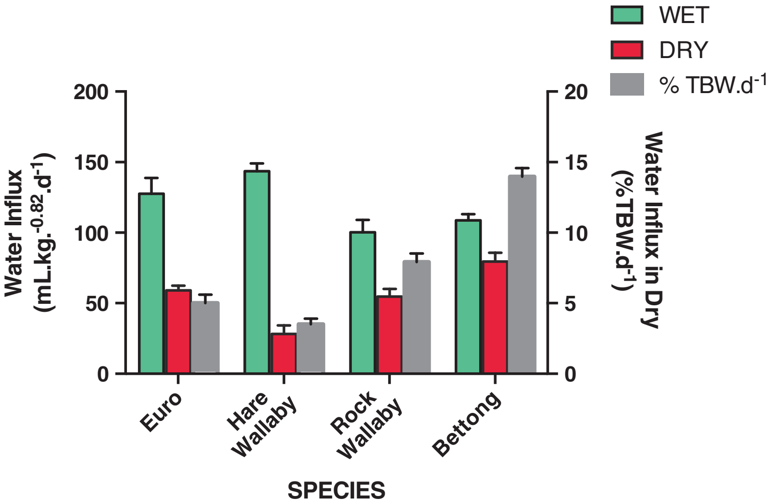

Fig. 8 Comparison between rates of water influx in mL.kg$^{-0.82}$.day^{-1} in wet and dry seasons in four marsupial species on Barrow Island in the arid Pilbara region of Western Australia. Species are the Barrow Island euro, *Macropus robustus isabellinus;* spectacled hare-wallaby, *Lagorchestes conspicillatus*; black-flanked rock wallaby, *Petrogale lateralis*; and the Burrowing bettong, *Bettongia lesueur*. Rates of water loss in the dry season are also expressed on the right-hand ordinate as a percentage of the total body water content exchanged per day (% of total body water content.day^{-1}) (calculated with data from King and Bradshaw 2008, 2010)

in 'Kangaroo Equivalents' (Fig. 9). Interestingly, the much smaller but very abundant golden bandicoots have an energetic impact larger than the euros, followed by hare wallabies, then the giant varanid lizard, the perentie, and then burrowing bettongs. The energetic impact of other small vertebrates, including native rodents, dragon lizards and the spinifex bird, is trivial in comparison.

The Barrow Island Euro

Seasonal variation in water and electrolyte balance were studied over a 5-year period in the euro, and plasma levels of ADH (lysine vasopressin) were also measured for the first time in this species (King and Bradshaw 2008). Plasma ADH levels increased with plasma osmolality and were negatively correlated with total body water content. Mean concentrations ranged from 2.1 ± 0.6 pg.mL^{-1} in June 1995, the wettest year of the study, to 16.7 ± 4.6 pg.mL^{-1} in November 1994, following an 8-month drought, when total rainfall for that year was only 54 mm. This was composed of 50 mm in February and 4 mm in December. The average annual rainfall for Barrow Island is 320 mm (Moro and Lagdon 2013).

A detailed analysis of changes in body condition, haematology and hormone levels of Barrow Island euros in November 1994 found significantly elevated

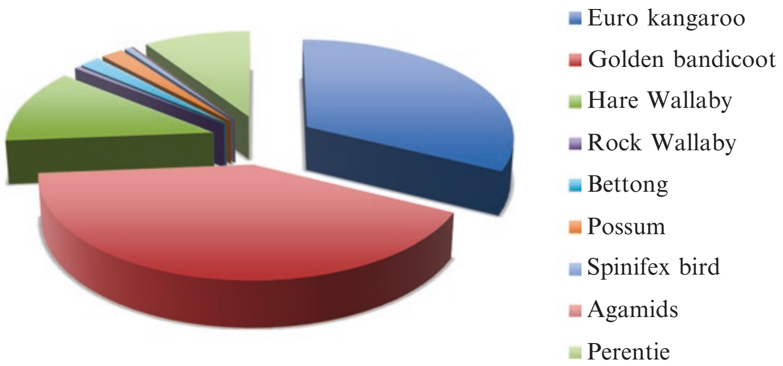

Fig. 9 Partitioning of energy turnover by a suite of vertebrate taxa on Barrow Island in the Pilbara region of Western Australia. Field metabolic rates (FMR) of the various taxa, measured in CO_2 production in litres per animal, have been normalised to 'Kangaroo Equivalents' (KE) with 1 KE = daily CO_2 production of the euro, *Macropus robustus isabellinus*, and then multiplied by the population estimate for each species. (Produced with data from Nagy and Bradshaw 2000; Short and Turner 1991, 1993; A.A. Burbidge (pers. com.), Ambrose et al. 1996; Green et al. 1986)

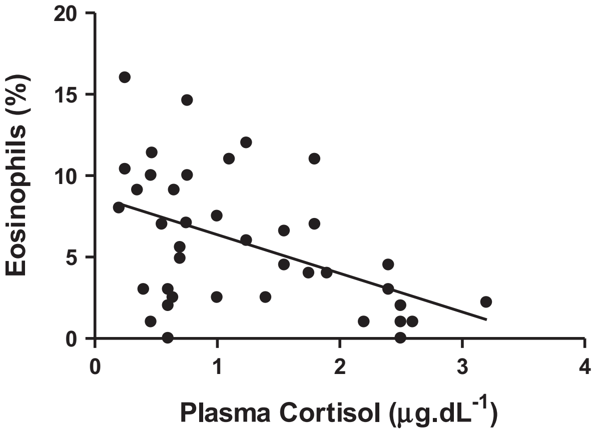

Fig. 10 Regression of eosinophil counts, expressed as a percentage of total leucocytes, against plasma cortisol levels in µg.dL^{-1} in the Barrow Island euro, *Macropus robustus isabellinus*. The negative regression is statistically significant with $r^2 = 0.201$ and $P = 0.0037$. (Adapted from King and Bradshaw 2010)

plasma osmolality, plasma cortisol levels and plasma ADH and a reduced eosinophil count (King and Bradshaw 2010). Plasma cortisol levels and eosinophil counts were also significantly negatively correlated (Fig. 10), suggesting that the exposure was chronic and compromising the euros' immune capabilities (Sapolsky et al. 2000; Davis et al. 2008). Euros suffered from a regenerative normative hypochromic anaemia, not found in mainland animals, compromising their respiratory capacities and severely limiting any sustained activity (Billiards et al. 1999). Despite their small body size, and high surface/volume ratio, the euros have a low rate of water turnover in the dry time of the year and a water economy index (WEI = water influx/FMR) characteristic of arid-adapted marsupials (Nagy and Bradshaw 2000). Unfortunately, rates of water influx were not measured on the November 1994 field trip, but the body mass and condition index of the euros was not decreased, suggesting that the increased plasma osmolality may not have been the result of simple dehydration. Levels of ADH, although elevated, were nowhere near as high as those measured in quokkas from the West End of Rottnest Island, suggesting that the euros were experiencing a very high 'allostatic load' but had not yet reached 'allostatic overload' (King and Bradshaw 2010).

Desert Wallabies

Seasonal changes in water and electrolyte balance and kidney function have been studied in two species of wallabies in the arid Pilbara region of Western Australia: the spectacled hare-wallaby, *Lagorchestes conspicillatus*, again on Barrow Island, and Rothschild's rock wallaby, *Petrogale rothschildi*, on Enderby Island in the Dampier Archipelago (Fig. 11) (Bradshaw et al. 2001). Although both species are nocturnal feeders, they differ markedly in the nature of their daytime refugia. The hare wallaby shelters in large clumps of spinifex (*Triodia* spp.) during the day, whereas the rock wallaby shelters in caves and rock piles. Temperatures in the spinifex clumps often exceed 40 °C during the summer months, and the air is very dry, whereas cave sites are much cooler, with high constant humidity.

Data on rates of water turnover and water and electrolyte homeostasis for the two wallabies during the driest part of the year are presented in Table 3. There are no significant differences between plasma electrolyte concentrations, plasma osmolality and total body water content for the two species, indicating that they are both effectively maintaining homeostasis at this time. Rates of water influx, however, differ substantially, and the figure of 27.5 ± 2.0 $\text{mL.kg}^{-0.82}\text{day}^{-1}$ is the lowest rate of water turnover yet reported for any mammal worldwide.

A comparison of kidney function in the field during the dry and the wet months of the year revealed major differences between the two species (Fig. 12) (Bradshaw et al. 2001; Bakker and Bradshaw 1989). Hare wallabies show a pronounced antidiuresis in the dry season, accompanied by a large increase in urine concentration (U/P_{OSM}) and a very significant increase in plasma ADH levels. This is a standard response from a mammal and what one would expect when challenged by water deprivation. The rock wallabies, on the other hand, respond with an antidiuresis (not as effective), but there is no change in the concentration of the urine being elaborated (U/P_{osm}) nor any significant increase in plasma ADH levels. A detailed analysis of kidney function, involving measurement of rates of glomerular filtration and renal plasma flow (RPF), revealed that the rock wallabies in the dry season were reducing rates of blood flow to the kidney and hence reducing glomerular filtration (GFR) rather than increasing the rate of water reabsorption from the renal tubules (Bradshaw et al. 2001). This marked difference in renal function between the two species is shown in Fig. 13.

Rothschild's rock wallaby is unique in being the first mammalian species in which the lack of a hormonal antidiuretic response to water deprivation has been

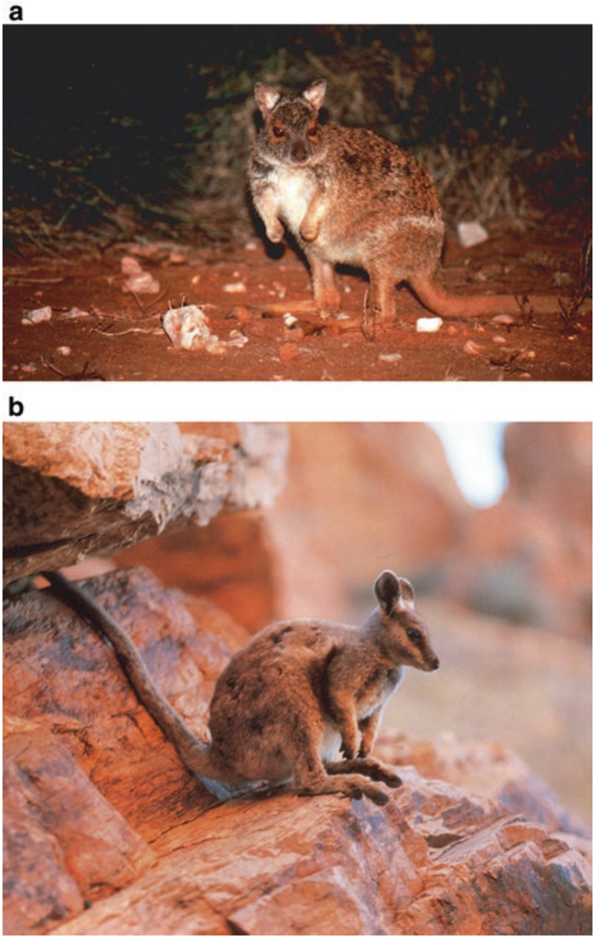

Fig. 11 Photographs of (**a**) the spectacled hare-wallaby, *Lagorchestes conspicillatus*, on Barrow Island and (**b**) Rothschild's rock wallaby, *Petrogale rothschildi*, on Enderby Island in the Pilbara region of Western Australia

Table 3 Water metabolism and homeostasis during the dry season of spectacled hare-wallabies (*Lagorchestes conspicillatus*) on Barrow Island and Rothschild's rock wallaby (*Petrogale rothschildi*) on Enderby Island in the arid Pilbara region of Western Australia

Species	Body mass (kg)	Total body water (%)	Water influx (mL.kg$^{-0.82}$.day^{-1})	Plasma sodium (mmol.L^{-1})	Plasma potassium (mmol.L^{-1})	Plasma osmolality (mOsm.kg^{-1})
Hare wallaby	2.31 ± 0.06	76.4 ± 1.60	27.5 ± 2.0	151.9 ± 1.1	5.6 ± 0.2	287.3 ± 2.1
Rock wallaby	3.32 ± 0.12	78.2 ± 1.69	68.9 ± 5.2	145 ± 0.86	5.17 ± 0.2	291.3 ± 2.5

Adapted from Bradshaw et al. (2001)

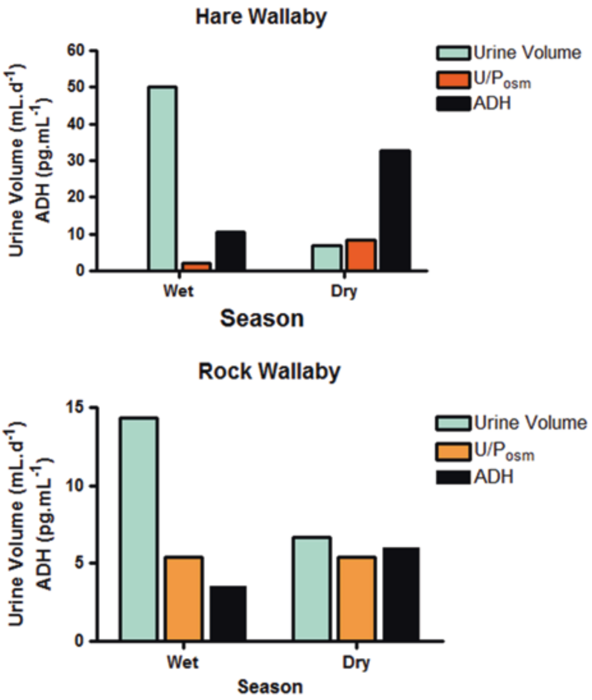

Fig. 12 Renal function in wet and dry seasons in arid-zone wallabies in the Pilbara region of Western Australia. Rates of urine production in mL.day^{-1}, urinary concentrations in U/P$_{OSM}$ and plasma levels of antidiuretic hormone (ADH = lysine vasopressin) in pg.mL^{-1} are shown for spectacled hare-wallabies (*Lagorchestes conspicillatus*) on Barrow Island and Rothschild's rock wallaby (*Petrogale rothschildi)* on Enderby Island. (Adapted from Bradshaw et al. 2001)

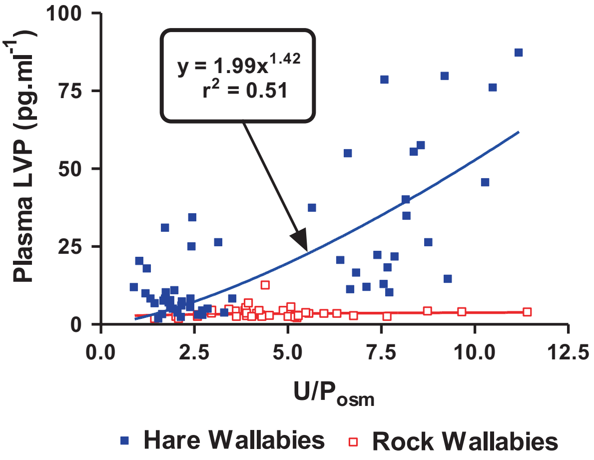

Fig. 13 Variation in circulating levels of lysine vasopressin (LVP) in hare wallabies and rock wallabies as a function of the concentrating activity of the kidney (U/P$_{osm}$). (Adapted from Bradshaw et al. 2001)

detected. Despite this, it is clear that the species is able to deal effectively with the challenges of its arid environment and it owes this to the protection provided by its cave or rockpile habitat. Its response to water deprivation is reminiscent of that often seen in reptiles, with a reduction in glomerular filtration, rather than an increase in reclamation of filtered water (Dantzler and Bradshaw 2009; Ford and Bradshaw 2006). Lysine vasopressin is present in the plasma of the rock wallaby, but further work is needed to establish whether it acts via cyclic-AMP to increase water permeability of the renal tubules in this species or whether it has lost this primary function (Morel 1983). There is no evidence that either species of wallaby experiences ‘allostatic overload’ or stress, even during the longest drought recorded in 1994 on Barrow Island. The vital role of habitat in enabling the rock wallaby to persist in this arid habitat is paramount, however, and without a cool, humid refuge to which it can retreat during daylight hours, it would be unable to survive. By way of contrast, the hare wallaby provides another example of the extent to which physiological adaptations can facilitate survival and persistence in challenging environments (Bradshaw 1992, 2007).

Seasonal Deficiencies of Nitrogen, Phosphorus and Energy

The last of the major factors confronting animals that inhabit the arid zone is the one least documented, despite its importance. It is primarily a concern of herbivorous species, as the food of carnivores is generally much more nitrogen, phosphorus and energy-dense than plant products (Prosser et al. 1950; Withers 1992). Nitrogen in the form of protein varies seasonally in most plants, falling to extremely low levels in arid-zone species. By reconstituting the diet of free-ranging macropods from faecal analysis of epidermal fragments (Storr 1963), the nitrogen concentration as percent dry weight of the diet of euro (*Macropus robustus)* and red kangaroos (*Macropus rufus*), for example, was found to range from 1.6 to as low as 0.8% (Main 1970). Nitrogen levels in spinifex grasses (*Triodia* spp.) fall to even lower levels and present a major challenge for the maintenance of nitrogen balance and growth in arid-zone marsupials.

A series of studies on the energetics of arid-zone kangaroos, carried out at the Fowlers Gap research station in New South Wales, has highlighted the large difference between marsupial and eutherian herbivores. Both the basal metabolic rate and field metabolic rate (FMR) of kangaroos are lower than those of similar-sized eutherian mammals, such as sheep, and a ‘dry sheep equivalent’ (DSE) of 0.7 is usually used when calculating rates of resource utilisation (Munn et al. 2009; Dawson and Munn 2007). This difference has usually been attributed to the significantly lower basal metabolic rate of marsupials, compared with that of eutherian mammals (Dawson and Hulbert 1970), but has been tempered somewhat by the compilation of data on the field metabolic rate (FMR) of free-ranging marsupials. Whereas the allometric exponent of FMR with body mass in eutherians is 0.75 (similar to that of basal metabolic rate), in marsupials the exponent for FMR is much lower at 0.69

(Nagy 2005; Nagy et al. 1999; Riek and Bruggeman 2013). This means that the FMR of small marsupials is higher than that of equivalent-sized eutherian mammals but substantially lower in the case of large marsupials, such as kangaroos. A recent analysis of FMR in Australian marsupials revealed strong phylogenetic correlations, with macropods having significantly lower FMRs than carnivorous marsupials (Riek and Bruggeman 2013). FMR also scales positively with body mass in marsupials, in contrast to the situation in eutherian mammals where total population energy use is independent of species' body mass (Munn et al. 2013).

As well as having lower rates of energy turnover in the field, it has also been suggested that marsupials have lower protein requirements in their diet than eutherians, because there should be a close relationship between basal metabolic rate and rates of whole-body protein synthesis (Hume 1999). Few species have been studied to date, but rates of protein synthesis in the tammar wallaby (*Macropus eugenii*), the Parma wallaby (*M. parma*) (White et al. 1988), the wombat (*Vombatus ursinus*) (Barboza et al. 1993) and the honey possum (*Tarsipes rostratus*) (Bradshaw and Bradshaw 2001) are all well below levels reported in eutherians (Waterlow 1984). Nitrogen cycling to the fermentative foregut of macropods has also been posited as one of the mechanisms employed by arid-zone herbivores to offset the low levels of protein in plants available in summer (Kinnear et al. 1979).

Laboratory studies with a number of species of macropods have shown that they are resistant to the usual nitrogen-mobilising diabetogenic effect of the steroid hormone cortisol (Griffiths et al. 1969; McDonald and Bradshaw 1981). This is not a general marsupial characteristic, however, as cortisol injections increase nitrogen excretion and elevate blood glucose levels in brush-tailed possums (*Trichosurus vulpecula*) (McDonald and Than 1976; McDonald 1977). Cortisol is a catabolic steroid released in response to stressors, such as starvation, and stimulates gluconeogenesis through the breakdown of tissue protein with the release of nitrogen that is normally excreted. In the case of arid-zone macropods, however, the nitrogen would appear to be reabsorbed through the kidney, rather than excreted, and redirected to the microbial population in the animal's foregut, where it is synthesised into bacterial protein and subsequently digested (Kinnear and Main 1975). This would appear to be a very effective adaptation that may have evolved in these species to offset the problem of seasonal deficiencies in dietary protein (Kinnear and Main 1979). Little is known of the precise mechanism of urea recycling, however, although a study of the effects of dehydration and protein deficiency in the spectacled hare-wallaby found that relative urea clearance (C_{UREA}/C_{IN}) is positively correlated with the rate of urine production (Bakker and Bradshaw 1983). Both dehydration and protein deficiency resulted in a decrease in the glomerular filtration rate (measured as inulin clearance C_{IN}) and a decrease in urea clearance with a fall in nitrogen excretion. Exposure to high-protein diets in this desert wallaby resulted in a 'protein diuresis', and the authors of the study suggest that urea recycling in this macropodid is obligatory and reflects the low level of protein in its spinifex-dominated diet (Bakker and Bradshaw 1983).

Another important discovery is the major difference in nitrogen metabolism of marsupials compared with that of eutherian mammals involving endogenous urinary

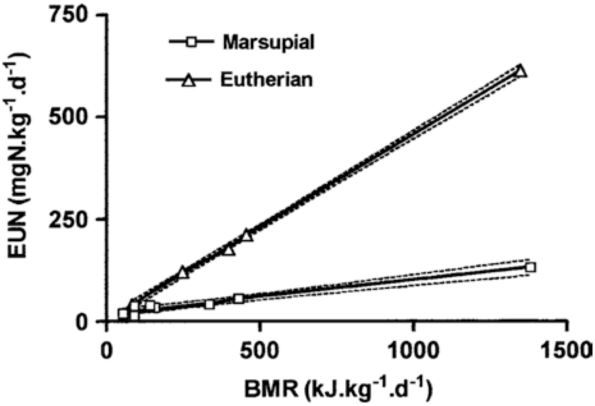

Fig. 14 Relationship between endogenous urinary nitrogen (EUN in mg N $kg^{-1}d^{-1}$) excretion and heat production (kJ kg^{-1} day^{-1}) in eutherian (open squares) and marsupial (open triangles) mammals. Data for eutherians are taken from Smuts (1935) and data for marsupials from Hume (1999) and Bradshaw and Bradshaw (2001). The linear regression equation for eutherians is EUN = 0.45BMR + 4.44, r2 = 0.999 and for marsupials is EUN = 0.08BMR + 19.24, r2 = 0.96. (Adapted from Bradshaw and Bradshaw 2001)

nitrogen excretion (EUN). EUN is estimated by regressing urinary nitrogen excretion against truly digestible nitrogen intake and extrapolating the curve to zero intake, where the measured loss is taken as obligatory. In eutherian mammals it is well established that approximately 2 mg of EUN is excreted for each kcal (4.184 kJ) of basal heat production (Smuts 1935), and it has been assumed that the lower EUN found in marsupials is a direct consequence of their lower basal metabolic rate (Hume 1999). When data from a range of marsupials are examined, however, a highly significant, but very different, relationship from that observed in eutherian mammals is found (Fig. 14). When expressed per kcal of heat production, marsupials excrete only 0.34 mg of nitrogen, not 2 mg, and this figure is, of course, corrected for the difference in basal metabolic rate (Bradshaw and Bradshaw 2001). Such a fundamental biochemical difference between marsupials and eutherian mammals is unexpected, and its mechanism and significance remain to be explored, but it does suggest that the separate dictates of marsupial evolution have resulted in a different balance between energy and nitrogen metabolism from that in eutherians.

Conclusion

Ecophysiology is the study of the physiological responses of organisms to the ecological dictates of their environment. It involves the study of animals in their natural environment, rather than in the confines of laboratories. An operational definition of stress as ‘the physiological resultant of demands that exceed an animal’s homeostatic capacities' has been used to search for instances of stress in arid-zone marsupials due to high temperatures and lack of water. The recent use of body temperature loggers in free-ranging animals has shown that 'adaptive heterothermy' and selective brain cooling, first described in camels and African ungulates, do not occur in kangaroos, which maintain normothermia over a wide range of ambient temperatures. Torpor, on the other hand, is widely employed by arid-zone marsupials, especially those with low body mass, to offset the low night temperatures also characteristic of the arid zone. Torpor in marsupials, however, is not a stress but an adaptive regulated response to an unfavourable energy balance, whereby normal homeostatic maintenance of body temperature is uncoupled, allowing body temperature to fall with significant savings in energy expenditure. Significant perturbations of water and electrolyte balance in arid-zone marsupials are also rare, and, even in the driest year ever recorded on Barrow Island, only one species, the Barrow Island euro *Macropus robustus isabellinus*, showed signs of an increased allostatic load, but not 'allostatic overload', or stress. The spectacled hare-wallaby, *Lagorchestes conspicillatus*, serves as an exemplar of the level of adaptation by some arid-zone marsupials, having the lowest rate of water loss yet recorded for any mammal worldwide. This contrasts with the Rothschild's rock wallaby, *Petrogale rothschildi*, which lacks hormonal control over water loss by the kidney but survives in a similar habitat to that of the hare wallaby by its reliance during daylight hours on cool and humid caves and rockpile shelters to conserve water. Nutritional adaptations are also evident; these aid with seasonal deficiencies of nitrogen and energy in herbivorous species. The lower field metabolic rate of arid-zone compared with mesic species of marsupials, especially macropods, results in lower levels of food intake, and urea recycling helps offset exceptionally low protein levels in the diet. Rates of protein turnover also appear to be lower in marsupial than in eutherian mammals, and the linkage between obligatory nitrogen loss from the body in the form of endogenous urinary excretion and basal energy production is almost six times lower in marsupials. Arid-zone marsupials thus appear physiologically and behaviourally well buffered to withstand the rigours of their habitat, but the environmental changes wrought by European colonisation of the continent increasingly put these adaptations to the test. If the predications of current climate change scenarios do eventuate, stress responses seen in species such as the Rottnest Island quokka will likely in the not-too-distant future be observed in arid-zone species.

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Glossary

CWR The critical weight range of 35 g–5.5 kg for which Australian marsupials have been found most vulnerable to extinction.

Stress An unfavourable body condition originally defined by Hans Selye as ‘a state of non-specific tension in living matter’.

Stressor An environmental factor that will provoke stress if sustained.

Allostasis Originally conceived as ‘maintaining stability through change’ and introduced into the medical literature to deal with situations, such as fever, where the body changes to a new regulated state, with a change in the set point for body temperature. The concept has been developed by biologists who have introduced the concept of ‘allostatic load’, which can be monitored through changes in circulating levels of adrenal corticosteroids.

Glucocorticoids Steroid hormones secreted by the adrenal glands in vertebrates that are released under stressful conditions and act to mobilise glucose reserves in the body.

Allostatic overload A condition equivalent to chronic stress.

‘milieu intérieur’ The ‘internal environment’ of animals identified by the French physiologist, Claude Bernard in 1878, as the closely regulated condition common to all vertebrate animals and on which their independence from the external environment depends.

Homeostasis (also homoiostasis) The term introduced by the American physiologist, Walter Cannon in 1929, to describe the process by which the ‘internal environment’ of vertebrates is protected from change.

ADH Antidiuretic hormone, which is secreted by the posterior lobe of the pituitary gland and, when released into the blood, stimulates reabsorption of water from the kidney of vertebrates, reducing urine production and assisting in coping with water deprivation and dehydration.

Heterothermy Significant variation throughout the day in body temperature – as opposed to homeothermy in which body temperature is maintained between narrow limits.

Adaptive heterothermy The process originally described in desert camels whereby they contend with excessive heat by allowing their body temperature to rise, thus storing heat that may then be lost at night by radiation.

Carotid rete A group of specialised blood vessels in the base of the brain of some mammals that operates to cool warmed blood flowing to the brain by countercurrent exchange.

Crepuscular Animals that commence their activity at dawn and dusk.

Torpor A condition in which an animal ceases activity for a period of time and is associated with a significant fall in both body temperature and metabolic rate. Torpor may last for hours or days and is truncated by spontaneous arousal of the animal with a rapid rise in its body temperature back to normal levels.

Leptin Often known as the ‘satiety hormone’, is a hormone produced by fat cells that helps to regulate energy balance by inhibiting hunger.

Ghrelin Also called the 'hunger hormone' is a peptide hormone, made up of a small number of amino acids, that is produced by cells in the intestinal tract and acts on the central nervous system to increase appetite.

Arcuate nucleus A group of nerve cell bodies in the hypothalamus of the brain that contain important neurotransmitters involved in controlling hormone release from the pituitary gland.

Neuropeptide Y Neurones (NPY) are also located in the arcuate nucleus and are important in the regulation of appetite.

Tanycytes Special cells found in the brain and on the floor of the fourth ventricle, which have processes extending deep into the hypothalamus. It is thought that their function is to transfer chemical signals from the cerebrospinal fluid to the central nervous system.

Thyroidectomy Removal of the thyroid gland.

Deiodinase Specific enzymes that break down the hormone thyroxin (T_4) by successively removing iodine molecules.

Iodothyronine (T_3) The active form of the hormone thyroxin, which has three molecules of iodine instead of four.

Pars tuberalis A group of cells located around the stalk of the pituitary gland that contain hormone-secreting cells.

Brown fat Highly metabolically active tissue found in eutherian mammals that produces heat to restore the normal body temperature of torpid mammals. Brown fat is not found in marsupials, which recover from torpor by shivering.

ADH (anti-diuretic hormone) A hormone secreted by the posterior lobe of the pituitary gland, which acts on the kidney to enhance reabsorption of water from the urine when animals are dehydrated or water deprived.

HPA axis (hypothalamo-pituitary-adrenal axis) Many of the hormones secreted by the pituitary gland (e.g. growth hormone, thyroid-stimulating hormone, adrenocorticotropic hormone (ACTH), etc.) are controlled by small molecule 'releasing factors' elaborated in the hypothalamus of the brain and transported to the pituitary gland by a dedicated portal blood supply. Stressors act via the HPA to activate secretion of hormones, firstly from the pituitary gland, which then go on to stimulate other glands in the body, such as the adrenals.

Doubly-labelled water This is water where the heavy isotope of oxygen (^{18}O) replaces normal oxygen (^{16}O) and hydrogen is replaced by either deuterium (^{2}H) or radioactive tritium (^{3}H). When injected into animals, the differential turnover of the two isotopes enables one to measure the rate of carbon dioxide production in free-ranging animals and hence their field metabolic rate (FMR).

Macropodids Marsupial species belonging to the family Macropodidae (large foot) which includes the kangaroos and wallabies.

Osmolality The osmotic pressure of a liquid that is a function the number of molecular components dissolved in the fluid (e.g. plasma or urine).

Water economy index (WEI) The ratio of water influx to FMR and a measure of animal's adaptation to low water regimes.

Eosinophil One type of white-blood cell and one of the immune system's components responsible for combating multicellular parasites and certain infections in vertebrates. Their numbers are often used as a measure of the competence of the animal's immune system.

Anaemia A reduction in the number of red cells in the blood, and its haemoglobin content, resulting in a lowering of the oxygen-carrying capacity of the blood.

U/P_{OSM} The ratio of the osmotic pressure of the urine relative to the plasma is a measure of the concentrating capacity of the kidney and increases when animals are water deprived.

Renal plasma flow (RPF) The rate at which blood flows through the kidneys.

Glomerular filtration rate (GFR) The rate at which blood is filtered in the kidney to produce urine. GFR is usually measured by the rate of clearance of inulin, a plant polysaccharide, and expressed as $C_{IN.}$

C_{OSM}/C_{IN} Relative osmolar clearance.

Dry sheep equivalent (DSE) The amount of energy that a marsupial requires per day compared with a similar-sized sheep. The figure of 0.7 is usually quoted and reflects the fact that the basal metabolic rate (BMR) of marsupials is approximately 30% lower than that of eutherian mammals.

'Diabetogenic' effect of steroid hormones So-called because steroids such as cortisol and corticosterone increase blood sugar levels when injected.

C_{UREA}/C_{IN} Relative urea clearance and the amount of urea cleared from the blood relative to the rate of glomerular filtration.

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