

School of Science  
Department of Environment and Agriculture

Comparative physiology of Australian echidnas (*Tachyglossus aculeatus*)

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## Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council Australian code for the care and use of animals for scientific purposes 8<sup>th</sup> edition (2013). The proposed research study received animal ethics approval from the Curtin University Animal Ethics Committee, Approval Number AEC\_2011\_47

Signature:

A handwritten signature in black ink, appearing to be 'J.R.', written in a cursive style.

Date: 13/09/2016

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Chapter 2 has been published in *Physiological and Biochemical Zoology*, as a multi-author paper of which I was the primary author and made the major practical and intellectual contribution.

## General Abstract

Early studies concluded that monotremes are primitive mammals, considering them as a somewhat ‘intermediate’ between the ‘lower’ reptiles and ‘higher’ placentals. Since these early studies there has been much debate surrounding the ‘primitiveness’ or otherwise of the monotremes, and their physiological capacity to withstand and adapt to environmental conditions, despite their broad geographic and climatic range. Here I provide evidence that although short-beaked echidnas (*Tachyglossus aculeatus*), the most widespread and common extant monotremes, retain many ‘primitive’ morphological features, few aspects of their physiology can be considered primitive, and they have considerable physiological plasticity to adapt to a variety of environmental conditions.

While echidnas have a low (basal body temperature =  $29.5 \pm 0.32$  °C) and labile (body temperature fluctuates up to 8.1 °C over an ambient temperature range of 22.5 °C) body temperature which is physiologically ‘primitive’, they are capable of precise thermoregulation. The low metabolic rate of echidnas ( $0.08 \pm 0.01$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) is largely a reflection of their low body temperature and may be advantageous for a low-energy lifestyle, which has resulted from a myrmecophagous diet, armoured morphology, semi-fossorial habit and heterothermic thermoregulatory strategy. Despite retaining some ‘primitive’ traits, aspects of echidna’s physiology indicate that they also have typically mammalian regulatory systems, including a previously unrecognised ability to enhance evaporative heat loss at high ambient temperatures, as do ‘higher’ mammals. Laboratory studies demonstrate that echidnas can regulate their body temperature over a wide range of ambient temperatures. Field studies have further supported this by demonstrating that echidnas physiologically withstand environmental extremes, and utilise daily heterothermy seasonally, adjusting body temperature fluctuations depending on environmental conditions. In addition to daily heterothermy, echidnas even in a semi-arid woodland also utilise multi-day torpor to reduce thermoregulatory costs during periods that are energetically expensive, although the duration of this appears to be facultative rather than seasonal as in cooler parts of their distribution. Echidnas also have

considerable physiological plasticity that allows them to accommodate varying daily, seasonal and geographical environmental demands. As a result of this plasticity, there are significant differences in the physiology of the two most distinct sub-species of short-beaked echidna (*T.a. acanthion* and *T.a. setosus*). *T.a. setosus*, from the cooler and wetter climate of Tasmania, had a significantly higher body temperature, metabolic rate and evaporative water loss than *T.a. acanthion*, from Western Australia.

Short-beaked echidnas have a combination of 'primitive' ancestral and derived physiological traits. They are effective thermoregulators that have adapted to a low energy lifestyle, inhabiting in a wide range of environments. Echidnas are physiologically plastic and are able to adapt to a range of environmental demands.

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## General introduction

The variation in physiological parameters and how they relate to evolutionary, ecological and life-history parameters is one of the key concepts in comparative physiology. Comparative physiology describes the mechanisms of adaptation to a wide range of environments, and thus contributes to broad scale ecosystem impacts (Prosser 1991). Metabolic rate (MR) is a measure of the rate at which organisms take up, transform and expend energy and is the most fundamental physiological parameter (Brown *et al.* 2004). MR provides a basis for linking the biology of individual organisms to the ecology of populations, communities and ecosystems, and sets the pace of life (Brown *et al.* 2004). There are a number of influencing factors, the most important of which is body mass. The reason body mass has the greatest influence on BMR is that it has a huge range, varying in adult mammals from 1.7g to 160000kg (McNab 2008). Animals with a larger mass must have a greater MR than smaller animals, however this relationship is not linear and has a slope of approximately 0.75 (Kleiber 1932; Withers 1992). Diet also has a considerable impact on mammalian MR. For example, folivores, who feed primarily on leaves, have a much lower rate of metabolism than expected for their size (McNab 1980, 2002). Some of this reduction in metabolism may reflect the low nutritional value or the toxic compounds present in leaves, but some of it is also associated with the largely sedentary lifestyle of folivores (McNab 1980). Myrmecophages and termitivores also have very low rates of metabolism. Ants and termites have low digestibility and energy content due to their high chitin and ash content (McNab 1980; Redford and Dorea 1984; Cooper and Withers 2004). Feeding on ants and termites also inevitably results in the ingestion of large quantities of detritus, which further reduces the energy density of this diet, meaning a myrmecophagous diet is energetically limiting. In addition to the low energy and poor digestibility, the temporal and spatial distribution of this food source may also influence a low MR, as well as the chemical defences of ants and termites (McNab 1980;

Redford and Dorea 1984). In contrast to these diets which are associated with a low MR, carnivores, grazing ungulates and many lagomorphs have high metabolic rates (McNab 1980), due to these diets being more abundant, more easily digested and/or requiring a higher level of metabolism to obtain or digest their food. Spatial and temporal heterogeneity in food availability may also result in a low BMR (McNab 1979a, 2002); if food is not readily available a higher BMR cannot be supported. Larger species amplify the effect of food habits on BMR due to larger mammals requiring a higher level of energy and nutrition than smaller mammals. Therefore larger species are more likely to encounter energetic and nutritional limitations imposed by food (McNab 2002). Further studies of MR in relation to diet would be beneficial as diet, phylogeny and climate are so closely correlated it is often hard to distinguish which is affecting MR. For example, manatees have a low BMR compared to arctic/cold-temperate aquatic mammals, but they are also herbivorous, so their lower BMR may be a reflection of food habits as much as their warm water habitat (McNab 2002). Further research using distinct groups of mammals would be of benefit to further examine dietary impacts on BMR.

Body temperature ( $T_b$ ) drives biochemical reaction rates, metabolic rates, and nearly all rates of biological activity (Brown *et al.* 2004). The 'normal' functional  $T_b$  varies among species and taxonomic groups, with any given species operating over some subset of this temperature range (Brown *et al.* 2004).  $T_b$  has a considerable influence on metabolic rate, though this is often less clear for endotherms as they tightly regulate  $T_b$  (Brown *et al.* 2004; Withers 1992). However, the effect of  $T_b$  on MR can be more clearly seen in mammals that utilise torpor as an energy conservation strategy to escape unfavourable climatic conditions or when food is scarce (Geiser 2004; Withers 1992). Body temperature has an exponential effect on MR. For example, a hibernating mammal that reduces its  $T_b$  from 37°C to 10°C has a resulting decrease in MR approximately 50 fold (Withers 1992). By correcting  $T_b$  to 38°C, much of the taxonomic difference in basal metabolic rate (BMR) is explained. However, some groups of endotherms with more substantial differences in BMR, still show considerable differences after correcting  $T_b$  to a common value of 38°C e.g. shrews and passerine birds, which have high BMR's, and monotremes and ratite birds, which have low BMR's (Withers 1992).  $T_b$  of mammalian

taxa is an important parameter in the study of evolution; 'primitive' mammals tend to have a lower  $T_b$  (e.g. monotremes), compared to 'higher' mammals (e.g. placentals) which has been linked to their life-history. Taxonomic affiliation is correlated with most factors influencing BMR (McNab 2002), and it is therefore essential that a wide variety of species are studied to allow better interpretation of physiological responses.

Modification of insulation is one of the main means by which mammals can manipulate the interrelationship between metabolism,  $T_b$  and  $T_a$  (Scholander *et al.* 1950). Modification of insulation in response to climate, both seasonal and geographical is one way mammals adjust their energetics in response to climate, but climate may also influence the energetics of mammals (Irving *et al.* 1955; McNab 1980; Chown *et al.* 2004). For example, with prolonged exposure to cold conditions in the laboratory, many small mammals increase their rate of metabolism (McNab 1980). Many mammals that inhabit desert areas (e.g. heteromyid rodents) have low BMR's, especially if their diet consists of dry foods such as seeds (Carpenter 1966; McNab 1979a). A low BMR reduces water exchange which is important for mammals living in an environment where free water is often unavailable. Aquatic mammals generally have high BMR's to compensate for the high rate of heat loss in water (McNab 1978a; Dawson *et al.* 1979; Fish 1979; McNab 1980; Lavigne *et al.* 1986; McNab 2008), which has a thermal conductivity that is 24 times higher than that of air (McNab 2002). For example, the platypus has a low  $T_b$  similar to the other species of monotremes, however a MR that is more comparable to that of placental mammals (Dawson *et al.* 1979; Frappell 2003), presumably due to the thermally demanding semi-aquatic lifestyle of the platypus. Some studies have examined interspecific variation in BMR due to habitat, but few studies have focused on the intraspecific variation in the physiology of geographically widespread mammals. While broad-scale interspecific studies are important as they demonstrate how large environmental variation influences physiological traits (e.g. the influence of climatic variability on continental and hemispheric variation in mammalian MR; Lovegrove 2000; Chown *et al.* 2004), smaller-scale interspecific studies are also crucial to our understanding of the plasticity of a species.

Fossorial and burrowing mammals spend a large portion of time in an atmosphere saturated with water, with limited opportunities for heat dissipation, and that may be hypercapnic and hypoxic, and as such, generally have low a BMR (with the exception of those weighing <60g), presumably to prevent excess heat storage and limit gas exchange requirements (McNab 1979b, 2002; Lovegrove 1986; Frappell *et al.* 1994). Semi-fossorial species also tend to have a short inspiration time to total breath time compared to non-fossorial mammals (Frappell *et al.* 1994). Another specialisation linked to a low BMR in mammals is armour. Intermediate-size mammals in particular, that have low running speeds and endurance and need to forage away from cover, have evolved armour to reduce predation (Lovegrove 2001). This adaptation is associated with a low BMR as there is a lack of selection for locomotory performance (Lovegrove 2001). It has previously been thought that the low BMR of larger armoured animals is mostly attributed to their primitive phylogenetic position (McNab 1984). However, more recent studies indicate that this is not necessarily the case, as the phylogeny of armoured animals ranges from the most ancestral mammals (monotremata), to quite recently evolved groups (Pholidota), so their low BMR cannot be attributed to a primitive phylogenetic position (Lovegrove 2001).

Echidnas provide an ideal model with which to study broader scale questions relating to plasticity and evolution of mammalian physiology as they are a geographically wide—spread species with a long life-history that have retained some primitive mammalian characteristics while also being capable of survival in a wide range of habitats. Echidnas are a unique and iconic Australian mammal, belonging to the small and specialised order



**Figure 1.1:** Hind claws of *T.a. setosus* from Tasmania (left) and *T.a. acanthion* from W.A. (right). Note the long claws on digit II and III for *T.a. setosus* compared to the long claw on digit II and short claw on digit III for *T.a. acanthion*.

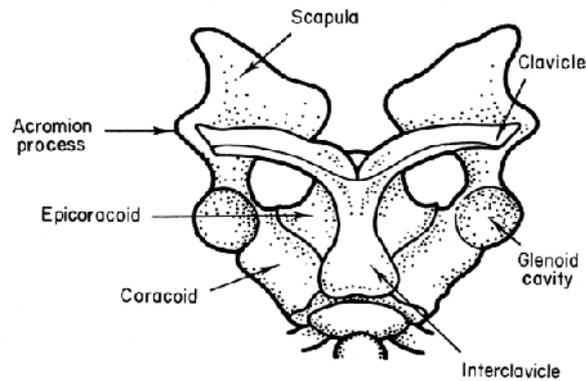
Monotremata. There have been many and varied studies examining the physiology of short-beaked echidnas over a long period of time, which has fuelled much debate concerning aspects of the physiology and ‘primitiveness’

of this group (Brice 2009). This thesis examines the physiological characteristics and adaptations of short-beaked echidnas (*Tachyglossus aculeatus*) to shed some light on this topic. Energy metabolism, thermoregulatory strategies and water balance are examined for the most wide-spread sub-species (*Tachyglossus aculeatus acanthion*) to better examine the physiological adaptations of typical echidnas in an ecological and phylogenetic context.

Short-beaked echidnas (*Tachyglossus*) represent one of three extant genera of monotreme. The order Monotremata is the only group of subclass Prototheria that has survived to the present (Van Dyck and Strahan 2008). It is thought that Prototherians diverged very early in the evolution of mammals, possibly early in the Jurassic period (~200 million years ago), long before subclass Theria separated into eutherians and marsupials (Van Dyck and Strahan 2008). Order Monotremata most likely evolved from a pre-Cretaceous origin independently of the Theria, and as a result have a range of ancestral and derived features (Brice 2009). The other two genera of monotreme are *Zaglossus* (long-beaked echidnas) and *Ornithorhynchus* (platypus). Long-beaked echidnas are larger than the short-beaked echidna, and there are currently three recognised extant species; *Zaglossus bruijini*, *Zaglossus attenboroughi* and *Zaglossus bartoni*. The platypus belongs to a separate family and is represented by one extant species, *Ornithorhynchus anatinus* (Helgen *et al.* 2012). The platypus differs from the echidnas as it is semi-aquatic, possessing a duck-like bill and webbed fore-feet (Grant 1989). All three genera are oviparous, and are the only mammals that use this more primitive form of reproduction. This primitive life history has led many to assume that echidnas also have a primitive physiology, which has been central to much debate. Here I aim to further shed light on this topic by investigating the physiology of short-beaked echidnas using modern laboratory and field techniques and incorporating comparative studies to determine if plasticity to environmental conditions is evident, in terms of geographic and seasonal variation in physiology and thermolability.

First documented by Shaw (1792), the short-beaked echidna was named *Myrmecophaga aculeata*, and was thought to be related to the South American ant-eater. The origin of this original specimen is not known, but it was most likely the 'porcupine' caught near Port Jackson in 1771 by Governor Arthur Phillip. It was first thought that echidnas might

form a link between the old world porcupines (which are rodents) and the edentate anteater *Myrmecophaga*. It was not until 1802 when a specimen was dissected, that its relationship with the platypus was recognised (Dawson 1983). Since the original discovery, the species has been re-named four times (reflecting changes in taxonomy); of the currently accepted nomenclature is *Tachyglossus aculeatus* (Iredale and Troughton 1934).



**Figure 1.2:** Ventral view of the short-beaked echidna's pectoral girdle (Griffiths 1992).

Perhaps the most striking feature of the short-beaked echidna is a dorso-ventrally compressed body covered in spines and hair. They are able to roll into a tight ball with only their sharp spines exposed, to avoid predation which limits their locomotory function (Clemente *et al.* 2016). Their skeletal structure reflects aspects of both reptilian and mammalian lineages. Perhaps the most primitive morphological character of echidnas is their pectoral girdle (Figure 1.2), which has similarities to that of therapsid reptiles (Griffiths 1989). The epipubic

bones are retained in the pelvis, as they are in marsupials. Their sprawling posture is a result of both the humerus and femur projecting horizontally at right angles to the body (Griffiths 1989), neither typically reptilian nor mammalian. Such



**Figure 1.3:** Pelage of *T.a. setosus* from Tasmania (left) and *T.a. acanthion* from W.A. (right). Note the dense fur and sparse spines of *T.a. setosus* compared to the dense covering of spines and minimal fur of *T.a. acanthion*.

primitive morphological characters lead to locomotory limitations (Clemente *et al.* 2016) and contribute to the common presumption that all aspects of this unique mammal are just that; primitive. While there is no doubt that short-beaked echidnas possess many primitive morphological features, I predict that their physiology is not as such, and encompasses many more ‘modern’ adaptations. They have not just survived, but thrived in an ever changing habitat and are seemingly quite plastic; adapting to extremes in climate from snow-capped mountains to hot deserts. I aim to further examine the physiology of short-beaked echidnas in a controlled environment to determine their physiological capabilities over a range of  $T_a$  (Chapter 1). By examining physiological responses to a range of  $T_a$ , it can be determined if echidnas display more a ‘primitive’, poorly regulated physiology or if in fact they are well adapted and have a more ‘modern’ physiology under precise control.

Short-beaked echidnas are one of the most widely distributed native Australian mammals and are the only species that has been recorded in all major terrestrial environments (Figure 1.4). They are found in every habitat type Australia-wide, including tropical savannahs, coastal heaths, alpine areas and deserts (Griffiths 1968; Abensperg-Traun 1991; Morrow *et al.* 2009; Nicol and Morrow 2012). The IUCN Red List lists short-beaked echidnas as a species of least concern with a stable population. Echidnas are



**Figure 1.4:** Distribution of short-beaked echidnas across Australia and Papua New Guinea. The dark grey areas indicate presence; across all of Australia.

armoured and have strong limbs to dig into soil when threatened so predation pressures are minimal. In addition to this, a specialised diet with little competition and a low energy lifestyle enables them to inhabit a wide range of habitats with little threat of predation or competition, meaning they are successful and abundant.

Despite this large and diverse geographical range, all Australian

short-beaked echidnas belong to a single species. There are five recognised subspecies, which differ primarily in morphological characteristics such as shape of the spines, length of the hind grooming claw and degree of hairiness (Figures 1.1 and 1.3; Griffiths 1968). It is however, still unclear if they differ with respect to their biology, in particular their physiology. These subspecies include *Tachyglossus aculeatus acanthion* (Western Australia, Northern Territory and arid zones of all mainland states), *Tachyglossus aculeatus aculeatus* (coastal regions and diving range slopes of southern Queensland, New South Wales, Victoria and South Australia), *Tachyglossus aculeatus lawesii* (Papua New Guinea), *Tachyglossus aculeatus multiaculeatus* (Kangaroo Island) and *Tachyglossus aculeatus setosus* (Tasmania). It has been hypothesised that differences in soil and climate between these locations have resulted in adaptive modification to the proportions of the claws and hair length over time, at the extremes of the species range (Griffiths 1968). *Tachyglossus aculeatus aculeatus* and *T.a. setosus* have a claws on the II and III digits that are almost the same length, while *T.a. acanthion*, *T.a. lawesii* and *T.a. multiaculeatus* have a claw on the III digit that is less than half the length of that on the II digit (Figure 1.1; Griffiths 1968). The sub-species with the greatest difference in pelage are *T.a. acanthion*, which has short, scarce hair with long stout spines, and *T.a. setosus*, which has soft, thick and woolly hair with relatively few spines (Griffiths 1968). It is for this reason that these two sub-species, with the greatest morphological differences, and most distinct climates, were chosen to examine geographic variation in echidna physiology and the potential adaptation to varying climate.

In addition to a unique phylogenetic history and the retention of many primitive morphological features, echidnas are also unique in that they are one of the very few Australian mammals that is myrmechopagous, feeding primarily on ants and termites (Abensperg-Traun 1988), although they will also occasionally also eat earthworms and scarab and moth larvae (Griffiths 1989). Termites are preferred in more arid areas (such as Western Australia; W.A.), despite there still being an abundance of ants, which is most probably due to termites having a much higher water content (up to 80%) than ants (64%; Griffiths 1989). Comparatively, termites make up a very small portion of the diet for echidnas living in more mesic areas such as Tasmania (Nicol, *pers. comm.*). Regardless

of where echidnas live, and on which prey type they specialise, they are seemingly able to obtain all their food and water requirements from ants and termites (Dawson 1983). Despite providing all their energetic requirements, ants and termites as a food source have a low digestibility due to their high chitin content (McClintock 1986; Cooper and Withers 2004), a low caloric density due to ingesting considerable quantities of detritus with food (McNab 1984), and reduce the duration of predator foraging bouts by soldier defence strategies (Redford 1987; Abensperg-Traun *et al.* 1991). Despite the low digestibility and energy content of ants and termites, a myrmecophagous diet also has several advantages such as large, easily exploitable food sources, low interspecific competition and a high moisture content (reducing the need for free water; Bentley and Schmidt-Nielsen 1967; Redford and Dorea 1984; Cooper and Withers 2004). This enables echidnas to inhabit a wide range of habitats and remain active throughout the majority of the year. Myrmecophagy is commonly associated with a low BMR and  $T_b$  (McNab 1984), which is evident for echidnas. This lowered BMR reduces the overall energy requirement which is important for mammals feeding on a diet with such a low energy density (McNab 1984). Associated with a low BMR is often a low field metabolic rate (FMR). As part of my study I measured the FMR of short-beaked echidnas, as they have a specialised diet of only ants and termites, it is therefore possible to obtain a full picture of the dietary requirements and water economy index (WEI).

While short-beaked echidnas have the ability to remain active year-round due to not experiencing considerable food shortages, the low energy density of their diet may contribute to a low energy lifestyle, for which they have adapted to reduce energy expenditure. One such adaptation is that they are opportunistic in their time of activity rather than remaining strictly diurnal/nocturnal throughout the year. They change from primarily nocturnal activity during summer to more diurnal activity during winter (Clemente *et al.* 2016); echidnas are active during periods of favourable ambient temperatures and climatic conditions (Griffiths 1989). During inactive periods, echidnas shelter in shallow depressions in leaf litter and at the base of trees and shrubs, as well as in hollow logs and subterranean shelters (Griffiths 1989; Abensperg-Traun 1991). As well as sheltering in more favourable microclimates, echidnas also reduce exposure to extreme

climatic conditions and conserve energy by utilising hibernation (Augee *et al.* 1970; Griffiths 1989). Echidnas, at least in the colder areas of their range, show a typical mammalian pattern of hibernation, whereby they frequently arouse to euthermia during long, deep bouts of hibernation throughout winter (Augee *et al.* 1970; Nicol and Andersen 2002). However, Nowak *et al.* (2016) found that W.A. echidnas use heterothermia plastically in response to environmental disturbance. In this study I examine patterns of  $T_b$  variation in W.A. short-beaked echidnas in both the laboratory and the field, to quantify the occurrence and extent of heterothermia for this sub-species, and determine if there is geographic variability in patterns of heterothermia for echidnas. Echidnas in the eastern states of Australia exhibit deep, prolonged hibernation that is strictly seasonal. However, seasonal hibernation has not been described for echidnas in W.A., and heterothermia appears more plastic, so patterns of  $T_b$  regulation may be quite different for amongst sub-species. Here I examine the physiological adaptations of short-beaked echidnas to further investigate the 'primitive vs. specialised' debate and to determine how plastic the physiology of this species is in response to daily, seasonal and geographic variation in environmental conditions. Energy metabolism (basal metabolic rate, respiratory ventilation and field metabolic rate), thermoregulation (body temperature regulation in the laboratory and the field and thermal conductance) and water balance (evaporative water loss, field water turnover rate and water economy index) of the short-beaked echidna in south-west WA were examined seasonally and compared with the other sub-species of echidna as well as to other mammals in general.

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# Thermoregulatory adaptations of short-beaked echidnas in Western Australia

## Abstract

Short-beaked echidnas have a more specialised and less primitive physiology than previously reported. I have confirmed previous measurements of low basal body temperature and metabolic rate of echidnas ( $29.5 \pm 0.32$  °C and  $0.08 \pm 0.010$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> respectively) compared with other mammals. Echidnas are incredibly thermolabile, allowing body temperature to fluctuate up to 8.1 °C over an ambient temperature range of 22.5 °C (10 - 32.5 °C). Such physiological traits, that differ substantially from other mammals, are often considered 'primitive', but perhaps are a result of specialisation to their environment and low energy lifestyle rather than being primitive features of poor regulatory ability. Echidnas are in fact very effective thermoregulators. Rather than allowing body temperature to fluctuate widely with little control over a range of ambient temperatures, echidnas regulate tightly around what appears to be a discrete set point, which varies with ambient temperature. This controlled lowering of body temperature at low ambient temperatures saves considerable energy (up to 30% by dropping body temperature by 5.6 °C below basal). This is important given their low energy diet, fossorial habit and armoured anatomy, and presumably contributes to their success in a wide range of environments. The pattern of water loss over a range of ambient temperatures for echidnas is typical of most mammals, including a thermoregulatory increase at high ambient temperatures. This finding is not consistent with previous reports that suggested that echidnas do not increase evaporative water loss to thermoregulate. I found that echidnas do evaporatively dissipate heat at high ambient temperatures and therefore do have an effective mechanism for physiological heat loss and are not necessarily reliant only on behavioural thermoregulation in the heat. Noteworthy was my observation at high

ambient temperatures that air expired from the nostrils formed mucous bubbles, which broke on the snout, providing a moist layer over the blood sinus that presumably aids in cooling. Short-beaked echidnas are well adapted to their environment and have several physiological adaptations that allow them to thrive in a wide range of habitats. As such their standard physiology can provide information concerning the evolution of mammalian physiology and ecological adaptation.

## Introduction

Monotremes (echidnas and platypus) have a very 'primitive' phylogenetic position amongst mammals, diverging long before modern day therian (marsupial and placental) mammals (Dawson *et al.* 1978; Bininda-Emonds *et al.* 2007), and as such have generated considerable interest amongst comparative physiologists.

There has been considerable debate as to how the 'primitive' phylogenetic position of echidnas is reflected in their reproductive, thermal and ventilatory physiology. It is widely accepted that short-beaked echidnas (*Tachyglossus aculeatus*) have a low basal rate of metabolism (BMR;  $-0.15 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) and body temperature ( $T_b$ ;  $-31^\circ\text{C}$ ); however their thermoregulatory abilities over a wide range of  $T_a$ 's are still unclear. Early physiological studies (De Miklouho Maclay 1883; Sutherland 1896) suggested that short-beaked echidnas are physiologically very 'primitive' due to their low and variable  $T_b$ , which reflected an intermediate state between the 'lower' reptiles and 'higher' mammals. Martin (1903) obtained a more complete picture of the short-beaked echidna's physiology by exposing them to a range of ambient temperatures ( $T_a$ ), and concluded that echidnas have a low  $T_b$  and are poor thermoregulators as their attempts at homeothermy largely failed. Their  $T_b$  fluctuated by up to  $10^\circ\text{C}$  when exposed to  $T_a$  of  $5\text{-}35^\circ\text{C}$ , and higher  $T_a$ 's were fatal. However, although he measured echidnas over a broad range of  $T_a$ s, he only measured three individuals (two of which were juveniles) and only exposed them to these  $T_a$  for 1.5-2 hours. Later studies by Robinson (1954) and McMurchie and Raison (1975) also concluded that short-beaked echidnas are poor homeotherms, unable to maintain a constant  $T_b$  with fluctuating  $T_a$ . However, Schmidt-Nielsen *et al.* (1966) concluded that

echidnas are generally very effective thermoregulators. When exposed to low  $T_a$  (as low as 0°C) echidnas maintained their  $T_b$  within the usual range (30-31°C), and when exposed to high  $T_a$  (30°C or above), their  $T_b$  increased by several degrees. However, many of these early findings are somewhat questionable due to methodological issues such as measurement duration (Cooper and Withers 2009; Page *et al.* 2011; Connolly and Cooper 2014) and sample size. Brice (2009) later concluded that echidnas are highly specialised mammals, with a combination of both ancestral and derived characters, and that labelling monotremes as physiologically 'primitive' is somewhat of an oversimplification (Brice 2009).

Despite conflicting opinions of the echidna's thermoregulatory 'primitiveness' and homeothermic capability, all studies agree that echidnas have a very low basal  $T_b$ . The general consensus is that echidnas maintain their  $T_b$  at 30-32°C when exposed to  $T_a$  within their thermoneutral zone (20-25°C; Martin 1903; Schmidt-Nielsen *et al.* 1966; Dawson and Hulbert 1970; Augee 1976; Dawson *et al.* 1979; Frappell *et al.* 1994; Nicol and Andersen 2007). This  $T_b$  is considerably lower than that of other mammals, with marsupials generally maintaining  $T_b$  around 35.5°C and placentals generally maintaining  $T_b$  around 38°C (Dawson and Hulbert 1970; Tyndale-Biscoe 2005). This low  $T_b$  supports the assertion that echidnas are physiologically 'primitive', as well as being anatomically/phylogenetically primitive (Schmidt-Nielsen *et al.* 1966). Their 'sister' monotreme, the platypus, also has a low  $T_b$ , but appears less thermolabile than echidnas, and are thought to be more 'advanced' than echidnas (Martin 1903; Robinson 1954; Smyth 1973; Grant and Dawson 1978).

The metabolic rate of echidnas is also considerably lower than that of most other mammals. It is generally accepted that echidnas have a BMR approximately 30% of the expected rate for a generalised mammal of a similar size (Dawson and Hulbert 1970; Augee 1976; Dawson *et al.* 1979; Frappell *et al.* 1994). This low BMR may be due to a range of interacting factors, and confounding the effects of body mass, temperature and phylogeny. Food habits, fossoriality, spines ('armour') and the use of torpor also account for considerable variation in mammalian BMR (White and Seymour 2004; McNab 2008). Echidnas have a low energy diet (ants and termites), precluding a high metabolic

rate; myrmecophagy is associated with a low BMR for mammals in general (McNab 1984; Cooper and Withers 2006; McNab 2008). Armoured animals also generally have a low BMR, reflecting their sedentary defensive strategy (Lovegrove 2000, 2001), as do mammals that use torpor (Cooper and Geiser 2008) and those that burrow (McNab 1979); echidnas encompass all of these low energy characteristics.

Contributing to the notion that echidnas have a 'primitive' physiology is the fact that they do not appear to use evaporative cooling at high  $T_a$ . They appear to lack sweat glands (Martin 1903; Schmidt-Nielsen *et al.* 1966; Augee 1976) and some early studies (Martin 1903) also concluded they lack the ability to make vasomotor adjustments (eg. vasoconstriction, vasodilation). This suggests a very primitive capacity for thermoregulation in the heat, which necessitates inactivity during hot daytime temperatures (Martin 1903; Robinson 1954). In contrast, both long beaked echidnas (*Zaglossus bruijini*) and platypus (*Ornithorhynchus anatinus*) sweat to increase their evaporative heat loss (Augee 1976; Dawson *et al.* 1978). It is of interest that short-beaked echidnas appear to be the only extant species of monotreme that does not utilise sweating as a method to dissipate excess heat, suggesting that if short-beaked echidnas lack sweat glands then this is a derived rather than primitive characteristic. The only physiological strategy that short-beaked echidnas appear to have to prevent over-heating is to have a low metabolic rate and thus metabolic heat production. Consequently, echidnas are thought to rely heavily on behavioural strategies for thermoregulation in the heat. The activity patterns of echidnas are somewhat opportunistic and inversely correlate with  $T_a$ . Echidnas avoid thermal extremes by sheltering in burrows and caves, and becoming primarily nocturnal during the hot summer months (Robinson 1954; Augee *et al.* 1975; Abensperg-Traun and De Boer 1992; this study, chapter 6). However, the  $T_a$ 's of echidna's shelters in W.A. (caves, burrows and logs) can exceed what has been reported as their lethal  $T_a$  (~37°C; Martin 1903) so these echidnas must presumably have some physiological mechanism to tolerate high  $T_a$  (Brice *et al.* 2002).

Few studies have examined the respiratory ventilation of echidnas. Early studies found that monotremes have a respiratory frequency ( $f_R$ ) that is much slower than that of marsupials (Martin 1903; Robinson 1954; Parer and Hodson 1974; Frappell *et al.* 1994),

and does not differ greatly over a range of  $T_a$ , except at high  $T_a$  where  $f_R$  increases (Robinson 1954). Values for tidal volume ( $V_T$ ) and minute volume ( $V_I$ ) of echidnas have been somewhat variable between studies. Parer and Hodson (1974) found that both were considerably lower than for other mammals of a similar body size, which presumably is related to their low  $T_b$  and BMR. Bentley *et al.* (1967), however, found slightly higher values than Parer and Hodson (1974) and concluded these were within the normal expected range. Differences in minute volume ( $V_I = f_R \times V_T$ ) between the two studies may be explained by difference seen in  $f_R$ . Oxygen extraction ( $EO_2$ ) is comparable and not out of the 'normal' range seen in mammals (Bentley *et al.* 1967).

In this chapter I investigated the thermal, metabolic, hygric and ventilatory physiology of short-beaked echidnas to provide the first comprehensive standardised physiological data set over a wide range of ambient temperatures. My study will allow better comparison of this unique species with other mammals and provide a clearer picture of echidna physiology in an evolutionary context. The hygric physiology of echidnas, in particular, is poorly documented and my study has the first complete hygric data set obtained using modern methodology, and will shed some light on how echidnas are able to physiologically regulate  $T_b$ , especially at high  $T_a$ 's. I predict that echidnas are physiologically better adapted to tolerate a wide range of ambient conditions than previously appreciated. They are the most widespread Australian mammal and are still very abundant across Australia, including hot, dry environments, despite changing environmental conditions. They are likely to possess physiological characteristics that complement their behavioural adaptations, and allow them to withstand a variety of environments.

## Materials and Methods

Seven reproductively-mature short-beaked echidnas (*Tachyglossus aculeatus acanthion*) were captured by hand at Dryandra Woodland (31° 46' S 117° 1' E), 170km south-east of Perth, Western Australia. The echidnas were housed in a custom-built outdoor enclosure at Curtin University, Bentley (32° 0' S 115° 53' E), where they experienced

natural weather variation and photoperiod. All the echidnas were housed together in a large (10m x 9m) enclosure except prior to experimentation. The night preceding experimentation, 1-2 echidnas were moved into a small enclosure (approximately 10m x 1m) and fasted to ensure they were in a post-absorptive state. Echidnas were maintained on a diet consisting of tinned cat food, flaky bran, insectivore powder (Wambaroo, Glen Osmond S.A.), primate omnivore powder (Specialty Feeds, Glen Forrest W.A.), primate leaf eater powder (Specialty Feeds, Glen Forrest W.A.) and water blended to make a smooth mash. Fresh drinking water was provided *ad libitum*. Four of the echidnas had been housed in captivity for several months prior to experimentation, while three of the echidnas were captured and released back into the wild within a week.

Experiments were carried out during late summer during the day from approximately 0500h to 1400h (exposed to one temperature per day), which is the echidnas' inactive phase, at  $T_a$  of 10°C, 15°C, 20°C, 25°C, 27.5°C, 30°C and 32.5°C. Echidnas were measured for a minimum of six hours at the highest and lowest  $T_a$ 's, and a minimum of eight hours at all other  $T_a$ 's. Metabolic rate (MR), measured as rates of oxygen consumption ( $VO_2$ ) and carbon dioxide production ( $VCO_2$ ), was measured simultaneously with evaporative water loss (EWL) using open-flow respirometry. Each echidna was weighed, then placed in a metabolic chamber (~32cm x 32cm x 32cm perspex box) located inside a constant temperature room. Air flowed through the chamber (dried using Drierite) at a constant rate of 11.5 to 12.8 L min<sup>-1</sup>, regulated by an Aalborg GFC37 mass flow controller. Excurrent air passed over a Vaisala MNP45A thin film capacitance relative humidity (RH)/ $T_a$  probe, with a subsample flowing through a column of Drierite before  $O_2$  and  $CO_2$  were measured with a Sable Systems Foxbox-C. The data from the Foxbox and RH/ $T_a$  probe were recorded using a custom written Visual Basic (VB) programme (P. Withers) that logged the data every 10 sec. Six echidnas were measured at each  $T_a$ , in random order. Immediately after removal from the chamber,  $T_b$  was measured using an Omega-HH25TC meter with a plastic-tipped thermocouple inserted approximately 6cm into the cloaca (lubricated with Vaseline). Baseline values of background  $CO_2$ ,  $O_2$  and  $H_2O$  vapour were recorded for at least 30 min before and after each experiment, as well as for 15-20 min at least once during experimentation to account

for O<sub>2</sub> drift due to T<sub>a</sub> and pressure changes. VO<sub>2</sub>, VCO<sub>2</sub> and EWL were calculated after Withers (2001), using a custom written Visual Basic programme (P. Withers). Minimal values were calculated by averaging data for a 20 min period where the echidna was resting and inactive and the values had become minimal and steady.

The mass flow-meters were calibrated using a Gilian Gilibrator 2, traceable to a national standard. The oxygen analysers were two-point calibrated using compressed nitrogen (0% O<sub>2</sub>) and dry ambient air (20.95% O<sub>2</sub>). A certified gas mix (0.53% CO<sub>2</sub>; BOCS, Perth, Western Australia) and compressed nitrogen (0% CO<sub>2</sub>) were used to calibrate the CO<sub>2</sub> analysers. The calibration of the relative humidity probes was confirmed using 1% RH air (dried using Drierite to approximately 0.005 mg L<sup>-1</sup>) and 100% RH air (saturated by breathing on the probe).

Ventilatory data were measured for each echidna at each temperature using whole-body plethysmography (Malan 1973; Withers 1977; Dawson *et al.* 2000), simultaneous to metabolic rate/EWL measurement. A custom-built MPX2010 pressure transducer detected pressure changes due to warming and humidifying of inspired air. A Pico ADC11 A/D converter monitored the voltage output from the pressure transducer, which was recorded by a computer every 10ms for the duration of the experiment (starting approximately two hours after placing the animal in the chamber), using Pico Logger software. Ventilatory variables ( $f_R$ , breaths min<sup>-1</sup>; V<sub>T</sub>, ml; V<sub>I</sub>, ml min<sup>-1</sup>; and EO<sub>2</sub>, %) were calculated after Malan (1973) and Cooper and Withers (2004b), using the average of several minutes where the animal was breathing most steadily and consistently and metabolic rate was minimal. The open-system plethysmography traces were mathematically converted to a closed system to account for the time course of breaths, and calibrated by injecting 3ml of air into the chamber with a syringe, after Szewczak and Powell (2003). Respiratory variables were calculated using a custom written Visual Basic data analysis programme (P. Withers).

Respiratory exchange ratio (RER) was calculated as VCO<sub>2</sub>/VO<sub>2</sub> and was used to determine the conversion of O<sub>2</sub> and CO<sub>2</sub> to joules after Withers (1992). Wet thermal conductance (C<sub>wet</sub>; J g<sup>-1</sup> h<sup>-1</sup> °C<sup>-1</sup>) was calculated as MR(joules)/(T<sub>b</sub>-T<sub>a</sub>), while C<sub>dry</sub> was calculated as

$[\text{MR}(\text{joules}) - \text{evaporative heat loss (EHL; joules)}] / (T_b - T_a)$ . EHL ( $\text{J g}^{-1} \text{h}^{-1}$ ) was determined as  $\text{EWL} \times 2.4$  (McNab 2002). MWP ( $\text{J g}^{-1} \text{h}^{-1}$ ) was calculated after Withers (1992), using RER as an indication of the ratio of glucose and lipids being metabolised, which determines water production. Relative water economy (RWE) was calculated as  $\text{MWP}/\text{EWL}$ . The point of relative water economy (PRWE) is the  $T_a$  at which water balance is achieved *i.e.* the  $T_a$  where  $\text{MWP} = \text{EWL}$ .

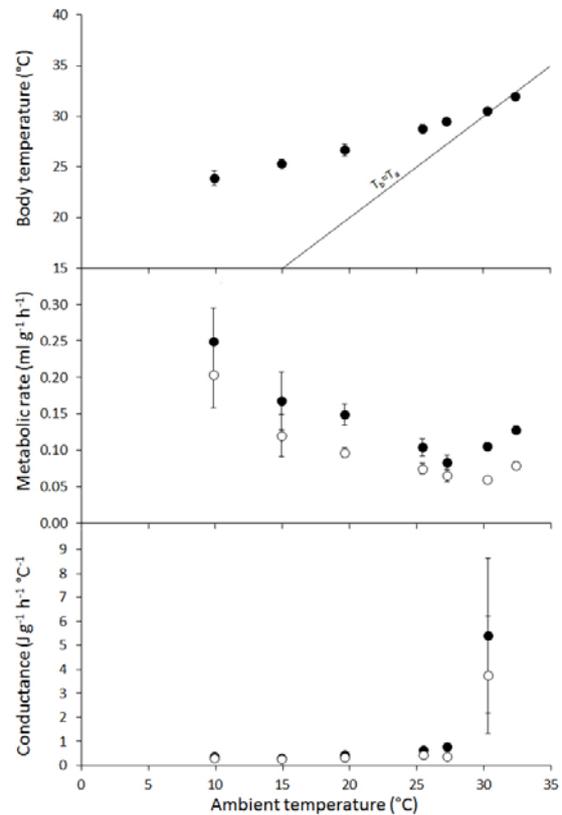
All values are presented as mean  $\pm$  SE with N being the number of individuals and n the number of measurements. Lmer and lmerTest libraries in R (R Core team 2016) were used to examine the influence of  $T_a$ , season (summer, autumn, winter, spring), geographic location and captivity on physiological variables. BMR,  $T_b$ , EWL, and  $C_{\text{wet}}$  were compared to values recorded for all other mammals, including 20 species of myrmecophages, using linear regressions of  $\log_{10}$  transformed variables (except  $T_b$ , which was not logged). Data for comparative analyses were obtained from McNab (2008) for BMR, White and Seymour (2004) for  $T_b$ , Van Sant *et al.* (2002) for EWL, and Bradley and Deavers (1980) for  $C_{\text{wet}}$ . The presence of a phylogenetic signal was examined for all physiological variables using  $K^*$  and P (Blomberg *et al.* 2003), and autoregression (Cheverud and Dow 1985; Rohlf 2001) was used to render the variables independent of phylogeny, with a phylogeny based on the mammal super-tree of Bininda-Emonds *et al.* (2007), using a custom written Visual Basic programme (P. Withers). I acknowledge that there are data for only three species of monotreme, two of which are closely related, which may impact on the phylogenetic analyses. However, these analyses are necessary to make any inference concerning environmental adaptation independent of evolutionary influence on physiological traits. Conformation by echidnas and other myrmecophages to the conventional and phylogenetically independent allometric relationships was tested using the 95% prediction limits for the regressions after Cooper and Withers (2006).

## Results

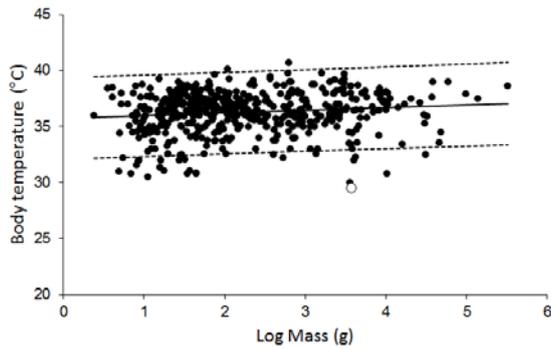
The mean body mass of short-beaked echidnas over all experiments ( $N = 7$ ,  $n = 42$ ) was  $3.53 \pm 0.068$  kg, ranging from 2.77 kg – 4.72 kg. Echidnas rested quietly in the metabolic chamber during experimentation, and were observed adjusting their posture with changing  $T_a$ . At  $T_a$ 's within their thermoneutral zone, echidnas rested on their feet, loosely curled. At low  $T_a$ 's, echidnas rested tightly curled on their feet, often shivering. At the highest  $T_a$ , echidnas sprawled out across the chamber, lying flat on their stomachs with limbs and beak outstretched. At high  $T_a$ 's echidnas were also observed 'blowing bubbles' from their nostrils. Expired air would form a mucous bubble on the tip of the snout, which would break, creating a moist layer across this highly vascular region (pers. obs.).

### *Thermal physiology*

The  $T_b$  of short-beaked echidnas ranged from  $23.9 \pm 0.72$  °C at  $T_a = 10$  °C to  $32.0 \pm 0.24$  °C at  $T_a = 32.5$  °C (Figure 2.1), and was significantly influenced by  $T_a$  ( $F_{6,30} = 49.8$ ,  $P < 0.001$ ).  $T_b$  was significantly different at all  $T_a$  except for  $T_a = 25$  °C and  $27.5$  °C ( $P = 0.192$ ), and  $T_a = 27.5$  °C and  $30$  °C ( $P = 0.111$ ). *A priori* contrasts indicated a significant positive linear relationship between  $T_b$  and  $T_a$  ( $P < 0.001$ ).

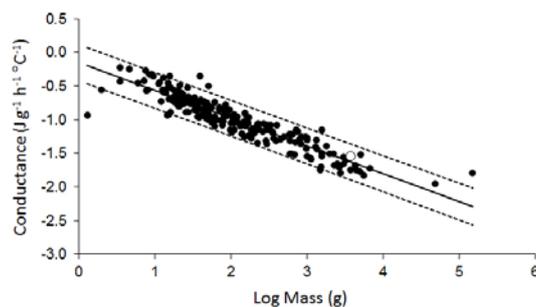


**Figure 2.1:** Body temperature (°C), metabolic rate (ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>, black circles, ml CO<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>, white circles), and wet (black circles) and dry (white circles) thermal conductance (J g<sup>-1</sup> h<sup>-1</sup> °C<sup>-1</sup>) of short-beaked echidnas at ambient temperatures from 10 °C to 32.5 °C during summer. Values are mean ± SE.  $n = 6$



**Figure 2.2:** Body temperature (°C) of 491 species of mammal (black circles; White and Seymour 2004) and short-beaked echidnas (white circle) as a function of body mass, with the regression line (solid) and 95% prediction limit bands (dashed).

When short-beaked echidnas were compared to these data they fell outside (below) the 95% prediction limits, indicating that the  $T_b$  of echidnas is significantly lower than expected for their size. A  $K^*$  of 0.331 indicated a weak phylogenetic signal for  $T_b$ , although it was highly significant ( $P < 0.001$ ). Correction for phylogeny removed the significant allometric effect on  $T_b$  ( $R^2 = 0.008$ ,  $F_{1,468} = 3.626$ ,  $P = 0.057$ ), but echidnas remained outside the 95% prediction limits.



**Figure 2.3:** Log transformed allometric relationship for wet thermal conductance of 230 species of mammal (black circles; Bradley and Deavers 1980) and short-beaked echidnas (white circle), with the regression line (solid) and 95% prediction bands (dashed).

Both  $C_{wet}$  and  $C_{dry}$  were relatively constant below  $T_a = 30$  °C (Figure 2.1). At  $T_a = 30$  °C and 32.5 °C, the calculation used to determine conductance was less reliable as  $T_b$  approximated  $T_a$ , and so the denominator approached 0, thus  $MR/T_b - T_a$  approached infinity (at  $T_b = T_a$ ). As a result, there is no statistically significant effect of  $T_a$  on either  $C_{wet}$  ( $F_{6,35} = 0.749$ ,  $P = 0.614$ ) nor  $C_{dry}$  ( $F_{6,35} = 0.723$ ,  $P = 0.634$ ). *A priori* contrasts showed no significant polynomial relationships between  $C_{wet}$  and  $T_a$  (linear  $P = 0.931$ , quadratic  $P = 0.622$ ), but when  $T_a = 30$  °C and 32.5 °C were excluded from the analysis, a significant linear relationship ( $P = 0.004$ ) was evident, indicating that thermal conductance does increase, albeit slightly, with increasing  $T_a$  below 30 °C.

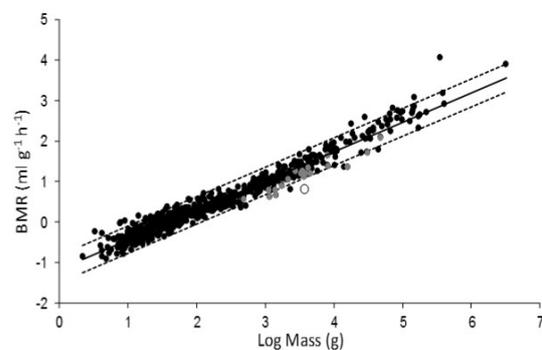
There was a significant but weak linear relationship between  $T_b$  and body mass for 491 species of mammal (eutherians, placentals and monotremes;  $R^2 = 0.016$ ,  $F_{1,489} = 7.72$ ,  $P = 0.006$ ; Figure 2.2). When short-beaked echidnas were compared to these data they fell outside (below) the 95% prediction limits, indicating that the  $T_b$  of echidnas is significantly lower than expected for their size. A  $K^*$  of

Both  $C_{wet}$  and  $C_{dry}$  were relatively constant below  $T_a = 30$  °C (Figure 2.1). At  $T_a = 30$  °C and 32.5 °C, the calculation used to determine conductance was less reliable as  $T_b$  approximated  $T_a$ , and so the denominator approached 0, thus  $MR/T_b - T_a$  approached infinity (at  $T_b = T_a$ ). As a result, there is no statistically significant effect of  $T_a$  on either  $C_{wet}$

A significant allometric relationship ( $R^2 = 0.93$ ,  $F_{1,228} = 2927$ ,  $P < 0.001$ ) was apparent for the  $C_{\text{wet}}$  of 230 mammals (eutherians, placentals and monotremes; Figure 2.3). When compared to the regression,  $C_{\text{wet}}$  of echidnas was 94% of the predicted value for a mammal of their size, which was within the 95% prediction limits. There was a highly significant phylogenetic signal ( $P < 0.001$ ) for  $C_{\text{wets}}$ , although a  $K^*$  of 0.292 indicates that this signal was less than expected from the phylogeny. When the data were corrected for phylogeny, a significant regression was still present ( $R^2 = 0.879$ ,  $F_{1,171} = 1237$ ,  $P < 0.001$ ) and echidnas remained within the 95% prediction limits.

### *Metabolic physiology*

Metabolic rate was significantly influenced by  $T_a$  ( $F_{6,30} = 8.41$ ,  $P < 0.001$ ). MR ranged from  $0.08 \pm 0.010 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  at  $T_a = 27.5^\circ\text{C}$  to  $0.25 \pm 0.045 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  at  $T_a = 10^\circ\text{C}$  (Figure 2.1). MR did not differ significantly at  $T_a = 27.5^\circ\text{C}$  and  $25^\circ\text{C}$  ( $P=0.508$ ),  $30^\circ\text{C}$  ( $P=0.472$ ) or  $32.5^\circ\text{C}$  ( $P=0.131$ ), but at  $T_a = 27.5^\circ\text{C}$  MR was significantly lower than that at  $10^\circ\text{C}$  ( $P<0.001$ ),  $15^\circ\text{C}$  ( $P<0.001$ ) or  $20^\circ\text{C}$  ( $P=0.005$ ). Significant linear ( $P < 0.001$ ) and quadratic ( $P = 0.001$ ) patterns were evident, and when  $T_a = 30^\circ\text{C}$  and  $32.5^\circ\text{C}$  were removed from the analysis, only a linear pattern remained ( $P < 0.001$ ), indicating that MR steadily decreased with increasing  $T_a$ , and began increasing at  $T_a = 30^\circ\text{C}$ . The lowest MR (at  $T_a = 27.5^\circ\text{C}$ ) was considered to be BMR. The pattern of  $\text{CO}_2$  with  $T_a$  was very similar to that for  $\text{O}_2$ .  $\text{CO}_2$  production was lowest at  $T_a = 30^\circ\text{C}$  ( $0.06 \pm 0.003 \text{ ml CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) and highest at  $T_a = 10^\circ\text{C}$  ( $0.20 \pm 0.046 \text{ ml CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ). Respiratory exchange ratio (RER) ranged from  $0.58 \pm 0.025$  at  $T_a = 30^\circ\text{C}$  to  $0.84 \pm 0.144$  at  $T_a = 27.5^\circ\text{C}$ , but  $T_a$  had no significant influence on RER ( $F_{6,35} = 1.760$ ,  $P = 0.136$ ).

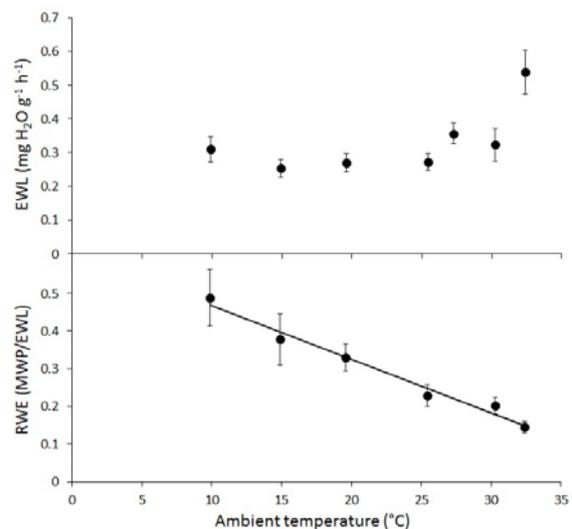


**Figure 2.4:** BMR of 617 species of mammals (black circles; McNab 1970), including myrmecophages (grey circles; McNab 1970) and short-beaked echidnas (white circle), with the regression line (solid) and 95% prediction bands (dashed).

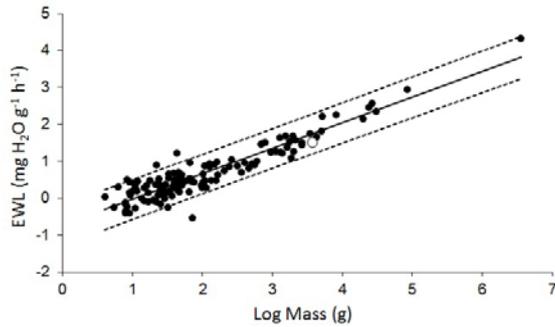
There was a significant linear relationship ( $R^2 = 0.957$ ,  $F_{1,615} = 13571$ ,  $P < 0.001$ ) between body mass and BMR for 617 mammal species (eutherians, placentals and monotremes; Figure 2.4). The BMR of short-beaked echidnas fell below the 95% prediction limits, indicating a significantly lower BMR than predicted for a mammal of their size (44% lower than expected). A  $K^*$  of 0.735 indicated moderate but highly significant ( $P < 0.001$ ) phylogenetic signal. When these data were corrected for phylogenetic history, the regression remained significant ( $R^2 = 0.925$ ,  $F_{1,608} = 7446$ ,  $P < 0.001$ ) and short-beaked echidnas remained below the lower 95% prediction limit. The BMR of 20 myrmecophagous mammals ranged from 2% to 41% lower than predicted, with no myrmecophages exceeding 100% of predicted. However, five species, *Tolypetus matacus*, *Manis crassicaudata*, *Manis tetradactyla*, *Myrmecophaga tridactyla* and *Tachyglossus aculeatus* fell below the 95% prediction limits for all mammals and so could be considered to have a statistically low BMR.

### Hygic Physiology

Ambient temperature had a significant effect on EWL ( $F_{6,30} = 6.11$ ,  $P < 0.001$ ; Figure 2.5). EWL was relatively stable and independent of  $T_a$  at  $T_a \leq 30$  °C (mean EWL =  $0.33 \pm 0.020$  mg H<sub>2</sub>O g<sup>-1</sup> h<sup>-1</sup>), but increased significantly ( $P = 0.003$ ) at  $T_a = 32.5$  °C ( $0.54 \pm 0.066$  mg H<sub>2</sub>O g<sup>-1</sup> h<sup>-1</sup>). There were significant linear ( $P = 0.003$ ) and quadratic ( $P < 0.001$ ) patterns between EWL and  $T_a$ , and when  $T_a = 32.5$  °C, 30 °C and 27.5 °C were removed from the analysis, only a linear pattern remained ( $P = 0.048$ ), indicating EWL increased slightly with  $T_a$ , then more markedly  $\geq 27.5$  °C.



**Figure 2.5:** Evaporative water loss (mg H<sub>2</sub>O g<sup>-1</sup> h<sup>-1</sup>) and relative water economy of short-beaked echidnas at ambient temperatures from 10 °C to 32.5 °C. Values are mean  $\pm$  SE.



**Figure 2.6:** EWL of several species of mammals (black circles; Van Sant *et al.* 2012) and short-beaked echidnas (white circle), with the regression line (solid) and 95% prediction bands (dashed).

A significant allometric relationship was found between body mass and EWL of mammals ( $R^2 = 0.87$ ,  $F_{1,133} = 872$ ,  $P < 0.001$ ; Figure 2.6). Short-beaked echidnas fell within the 95% prediction limits, indicating that their EWL was as expected for a mammal of similar size (86% of expected). A  $K^*$  of 0.506 indicates that there is moderate but highly significant phylogenetic

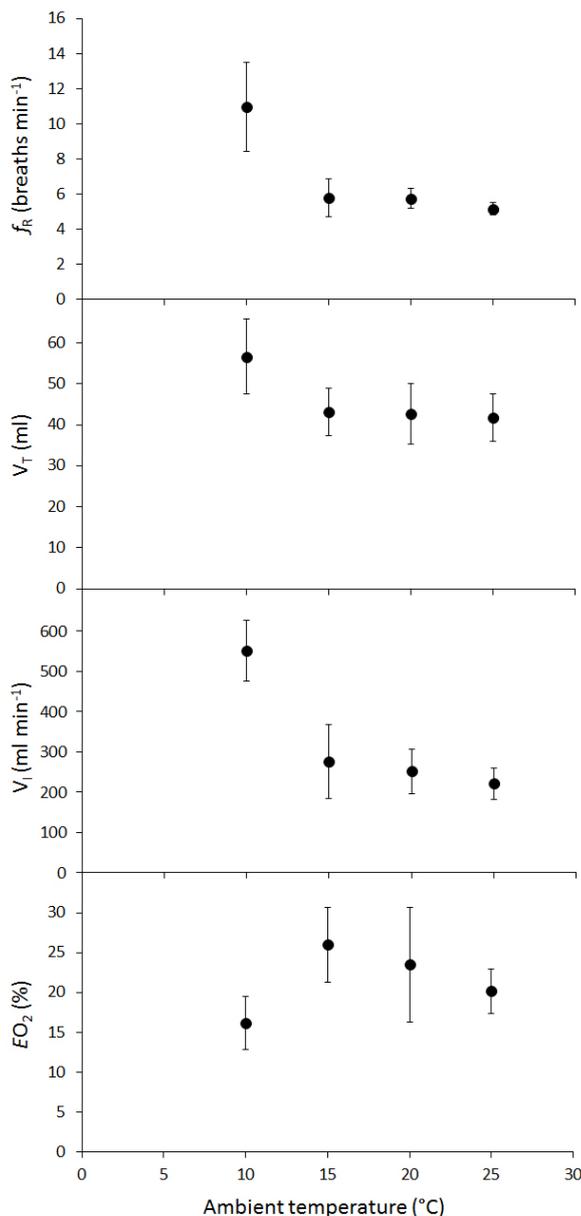
signal ( $P < 0.001$ ) for EWL. When the EWL data were rendered phylogenetically independent by autoregression, the allometric relationship remained ( $R^2 = 0.83$ ,  $F_{1,133} = 645$ ,  $P < 0.001$ ) and echidnas still remained within the 95% prediction limits.

Relative water economy ranged from  $0.145 \pm 0.015$  at  $T_a = 32.5 \text{ }^\circ\text{C}$  to  $0.487 \pm 0.075$  at  $T_a = 10 \text{ }^\circ\text{C}$  (Figure 2.5). There was a significant inverse linear relationship between RWE and  $T_a$  ( $\text{RWE} = -0.0152T_a + 0.621$ ;  $R^2 = 0.62$ ,  $F_{6,30} = 11.6$ ,  $P < 0.001$ ). The PRWE was calculated by extrapolation to be  $-24.9 \text{ }^\circ\text{C}$ .

By allowing their  $T_b$  to rise at high  $T_a$ , echidnas saved a considerable amount of water. At  $T_a = 30 \text{ }^\circ\text{C}$   $T_b$  rose to  $30.57 \text{ }^\circ\text{C}$ , saving  $1.62 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$  compared to maintaining a  $T_b$  of  $29.5 \text{ }^\circ\text{C}$ . At  $T_a = 32.5 \text{ }^\circ\text{C}$ ,  $T_b$  ( $32 \text{ }^\circ\text{C}$ ) approximated  $T_a$  so calculations of conductance became unreliable, resulting in similarly unreliable calculations of EHL and EWL savings.

## Ventilatory physiology

Ventilation of short-beaked echidnas was only measured at  $T_a \leq 25$  °C, as above this temperature  $T_b$  approximated  $T_a$  and so pressure changes from warming inspired air could not be reliably detected. Ventilatory frequency ( $f_R$ ) ranged from  $5.1 \pm 0.3$  breaths  $\text{min}^{-1}$  at  $T_a = 25$  °C to  $10.9 \pm 2.5$  breaths  $\text{min}^{-1}$  at  $T_a = 10$  °C. Ambient temperature had a significant influence on  $f_R$  ( $F_{3,20} = 3.66$ ,  $P = 0.03$ ; Figure 2.7). Ventilatory frequency was significantly



**Figure 2.7:** Ventilatory frequency (breaths  $\text{min}^{-1}$ ), tidal volume (ml), minute volume (ml  $\text{min}^{-1}$ ) and oxygen extraction (%) of short-beaked echidnas at ambient temperatures from 10 °C to 25 °C. Values are mean  $\pm$  SE.

higher at  $T_a = 10$  °C than all other temperatures ( $P = 0.009$ ), but there was no significant difference in  $f_R$  between any other temperatures ( $P > 0.756$ ).

Tidal volume ( $V_T$ ) of short-beaked echidnas was not significantly influenced by  $T_a$  ( $F_{3,15} = 1.03$ ,  $P = 0.405$ ; Figure 2.7). Ambient temperature significantly influenced minute volume ( $V_I$ ;  $F_{3,20} = 5.004$ ,  $P = 0.009$ ; Figure 2.7). Like  $f_R$ ,  $V_I$  was also significantly higher at  $T_a = 10$  °C than all other  $T_a$ 's ( $P = 0.003$ ). Oxygen extraction was not significantly influenced by  $T_a$  ( $F_{3,15} = 1.365$ ,  $P = 0.291$ ; Figure 2.7). A significant allometric relationship was found between body mass and  $f_R$  for a data set of 40 mammalian species ( $R^2 = 0.171$ ,  $F_{1,38} = 7.824$ ,  $P = 0.008$ ). The  $f_R$  of short-beaked echidnas was only 8% of predicted for a mammal of

similar size. Significant allometric relationships were present between mass and  $V_T$  ( $R^2 = 0.958$ ,  $F_{1,38} = 871$ ,  $P < 0.001$ ) and mass and  $V_I$  ( $R^2 = 0.841$ ,  $F_{1,38} = 200.8$ ,  $P < 0.001$ ). The  $V_T$  of echidnas was 129% of predicted for a similar sized mammal, but still fell within the 95% prediction limits. The  $V_I$  of echidnas was only 37% of predicted, however still fell within the prediction limits.

## Discussion

My study of the physiology of short-beaked echidnas over a wide range of  $T_a$ 's allows a broader understanding of their physiology than previous studies. My results suggest that echidnas are not physiologically 'primitive' as once thought, but indeed are capable but flexible thermoregulators. Echidnas have a very low energy lifestyle and thus have no need for a high MR,  $T_b$  or  $f_R$ . They have a low basal  $T_b$  and MR and breath slowly and deeply. Contributing to this frugal approach to energy expenditure is the great deal of thermolability displayed by echidnas, allowing their  $T_b$  to vary with  $T_a$  to save considerable amounts of energy. While allowing  $T_b$  to fluctuate with  $T_a$ , echidnas still retained precise thermoregulation around changing set-points, so this is a specialised adaptation rather than poor thermoregulatory ability. The dated belief that echidnas are poor thermoregulators has also led to the view that they have poor ability to deal with the heat. Many previous studies have concluded that echidnas are not able to tolerate high ambient temperatures, and rely on controlling heat production and behavioural adaptations to escape these extremes (Martin 1903; Robinson 1954; Griffiths 1968, 1978; Augee 1978) rather than augmented EWL. However, my results show that this is not the case.

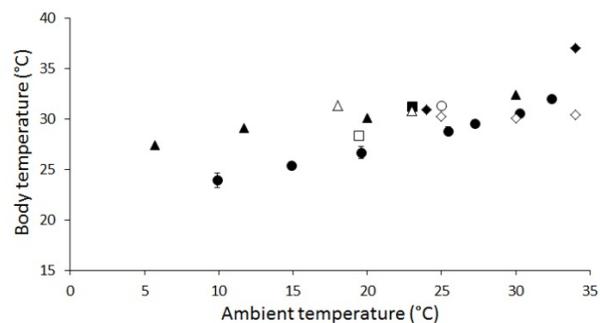
### *Basal physiology*

Basal  $T_b$  and BMR of short-beaked echidnas were significantly lower (44%) than expected for a mammal of similar size (body mass is a major determinant of both  $T_b$  and BMR in mammals (McNab1970)), as expected from previous studies (Martin 1903; Robinson 1954; Schmidt-Nielsen *et al.* 1966; Augee 1976; Dawson *et al.* 1978; Frappell *et al.* 1994;

Nicol and Andersen 2007; compared to the data set from McNab 2008; Figures 2.2 and 2.4). Despite the two monotreme families being long distinct from an evolutionary point of view (Dawson *et al.* 1979), they have similarly low  $T_b$ , *e.g.* short-beaked echidnas have a  $T_b$  of 29.5 °C (current study), long-beaked echidnas have a  $T_b$  of 31.7 °C (Dawson *et al.* 1978), and platypus have a  $T_b$  of 32.0 °C (Grant and Dawson 1978). Although it is generally recognised that comparative methods are required in an attempt to ‘correct for phylogeny’ (Rohlf 2001), the lower  $T_b$  of monotremes is consistent with the phylogenetic structure of mammals, with the first divergent lineage (monotremes) having the lowest  $T_b$ .

When the BMR and  $T_b$  of short-beaked echidnas were corrected for phylogeny, they were both still considerably lower than expected for a mammal of similar size, suggesting an influence of other aspects such as life history and ecology. The lower than expected BMR

of echidnas reflects, at least in part, their low  $T_b$ . In general,  $Q_{10}$  is about 2.5 (Guppy and Withers 1999) so BMR increases 10% for every 1 °C increase in  $T_b$ . However, I accounted for effects of  $T_b$  on MR using the conductance correction formula:  $MR/(C_{wet} \times (T_b - T_a))$ , where MR = actual MR,  $C_{wet}$  = actual  $C_{wet}$ ,  $T_b$  = predicted  $T_b$ , and  $T_a$  = actual  $T_a$ . This calculation is



**Figure 2.8:** Body temperature of short-beaked echidnas in this study (black circles) and previous studies (black triangles: Martin 1903, white triangles: Wardlaw 1914, black squares: Frappell *et al.* 1994, white squares: Robinson 1954, black diamonds: Schmidt-Nielsen *et al.* 1966, white diamonds: Augee 1976, white circles: Dawson *et al.* 1979).

used in preference to the calculation using the  $Q_{10}$  effect as this calculation takes conductance into consideration. The  $Q_{10}$  calculation assumes that conductance changes with  $T_b$ , which is not the case as insulation does not change if  $T_b$  is maintained at a higher temperature. Hence, by maintaining a low  $T_b$  of 29.5 °C (and a MR of 0.08 ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) at thermoneutrality, I calculate that echidnas save 73% energy compared to maintaining their  $T_b$  at 35.5 °C like marsupials (which would result in a MR of 0.31 ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>),

and save 79 % energy compared to maintaining their  $T_b$  at 38 °C like placentals (which would result in a MR of 0.41 ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>).

The BMR value for short-beaked echidnas measured here was significantly lower (1 sample t-tests;  $P \leq 0.005$ ) than previous studies (Martin 1903; Schmidt-Nielsen *et al.* 1966; Augee 1976; Dawson *et al.* 1979; McNab 1984; Bech *et al.* 1992; Frappell *et al.* 1994). This is most likely due to modernised methodology, and longer measurement duration in particular. I measured echidnas for a minimum of six hours at the upper and lower end of the  $T_a$  range, and for a minimum of eight hours at all other  $T_a$ , while other studies measured echidnas for under three hours. Experimental duration can significantly influence measurement of minimum values for physiological variables, with short durations significantly over-estimating BMR and other physiological variables (Cooper and Withers 2009; Page *et al.* 2011; Connolly and Cooper 2014). The difference may also reflect geographic variation; my study examined short-beaked echidnas from Western Australia, while other studies have examined echidnas from eastern Australia; this possible explanation is considered in chapter three.

Mammals that have a low BMR typically have consequently low respiratory ventilation *i.e.* a low  $f_R$  (Darden 1972; Cooper and Withers 2004b). This is evident for short-beaked echidnas;  $f_R$  was 5.1 breaths min<sup>-1</sup> at  $T_a = 25$  °C, which is 13% of that predicted compared to placentals ( $f_R = \text{body mass} \times 53.5^{-0.26}$ ; Stahl 1967) and 8% of the predicted value when compared to an all-mammal dataset I collated from published data (Darden 1972; Arieli and Ar 1979; Casey *et al.* 1979; Schlenker 1985; Hallam and Dawson 1993; Chappell and Dawson 1994; Frappell and Baudinette 1995; Dawson *et al.* 2000; Larcombe 2002; Cooper and Withers 2004b; Larcombe *et al.* 2006; Larcombe and Withers 2006; Larcombe *et al.* 2008; Cooper *et al.* 2009; Withers and Cooper 2009). Previous studies have reported slightly to considerably higher values (7.0 – 17.5 breaths min<sup>-1</sup>), which is most likely due to higher BMR and methodological differences including measurement duration and the use of restraints and/or tactile stimuli (Bentley *et al.* 1967; Augee *et al.* 1971; Parer and Hodson 1974; Frappell *et al.* 1994). Tidal volume of short-beaked echidnas in this study was significantly higher than previous studies ( $T = -3.776$   $P = 0.033$ ; Bentley *et al.* 1967; Parer and Hodson 1974; Bech *et al.* 1992; Frappell *et al.* 1994). Tidal

volume of echidnas from my study was  $41.6 \pm 5.7$  ml (STPD) at  $T_a = 25$  °C, which is 172% of that predicted for a mammal of similar size (compared to placentals; Stahl 1967) and 129% of predicted using the all-mammal data set. Again, this is most likely due to differences in measurement duration, with longer durations achieving a more accurate picture of true resting values (Page *et al.* 2011; Connolly and Cooper 2014). Many of the values used by Stahl (1967) were obtained from mammals that were restrained during measurement, which may affect ventilation (Cooper and Withers 2004b). Echidnas appear to breathe much slower than predicted, but also much deeper. Minute volume of echidnas was 24% of predicted (Stahl 1967), and 37% of predicted using the aforementioned more recent mammalian data set. The values obtained for  $V_I$  by previous studies (Bentley *et al.* 1967; Parer and Hodson 1974; Bech *et al.* 1992; Frappell *et al.* 1994) were not significantly different ( $P=0.939$ ) to those obtained here (lower  $f_R$  was counterbalanced by higher  $V_T$ ). Nevertheless, the echidna's  $V_I$  is lower than expected for a mammal of similar size, mainly due to a much lower  $f_R$  than expected (Bech *et al.* 1992; Figure 2.7). Resting mammalian  $EO_2$  is usually between 15 and 25% (Stahl 1967; Chappell 1985; Cooper and Withers 2004b), reflecting the consistent matching of  $V_I$  to  $VO_2$ . Echidnas have an  $EO_2$  (at  $T_a = 25$  °C) of 20.1%, which conforms to this expected range. Bentley *et al.* (1967) also reported that the  $EO_2$  of echidnas is comparable with placentals, and consistent with my findings.

The most influential factors affecting BMR are body mass (McNab 2008) and temperature (Withers 1992; Guppy and Withers 1999), but many other interacting factors may lower BMR, as seen here for echidnas. A low BMR may be interpreted as a general, primitive feature of monotremes, as the long-beaked echidna (*Zaglossus bruijnii*) has a similarly low BMR ( $0.08$  ml  $O_2$   $g^{-1}$   $h^{-1}$ ). However, the platypus has a higher BMR, of  $0.36$  ml  $O_2$   $g^{-1}$   $h^{-1}$ , which is similar to that of marsupials, although lower than that of placentals (Dawson *et al.* 1979; Frappell 2003). This can be attributed to the high energetic demands of their semi-aquatic lifestyle; aquatic and semi-aquatic mammals might in general have a BMR higher than a 'typical' mammal due to their thermally demanding environment (Dawson *et al.* 1979; Fish 1979; Dawson and Fanning 1981; Lavigne *et al.* 1986; McNab 2008). Echidnas have a number of other characteristics

related to their very low energy lifestyle. They are myrmecophageous, feeding primarily on ants and termites. This diet has a low energy density and poor digestibility (McNab 1984; Redford and Dorea 1984; Cooper and Withers 2004a), but feeding only requires relatively low energy expenditure. Termites are a concentrated food source, with a large number occupying a small area due to their colonial habits, meaning that minimal effort is required to feed. However, the length of feeding bouts by myrmecophages is limited due to the chemical defence strategies of ants and termites (Redford and Dorea 1984). The anti-predator strategy of echidnas also reflects its low energy lifestyle. Their spines provide excellent mechanical defence against predators; they curl into a tight ball of spines so no 'soft parts' are exposed, even if they are flipped over (Abensperg-Traun 1991). This, along with their ability to dig into soil and tightly clasp roots, means they can resist rather than escape from predators. This predator avoidance strategy of armoured and spiny animals is generally associated with a low metabolic rate (Lovegrove 2000, 2001). Mammals that use torpor as an energy conservation strategy generally have a lower BMR than similar-sized mammals that remain strictly homeothermic (Cooper and Geiser 2008; McNab 2008). Echidnas use both short-term torpor and long-term hibernation to balance their energy budget and avoid environmental extremes (Nicol and Andersen 2002; see chapter 5). Echidnas are semi-fossorial, raising their young in nursery burrows and spending much of the hot summer months sheltering in burrows, as well as hibernating in burrows during winter (Nicol and Andersen 2007). Fossorial mammals also tend to have lower-than-expected metabolic rates (McNab 1979, 2008).

### *Low $T_a$ physiology*

An interesting aspect of short-beaked echidna physiology is that  $T_b$  does not conform, over a wide range of  $T_a$ , to the pattern of relative constancy expected for an endothermic mammal (Figure 2.8). While  $T_b$  does not follow a completely 'typical mammalian' pattern with changing  $T_a$ , endothermic regulation of  $T_b$  with endogenous heat production is still apparent. Metabolic rate is highest at low  $T_a$ , decreasing linearly to the point of thermoneutrality at  $T_a = 27.5$  °C, and increasing thereafter as the animals start to become

heat challenged (Figure 2.1). Long-beaked echidnas have a similar pattern of MR with  $T_a$ , though the thermoneutral  $T_a$  at which BMR occurs is lower than for the short-beaked echidnas, at 20 °C (Dawson *et al.* 1978). This likely reflects the higher mass of the long-beaked echidnas and associated lower conductance.  $T_b$  increased linearly with increasing  $T_a$ , with a slope of 0.35. Thermoconformers, such as reptiles, have a  $T_b/T_a$  slope ( $\sim 1$ ) such that  $T_b$  follows  $T_a$  quite closely, whereas effective thermoregulators, such as most 'higher' mammals, maintain  $T_b$  near constant regardless of  $T_a$  and have a  $T_b/T_a$  slope close to 0 (Withers 1992). The  $T_b/T_a$  slope of 0.35 for echidnas, although closer to that of effective thermoregulators (0) than thermoconformers (1), nevertheless argues that echidnas are in fact less effective thermoregulators than other mammals. Although short-beaked echidnas are thermolabile at a range of  $T_a$ , my data suggest that they are quite precise thermoregulators at any particular  $T_a$ . Rather than allowing  $T_b$  to fluctuate widely at a particular  $T_a$ , echidnas appear to regulate  $T_b$  precisely around a certain  $T_a$  dependant set-point; SE of  $T_b$  is typically  $\pm 0.4^\circ\text{C}$ . All six individuals regulated  $T_b$  around a similar set-point at each particular  $T_a$ . So, the perception that echidnas are poor thermoregulators that allow their  $T_b$  to fluctuate widely with  $T_a$ , with an inability to precisely maintain a constant temperature is somewhat misinformed.

Short-beaked echidnas can save a substantial amount of energy because of their thermolability (in addition to having a low  $T_b$ ). For example, they save up to 30% energy at low  $T_a$  by reducing  $T_b$  by 5.6 °C from basal  $T_b$  (29.5 °C at  $T_a = 27.5$  °C) to 23.9 °C at  $T_a = 10$  °C. Metabolic rate is correspondingly reduced to 0.25 ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>, whereas if  $T_b$  was maintained at 29.5 °C their MR would be 0.36 ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>. Martin (1903) also noted the energy savings of allowing  $T_b$  to drop at low  $T_a$ . He calculated that his echidnas saved 800 Cal kg<sup>-1</sup> °C<sup>-1</sup> by this 'imperfect homeothermism'. Echidnas in my study saved considerably more energy (1589 Cal kg<sup>-1</sup> °C<sup>-1</sup> h<sup>-1</sup>) than Martin (1903) reported, but this more likely reflects advances in measurement techniques and accuracy since this early study. Utilising this thermolability to conserve energy therefore is in accord with their energy conservation strategies of a low basal  $T_b$  and BMR (Figure 2.1).

The increase in VO<sub>2</sub> of short-beaked echidnas at low  $T_a$  is accommodated by a proportional increase in V<sub>I</sub>; both MR and V<sub>I</sub> increase by 2.4 times from  $T_a = 25$  °C to 10

°C. The increase in  $V_I$  is explained by a 2-fold increase in  $f_R$  and 1.4-fold increase in  $V_T$  (Figure 2.7). Generally smaller mammals increase  $f_R$  rather than  $V_T$  and larger mammals increase  $V_T$  rather than  $f_R$  to accommodate increased  $VO_2$ , so a greater increase in  $f_R$  was somewhat unexpected (Larcombe 2003; Cooper and Withers 2004).  $EO_2$  decreases at  $T_a = 10$  °C, suggesting that perhaps the echidnas were a little unsettled at this cold temperature, which may explain the unexpected  $f_R$  and  $V_T$ .

For short-beaked echidnas, relative water economy increases linearly ( $R^2 = 0.98$ ) with decreasing  $T_a$  (Figure 2.5), as is generally observed for mammals (Cooper *et al.* 2005). Their PRWE is very low, about -24.9 °C, although this calculation of PRWE requires a considerable extrapolation of  $T_a$  down to PRWE (where RWE = 1). Generally, PRWE is dependent on body mass and  $T_a$ , with smaller mammals having higher values (Cooper *et al.* 2005), so echidnas would be expected to have a reasonably poor PRWE (about 0 °C) due to their size (Pusey *et al.* 2013). Mammals that are mesic-adapted also tend to have a lower PRWE than those that are arid-adapted (Cooper *et al.* 2005; Hinds and MacMillan 1985). However, the very low PRWE of echidnas, of -24.9 °C, is most likely a reflection of their low energy strategy and diet rather than being poorly adapted. Echidnas have a low MR (and thus MWP) but a normal mammalian EWL, so RWE is poor as they are losing relatively more water than they are producing metabolically. A diet of termites has a high water content and a relatively low energy content (Cooper and Withers 2004) so, relative to the quantity of termites that echidnas ingest to meet their energetic requirements, they take in a considerable quantity of water (see also chapter 4). Consequently, they do not need a high PRWE (ie. MWP/EWL) to maintain water balance, so it is not surprising that their RWE is very poor.

Postural adjustments were observed over changing  $T_a$ 's, with echidnas resting in a curled position below  $T_a = 30$  °C (becoming tighter curled as  $T_a$  decreased), which minimises SA:V ratio and reduces heat loss. There was a slight decrease in  $C_{wet}$  and  $C_{dry}$  at low  $T_a$ , consistent with possible minor changes in posture and vasomotion. At the lower  $T_a$  (10 °C and 15 °C) echidnas were also observed shivering to augment metabolic heat production.

### *High $T_a$ physiology*

Many previous studies have concluded that echidnas have poor ability to deal with the heat, with minimal physiological adaptations to aid in heat dissipation. However, my study shows that evaporative water loss,  $C_{\text{wet}}$  and  $C_{\text{dry}}$  of echidnas all conform to the typical mammalian pattern: remaining relatively stable at ambient temperatures below 30 °C, but sharply increasing above 30 °C, where  $T_b$  is also about 30 °C (Figures 2.1 and 2.5). Below this  $T_a$ , there is no need for echidnas to dissipate excess heat for cooling, whereas above the thermoneutral zone heat needs to be offloaded to the environment to avoid overheating.

Heat challenged mammals typically increase EWL to dissipate heat by various evaporative avenues (such as panting, licking, and sweating) and non-evaporative avenues (vasodilation, posture). However, previous studies have noted that echidnas do not pant, lick or sweat (Robinson 1954; Schmidt-Nielsen *et al.* 1966; Augée 1976) or undergo vasomotor adjustments (Martin 1903) in the heat, to cool themselves. Nevertheless, I found that evaporative water loss does increase substantially at high  $T_a$  (Figure 2.5) so they are presumably utilising some evaporative mechanism to dissipate excess heat. This inconsistency between studies may reflect the very different methodologies used. Robinson (1954) measured an EWL of about 1.2 mg g<sup>-1</sup> h<sup>-1</sup> at a  $T_a$  about 32 °C (by weight loss); echidnas were weighed at regular intervals during the experiment and urine/faeces were collected and water consumption measured. In my study, echidnas were not provided with water during experimentation and EWL was continuously measured by flow-through hygrometry, over a considerably longer period of time, to determine minimum values (about 0.5 mg g<sup>-1</sup> h<sup>-1</sup>). A moderate increase in  $f_R$  as well as a rise in cutaneous water loss could explain this rise in EWL (Tracy and Walsberg 2000); however, as  $T_a$  was so close to  $T_b$  for echidnas at these high  $T_a$  (30 °C and 32.5 °C), their breathing traces at these temperatures were un-analysable so I was unable to determine  $f_R$ ,  $V_T$  or  $V_I$ .

Although many previous studies have concluded that echidnas are not able to tolerate high ambient temperatures, and rely on controlling heat production and behavioural adaptations to escape these extremes (Martin 1903; Robinson 1954; Griffiths 1968, 1978;

Augee 1978), my and other recent studies show that this is not the case. Wilkinson *et al.* (1998) and Brice (2009) noted that echidnas were commonly found in logs during summer, in which temperatures varied as much as shaded  $T_a$  outside and often rose above the previously reported 'lethal temperature' (38 °C; Augee 1976) for echidnas, exceeding 35 °C for 10h and reaching 42 °C in hot weather (Brice *et al.* 2002). Brice (2009) did not further investigate the mechanisms which allow echidnas to tolerate these temperatures. Here, I have shown that at high  $T_a$  echidnas increase their EWL and lose heat by evaporation, just like most mammals.

Echidnas effectively use evaporative cooling, postural adjustments and 'bubble blowing' to cool themselves at high  $T_a$ . At the two higher  $T_a$ 's (30 °C and 32.5 °C), expired air from the nostrils formed bubbles in the nasal mucous, which then broke, moistening the tip of the snout which contains a blood sinus. This presumably allows echidnas to enhance evaporation and cool themselves rather than using licking or panting, as the tip of their snout is extremely vascular and distributes cooled blood. This 'bubble blowing' by echidnas may function in a similar way to the excess saliva produced by some rodents for evaporative cooling; rodents also do not sweat, though they pant and spread excess saliva to cool themselves (Collins 1973). A study by Proske *et al.* (1998) examining electroreceptors in anaesthetised echidnas noted that wiping the skin on the snout with a saline solution acted as a stimulus for the electroreceptors. It is possible that moistening of the snout by echidnas may also act to enhance electroreception and maintain a low resistance pathway between their electroreceptors and sources of electric current in the soil (Proske *et al.* 1998).

Dry thermal conductance is in part a physical constant, reflecting the insulative role of fur and subcutaneous fat, but it can also be altered by physiological (vasomotor) and behavioural (postural) means (Withers 1992). At high  $T_a$ , I observed echidnas to sprawl out across the chamber, presumably to increase SA:V ratio and dissipate excess heat (Stelzner and Hausfater 1986; Withers 1992). My results suggested there is a slight but significant increase in  $C_{dry}$  at higher  $T_a$ , indicating that echidnas might also use vasomotor adjustments to increase heat loss at high  $T_a$ . Martin (1903), who claimed that echidnas

did not undergo vasomotor adjustments, had a very small sample size and used out-dated methodologies, so there is a need to further investigate this.

### *Conclusion*

I suggest that the basal physiology of echidnas is a reflection of both their 'specialised' low energy lifestyle and their 'primitive' phylogenetic position. From an evolutionary point of view, all three extant groups of monotremes have a low  $T_b$ , so it could be argued that their low  $T_b$  is a 'primitive' character. Low  $T_b$  will impart a low BMR, through the  $Q_{10}$  effect of  $T_b$  on BMR. That both genera of echidnas have a significantly lower BMR than predicted for a typical mammal of a similar size might reflect in part the  $T_b/Q_{10}$  effect, but might also reflect phylogeny and life history characters as well. In terms of lifestyle, the short-beaked echidna has several traits consistent with a low BMR; echidnas are myrmecophageous, semi-fossorial, armoured, and utilise torpor/hibernation, as well as having a low  $T_b$ . Their low BMR could be interpreted as adaptive for a low energy lifestyle, rather than a phylogenetic character. The 'sister' monotreme, the platypus, has a higher BMR than echidnas and more like that expected for a mammal despite its low  $T_b$ ; its BMR is similar to that expected for a typical mammal of similar size. This is consistent with the observation that aquatic and semi-aquatic mammals generally have an elevated BMR due to their energetically demanding environment.

The pattern of thermoregulation for echidnas is most interesting; they are very thermolabile, but are effective thermoregulators. Thermolability *per se* is not necessarily a primitive feature, but can be a specialised and very effective energy conservation strategy for survival over a wide range of environments. Short-beaked echidnas are indeed found in every type of habitat across Australia, so they presumably are well adapted to a wide range of conditions. It has long been thought that echidnas are poorly adapted to deal with the heat, with no other strategy than minimising metabolic heat production and behaviourally avoiding  $T_a$  extremes. This appears to be somewhat misinformed, as echidnas do in fact augment heat loss by evaporation at high  $T_a$ , like most 'higher'

mammals. In addition to this, echidnas 'blow bubbles' at high  $T_a$ , to moisten and thus cool the vascular blood sinus at the tip of their snout.

My arguments for a specialised physiology of echidnas leads to the question of potential geographic and seasonal variation in their physiology. Sub-species of short-beaked echidnas from different geographic regions are morphologically distinct, so are their metabolic and thermoregulatory strategies also distinct and specialised to reflect an adaptation to different climates? Is there seasonal variation in their physiology in response to changing environmental conditions? These questions are addressed in chapter three.

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## Environmental correlates of echidna physiology:

### Are echidnas plastic?

#### Abstract

Physiological plasticity refers to changes in the phenotype of an individual in response to environmental conditions. Physiological plasticity, in terms of geographic variation, seasonal variation and captivity effects, was examined for short-beaked echidnas (*Tachyglossus aculeatus*). I examined physiological differences between two geographically and morphologically distinct sub-species: the Western Australia (*T. a. acanthion*) and Tasmanian (*T. a. setosus*) forms. *T. a. setosus*, from cooler wetter Tasmania, had higher body temperatures (1.9°C higher at  $T_a = 25^\circ\text{C}$ ), higher metabolic rates ( $0.12 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  at  $T_a = 25^\circ\text{C}$ ), and higher rates of evaporative water loss ( $0.06 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$  at  $T_a = 25^\circ\text{C}$ ) than *T. a. acanthion*. Interestingly, thermal conductance did not differ between the two sub-species as may be assumed from their difference in pelt. I also investigated seasonal variation in physiological parameters of *T.a. acanthion*. Body mass was highest in autumn (3816 g) and fell in winter (3512 g) due to decreased food intake associated with torpor and then mating. Body temperature was lowest ( $27.9^\circ\text{C}$ ) and evaporative water loss was highest ( $0.32 \text{ mg H}_2\text{O g}^{-1} \text{ h}^{-1}$ ) in summer. There were no seasonal effects of metabolic rate or conductance and captivity had little effect on the physiology of echidnas. Short-beaked echidnas show considerable physiological plasticity between geographic locations, with several physiological factors differing between the sub-species, yet show minimal seasonal plasticity, with only body mass and body temperature differing between the seasons.

## Introduction

Changes in an individual's phenotype in response to environmental conditions is described as physiological plasticity (Kingsolver and Huey 1998). There are three main types of physiological plasticity: genetic, developmental and acclimatory. Genetic plasticity occurs through natural selection whereby populations are selected for adaptations to local conditions. Developmental plasticity occurs when exposure to particular environmental conditions early in life determines an individual's physiological capacity. Acclimatory plasticity occurs over a relatively short period of time and is reversible, where individuals change their physiological responses with a changing environment (Tracy and Walsberg 2001; Tattersall *et al.* 2012). Plasticity can occur in response to a number of environmental and biological factors. Here I examine the physiological plasticity of the short-beaked echidna (*Tachyglossus aculeatus*) in terms of geographic and seasonal variation, and also consider possible captivity effects.

Understanding geographic variation within species aids in understanding the evolutionary origin of adaptations and distributional limits (Tracy and Walsberg 2001). Birds have a great deal of plasticity in their thermoregulatory strategies (Dawson 2003), however fewer studies have examined physiological plasticity in mammals (Mueller and Diamond 2001; Tracy and Walsberg 2001). Short-beaked echidnas provide an ideal model with which to study intra-specific geographic variation as they are the most widely distributed native mammal in Australasia, occurring in all habitat types from tropical savannah rangelands, to coastal heaths, to alpine areas (Griffiths 1968; Morrow *et al.* 2009; Nicol and Morrow 2012). Despite this wide geographic range and occupation of a wide diversity of habitats, all Australian short-beaked echidna are considered to belong to a single species. Five subspecies have been distinguished, mostly by morphological characteristics such as degree of hairiness, shape of the spines and the length of the hind grooming claw (Griffiths 1968). It is of interest to determine if these morphologically distinct geographic subspecies also differ in their physiology, and if these differences are consistent with adaptation to differing environmental conditions and other habitat characteristics.

Generally, mammals living in more arid environments, characterised by high ambient temperature ( $T_a$ ), low rainfall, high rainfall variability and low primary productivity, have a lower body temperature ( $T_b$ ), basal metabolic rate (BMR) and evaporative water loss (EWL) than species living in more mesic environments (Lovegrove 2000; Mueller and Diamond 2001; Tracy and Walsberg 2001; Williams *et al.* 2001; Lovegrove 2003; Withers *et al.* 2006; Cooper and Withers 2010; White and Kearney 2013). A low BMR and  $T_b$  may be important for species inhabiting hot, resource poor environments as energy expenditure and water loss is reduced. High  $T_a$  may also limit the sustained metabolism of endotherms (Speakman and Krol 2010). Mueller and Diamond (2001) correlated metabolic rate (MR) with net primary production for five species of deer mice (*Peromyscus spp.*) from varying locations characterised by very different rainfalls and productivities, and found that 82-90% of the variation in MR (after accounting for body mass effects) was explained by net primary productivity. This suggests that while geographic variation in physiology may correlate with several climatic factors, primary productivity, which is closely related to several of these factors, is one of the most influential (Mueller and Diamond 2001; Bozinovic *et al.* 2007,2009; White and Kearney 2013). Primary productivity may be a good measure of the differences in food availability that related species of similar dietary habits encounter. Several other studies, however, have suggested that environmental variability rather than environmental productivity is a more influential factor leading to lower MR in individuals inhabiting arid areas (Dobson and Crawley 1987; Lovegrove 2000). As Western Australia is hotter, drier and has a more variable climate than Tasmania it may be expected that echidnas living in Western Australia will have a lower BMR,  $T_b$  and EWL than those living in Tasmania.

Body mass differences also occur across a species' geographical distribution, and can confound interpretation of physiological adaptations. Body mass may be under selection from several interacting factors, such as differences in primary productivity and subsequent resource availability, or thermal differences between locations (resulting in variable heat dissipation or storage needs), in accordance with Bergmann's rule (Mayr 1963). Geographic races found in the warmer areas of a species' range are generally smaller, while those in cooler areas are larger (Mayr 1963; Kendeigh 1969). Increasing

body size to tolerate cold has some obvious physiological advantages such as reduction in relative energy required for maintenance, lower metabolic stress per degree drop in  $T_a$ , extension of the zone of thermoneutrality with a reduced lower critical temperature and lower extreme limits of tolerance. These advantages, however, may be offset by the need for a greater food intake (Kendeigh 1969). An alternative interpretation is offered by Speakman and Król (2010) who suggest that large body size may be disadvantageous in warmer conditions, as it will limit heat loss, leading to hyperthermia. So, we might expect that echidnas may be larger in Tasmania than Western Australia, reflecting the climatic variation between the two locations.

Seasonal acclimatisation (adjustments in physiology, behaviour or anatomy) may be triggered by changes in  $T_a$ , relative humidity (RH), photoperiod or food availability (Zheng *et al.* 2008; Cooper and Withers 2012). Changes in season result not only in changes in thermoregulatory strategies with the change in weather conditions, but also in availability of food and water, and for some species, changes in reproductive condition (Cooper and Withers 2012). Animals may respond to these seasonally changing environments by altering body mass, body fat, metabolic rate and behaviour (Zheng *et al.* 2008). Small and large mammals respond differently to season, with seasonal acclimatisation by large animals being dominated by increased insulation and body mass in winter, while smaller mammals reduce their energy requirements by utilising hibernation and torpor and reducing body mass (Heldmaier 1989; Lovegrove 2005). This reduction in body mass is most important for small mammals (<500g) as they reduce their overall energy expenditure due to a decrease in absolute metabolic rate (Lovegrove 2005). Numbats (*Myrmecobius fasciatus*), which are considerably smaller than echidnas (~516g) but have a similar diet, reduce their body mass in winter (Cooper and Withers 2012), while many other mammals that are closer in size to echidnas increase their body mass (Lovegrove 2005). A previous study examining short-beaked echidnas in Tasmania found that body mass followed a strong seasonal cycle; body mass of echidnas was lowest in winter and highest in summer (Nicol and Morrow 2012). Tasmanian echidnas enter longer bouts of hibernation than Western Australian echidnas (see chapter five), so it is of interest to determine if this influences variation in body mass.

Metabolic rate and other physiological variables are often also influenced by seasonal acclimatisation (Angilletta 2001). Increased thermogenic capacity in winter means many small animals have a higher mass-specific BMR (Zheng *et al.* 2008; Cooper and Withers 2012), and are often more resistant to cold in winter than in summer (Dawson *et al.* 1983). Myrmecophageous numbats have a significantly higher BMR,  $T_b$  and EWL in winter than in summer (Cooper and Withers 2012), although larger mammals tend to show a moderate decrease in BMR (Lovegrove 2005). Green *et al.* (1992) found that wild echidnas on Kangaroo Island had a lower field MR in winter than in summer. They attributed this to reduced activity and increased torpor use in winter. In addition to a lower MR, many mammals of a similar size range to echidnas (intermediate; 0.1-10kg) also lower their thermal conductance during winter (Lovegrove 2005). This is associated with an increased pelage thickness during winter, which is subsequently moulted for a thinner summer pelt in many species (Maurel *et al.* 1986). There are no data for seasonal moult for short-beaked echidnas.

Seasonal variation measured in captive animals must also take into account possible captivity effects that may confound any variations in physiology. Captive animals are not exposed to the same environmental pressures as wild animals; their diet is different and they do not have to forage for food, variation in food availability is greatly reduced, they often have lower levels of activity, predation pressures are removed, reproductive activity is usually controlled and they will often experience different weather conditions compared to wild individuals (Cooper and Withers 2012). These different conditions may have physiological consequences for animals held in captivity for any significant period of time. The physiological variables most likely to be influenced by captivity are those relating to diet and nutrition (Larcombe and Withers 2007; Cooper and Withers 2012). Thermoregulation is influenced by energy availability, with many captive animals having reduced occurrence, frequency and depth of torpor and hibernation (Geiser *et al.* 1990), and respiratory exchange ratio (the ratio of  $CO_2$  produced to  $O_2$  used) may vary if different foods are metabolised (Cooper and Withers 2012). However, many other standard physiological variables, measured at thermoneutrality, appear to be unaffected by captivity (Dawson and Carey 1976; Weathers *et al.* 1983; Wasser 1986; Larcombe and Withers

2007; Cooper and Withers 2012). Some studies have reported a difference in some of these variables between captive and wild individuals. However, many animals used in captive studies are being held because they are injured, while others may have been bred in captivity for many generations (Bradshaw 1974; Warkentin and West 1990; Geiser *et al.* 2000), so they are not representative of the effects of captivity on healthy, wild-caught animals. There has been a strong focus on the effects of captivity on birds (Skadhauge and Bradshaw 1974; Dawson and Carey 1976; Weathers *et al.* 1983; Wasser 1986; Warkentin and West 1990), with fewer studies examining mammals (Geiser *et al.* 1990; Larcombe and Withers 2007; Cooper and Withers 2012).

This chapter aims to determine the physiological plasticity of short-beaked echidnas. As echidnas are so widespread across Australia, I expect there to be some geographic variation in the physiology of the subspecies inhabiting different areas, consistent with the morphological variation that is apparent. It is therefore of interest to determine if echidnas from Tasmania and Western Australia, representing two of the most morphologically distinct sub-species, are also physiologically distinct. In this chapter I also examine seasonal plasticity in Western Australian short-beaked echidnas held in captivity for one year and measured each season. As these echidnas were in captivity for such a long period of time, captivity effects are also briefly examined. Seasonal plasticity is apparent for many mammals, and I expect that short-beaked echidnas will also show some level of seasonal plasticity, living in an area that has considerable climatic variation between seasons.

## **Materials and Methods**

### *Study sites*

Echidnas were collected at Dryandra Woodland in Western Australia and at Lovely Banks in Tasmania. Dryandra Woodland (31° 46' S, 117° 1' E), is located approximately 170km south-east of Perth, Western Australia. This area is characterised by a warm, dry, Mediterranean climate, with an average annual rainfall of approximately 500mm. Seasonal variation in temperature, rainfall and wind is marked, and mean daily temperatures are approximately 31°C in January and 5.6°C in August. Stockman Stud, at

Lovely Banks (42° 28' S, 142° 14' E), is approximately 50km north of Hobart, Tasmania. Seasonal variations in temperature are relatively small, with summer maxima of approximately 24°C and winter maxima of 11°C.

### *Western Australia*

Seven reproductively mature short-beaked echidnas (*Tachyglossus aculeatus acanthion*), not in breeding condition, were captured by hand at Dryandra Woodland to examine seasonal and geographic variation in body temperature ( $T_b$ ), metabolic rate (MR), wet ( $C_{wet}$ ) and dry ( $C_{dry}$ ) thermal conductance and evaporative water loss (EWL). The echidnas were housed in a custom-built (by myself and my supervisors and technicians) outdoor enclosure at Curtin University in Bentley, where they experienced natural variation in weather and photoperiod. Echidnas were maintained on a diet consisting of cat food, bran, insectivore powder, primate omnivore powder, primate leaf eater powder and water blended to make a smooth mash. Fresh drinking water was provided *ad libitum*. All the echidnas were housed together in the main enclosure (10m x 9m) except prior to experimentation. The night preceding experimentation, 1-2 echidnas were moved into a small enclosure (approximately 10m x 1m) and fasted to ensure they were in a post-absorptive state. Some of the echidnas had been held in captivity for several months prior to experimentation, while others were captured, measured daily, then released, to reduce time in captivity without food. This reduced any influence of captivity on the animals so the variation in physiology could more accurately be attributed to seasonal and geographic variation. Echidnas were measured during summer, autumn, winter and spring in 2012 to examine seasonal variation. The echidnas measured during spring 2012 were also used for the geographic variation studies. Three echidnas were measured during spring 2011, soon after introduction to captivity. Six echidnas were measured during Spring 2012, including the three measured in 2011 that had been in captivity over one year, two that had been in captivity since summer 2012, and one that was newly caught from the wild. A comparison between the two years allowed me to determine if there were any captivity effects.

## *Tasmania*

Six reproductively mature short-beaked echidnas (*Tachyglossus aculeatus setosus*) were captured by hand on a grazing property (Stockman Stud) at Lovely Banks in the Tasmanian Southern Midlands, to examine geographic variation for this sub-species in comparison with the Western Australian subspecies. These measurements were carried out during spring 2012. Two echidnas were captured, returned to the University of Tasmania and measured daily for six days, before being returned to the point of capture and another two echidnas collected. Echidnas were housed in an outdoor animal enclosure at the University of Tasmania, where they experienced natural variation in photoperiod and weather. Echidnas were provided with *ad libitum* water but no food.

## *Experimental procedures*

Measurement protocols and calculations were as per chapter two. The same equipment and measurement protocols were used in both W.A. and Tasmania. All values are presented as mean  $\pm$  SE with N being the number of individuals and n the number of measurements. The influence of  $T_a$ , season (summer, autumn, winter, spring), geographic location and captivity on physiological variables was determined by two-way analysis of variance (ANOVA), with *a priori* contrasts and Student-Newman-Keuls (SNK) post-hoc tests. Although desirable to account for multiple measurements of some individuals, repeated measures ANOVA was unsuitable to analyse the seasonal data as not all individuals were the same between seasons and there were less individuals than treatment groups.

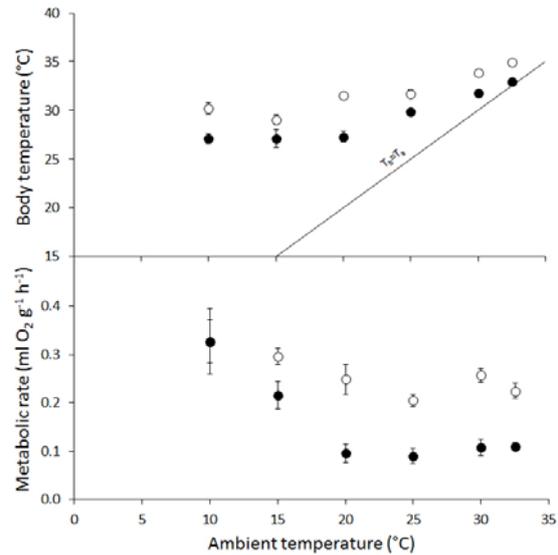
## Results

### *Comparison of sub-species*

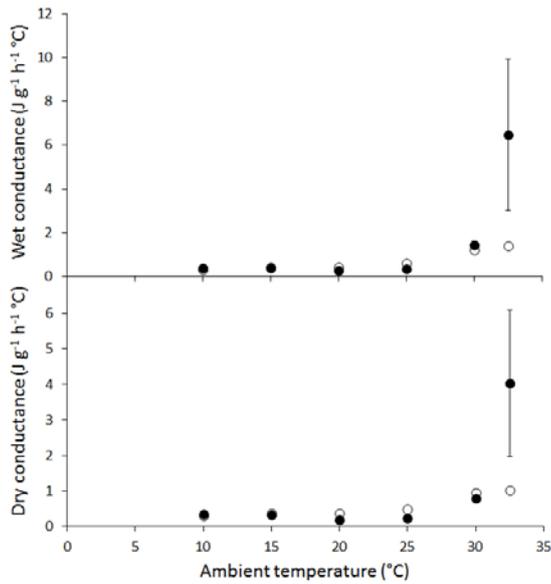
The mean body mass of W.A echidnas was  $3.44 \pm 0.009$  kg ( $n = 36$ ,  $N = 6$ ) and the mean body mass of Tasmanian echidnas was  $3.54 \pm 0.051$  kg ( $n = 36$ ,  $N = 6$ ). There was no significant difference in body mass between the two locations ( $F_{1,60} = 0.584$ ,  $P = 0.448$ ).

$T_b$  of short-beaked echidnas from Tasmania was significantly higher than for those from W.A. ( $F_{1,60} = 95.8$ ,  $P < 0.001$ ; Figure 3.1), with basal  $T_b$  (at  $T_a = 25^\circ\text{C}$ ) of Tasmanian echidnas being  $31.7 \pm 0.36^\circ\text{C}$  and W.A echidnas

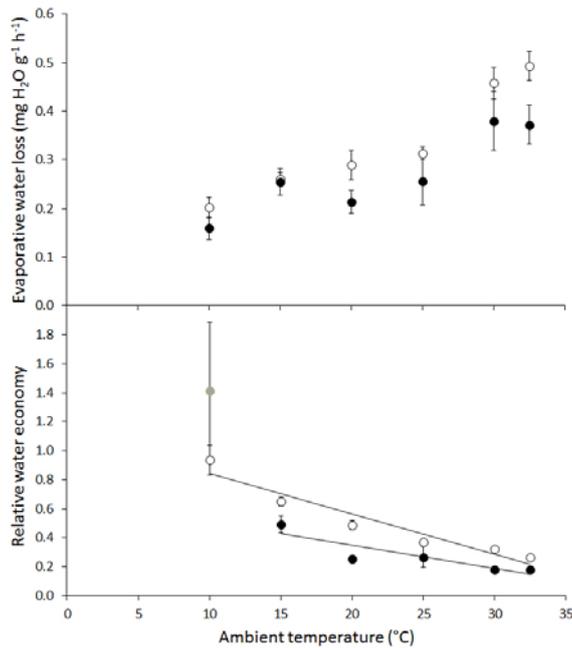
being  $29.8 \pm 0.29^\circ\text{C}$ .  $T_b$  was also significantly influenced by  $T_a$  for both sub-species ( $F_{5,60} = 55.4$ ,  $P < 0.001$ ), with  $T_b$  differing at all  $T_a$  above  $T_a = 20^\circ\text{C}$ .



**Figure 3.1:** Body temperature ( $^\circ\text{C}$ ) and metabolic rate ( $\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ), of short-beaked echidnas at ambient temperatures from  $10^\circ\text{C}$  to  $32.5^\circ\text{C}$  from Western Australia (black circles) and Tasmania (white circles). Values are mean  $\pm$  SE,  $N = 6$  for both groups



**Figure 3.2:** Wet and dry conductances ( $\text{J g}^{-1} \text{h}^{-1} \text{ } ^\circ\text{C}$ ) of short-beaked echidnas at ambient temperatures from  $10^\circ\text{C}$  to  $32.5^\circ\text{C}$  from Western Australia (black circles) and Tasmania (white circles). Values are mean  $\pm$  SE,  $N = 6$ .



**Figure 3.3:** Evaporative water loss ( $\text{mg H}_2\text{O g}^{-1} \text{h}^{-1}$ ) and relative water economy of short-beaked echidnas at ambient temperatures from  $10^\circ\text{C}$  to  $32.5^\circ\text{C}$  from Western Australia (black circles) and Tasmania (white circles). Grey circle is RWE at  $T_a = 10^\circ\text{C}$  for W.A. echidnas (this value was omitted from the analyses as it has a considerably larger SE than all other values). Values are mean  $\pm$  SE,  $N = 6$ .

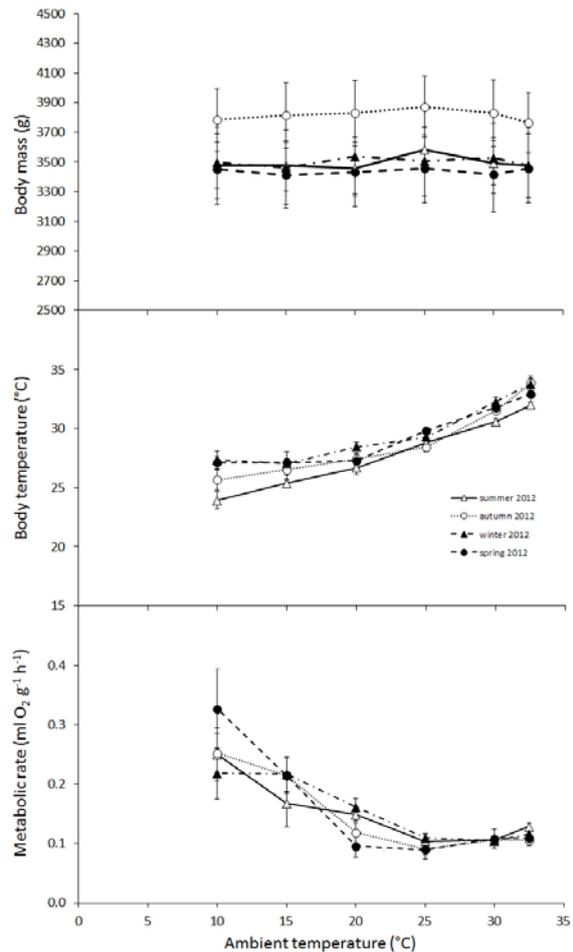
Both  $\text{VO}_2$  and  $\text{VCO}_2$  were significantly higher in Tasmanian echidnas than in W.A. echidnas ( $F_{1,60} = 36.8$ ,  $P < 0.001$ ;  $F_{1,60} = 15.6$ ,  $P < 0.001$ , respectively; Figure 3.1), although the metabolic response to  $T_a$  was similar in both sub-species.  $\text{VO}_2$  did not differ at  $T_a \geq 20^\circ\text{C}$  ( $P \geq 0.504$ ), but increased with decreasing  $T_a$  below this ( $P \leq 0.018$ ).  $\text{VCO}_2$  largely followed the same pattern, with  $\text{VCO}_2$  remaining constant at  $T_a \geq 15^\circ\text{C}$  ( $P \geq 0.056$ ). Respiratory exchange ratio was significantly higher for echidnas in W.A. than those in Tasmania ( $F_{1,60} = 20.2$ ,  $P < 0.001$ ) but was not significantly influenced by  $T_a$  ( $F_{5,60} = 0.91$ ,  $P = 0.478$ ).

$C_{\text{wet}}$  did not significantly differ between sub-species ( $F_{1,60} = 1.98$ ,  $P = 0.164$ ), but was significantly influenced by  $T_a$  ( $F_{5,60} = 4.06$ ,  $P = 0.003$ ; Figure 3.2).  $C_{\text{dry}}$  followed the same pattern; there was no difference between the sub-species ( $F_{1,60} = 1.37$ ,  $P = 0.246$ ), but  $T_a$  had a significant influence ( $F_{5,60} = 4.38$ ,  $P = 0.002$ ; Figure 3.2).  $C_{\text{wet}}$  ( $P < 0.011$ ) and  $C_{\text{dry}}$  ( $P < 0.007$ ) at  $T_a = 32.5^\circ\text{C}$  were significantly different to all other  $T_a$ , however, calculations of  $C_{\text{wet}}$  and  $C_{\text{dry}}$

at  $T_a = 32.5^\circ\text{C}$  were unreliable as  $T_b$  approached  $T_a$ . At the upper boundary of thermoneutrality ( $T_a = 25^\circ\text{C}$ )  $C_{\text{wet}}$  was  $0.59 \text{ J g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$  for Tasmanian echidnas and  $0.35 \text{ J g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$  for W.A. echidnas. Minimal  $C_{\text{wet}}$  as calculated from the slope of the MR line was  $0.008 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$  for Tasmanian echidnas, and  $0.023 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$  for W.A. echidnas.

EWL differed significantly between W.A. and Tasmanian echidnas ( $F_{1,60} = 10.9$ ,  $P = 0.002$ ), with those from Tasmania having a higher rate of EWL (Figure 3.3). EWL was also significantly influenced by  $T_a$  ( $F_{5,60} = 18.2$ ,  $P < 0.001$ ). EWL at  $T_a = 32.5^\circ\text{C}$  and  $T_a = 30^\circ\text{C}$  were not significantly different from each other ( $P = 0.681$ ) but were significantly higher than all other  $T_a$  ( $P < 0.001$ ). Polynomial contrasts indicate a significant linear relationship ( $P < 0.001$ ) between EWL and  $T_a$ .

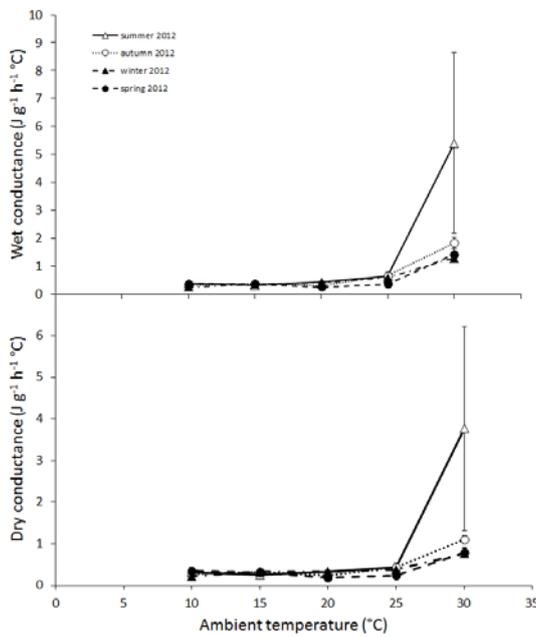
RWE was not significantly influenced by location ( $F_{1,60} = 0.23$ ,  $P = 0.630$ ), but was significantly influenced by  $T_a$  ( $F_{5,60} = 12.4$ ,  $P < 0.001$ ; Figure 3.3). An ANCOVA showed that the slopes of the RWE lines were not significantly different ( $F_{1,56} = 1.91$ ,  $P = 0.173$ ) between the two locations, though the intercepts were ( $F_{1,57} = 35.3$ ,  $P < 0.001$ ). The PRWE was  $-58.7^\circ\text{C}$  for WA echidnas ( $y = -0.0159x + 0.6643$ ;  $R^2 = 0.785$ ) and  $4.24^\circ\text{C}$  for Tasmanian echidnas ( $y = -0.0278x + 1.1179$ ;  $R^2 = 0.926$ ). RWE at  $T_a = 10^\circ\text{C}$  for W.A. echidnas was omitted from the analyses as the standard error was considerably larger than the rest of the data.



**Figure 3.4:** Body mass (g), body temperature ( $^\circ\text{C}$ ) and metabolic rate ( $\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ), of short-beaked echidnas at ambient temperatures from  $10^\circ\text{C}$  to  $32.5^\circ\text{C}$  during summer, autumn, winter and spring 2012. Values are mean  $\pm$  SE, N

### Seasonal variation in *W.A. echidnas*

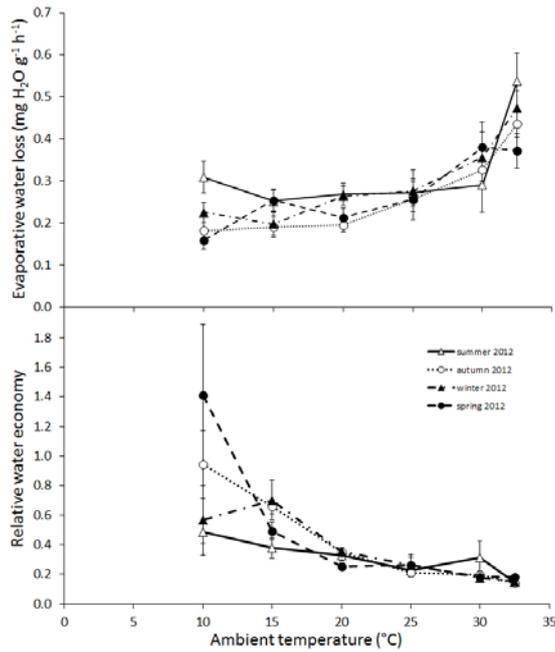
There was a significant difference in body mass of *W.A. echidnas* between seasons ( $F_{3,120} = 3.66$ ,  $P = 0.014$ ; Figure 3.4), with mass during autumn ( $3.81 \pm 0.081$  kg) being significantly higher than in winter ( $3.51 \pm 0.093$  kg;  $P = 0.017$ ), spring ( $3.44 \pm 0.089$  kg;  $P = 0.017$ ) and summer ( $3.49 \pm 0.064$  kg;  $P = 0.031$ ). There was no pattern with changing mass and  $T_a$  ( $F_{5,120} = 0.04$ ,  $P = 0.999$ ).



**Figure 3.5:** Wet and dry conductances ( $J g^{-1} h^{-1} °C$ ) of short-beaked echidnas at ambient temperatures from 10°C to 32.5°C during summer, autumn, winter and spring 2012. Values are mean  $\pm$  SE,  $N = 6$ .

Body temperature was significantly influenced by both season ( $F_{3,120} = 11.89$ ,  $P < 0.001$ ; Figure 3.4) and  $T_a$  in the laboratory ( $F_{5,120} = 102$ ,  $P < 0.001$ ).  $T_b$  differed between all seasons ( $P < 0.032$ ) except spring and autumn ( $P = 0.178$ ), and spring and winter ( $P = 0.235$ ), as spring was intermediate.  $T_b$  differed at all  $T_a$  ( $P < 0.019$ ) except at  $T_a = 15°C$  and  $10°C$  ( $P = 0.271$ ).

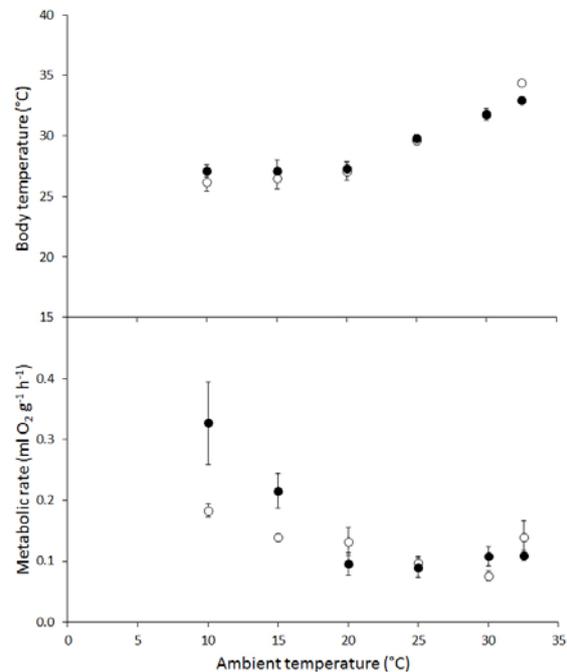
Metabolic rate ( $VO_2$ ) was not significantly influenced by season ( $F_{3,120} = 0.155$ ,  $P = 0.926$ ), but was significantly influenced by  $T_a$  ( $F_{5,143} = 24.8$ ,  $P < 0.00$ ; Figure 3.4), decreasing with increasing  $T_a$  to a thermoneutral  $T_a$  of 25°C, before increasing.  $CO_2$  production followed a similar pattern, with  $VCO_2$  also significantly influenced by  $T_a$  ( $F_{5,120} = 30.2$ ,  $P < 0.001$ ) but not season ( $F_{3,120} = 0.303$ ,  $P = 0.823$ ). Respiratory exchange ratio was not significantly influenced by season ( $F_{3,120} = 2.54$ ,  $P = 0.06$ ) nor temperature ( $F_{5,120} = 1.72$ ,  $P = 0.134$ ).



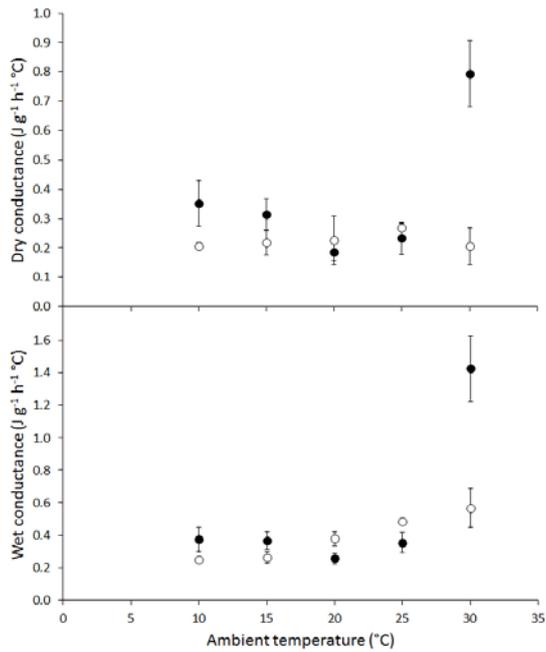
**Figure 3.6:** Evaporative water loss (mg H<sub>2</sub>O g<sup>-1</sup> h<sup>-1</sup>) and relative water economy of short-beaked echidnas at ambient temperatures from 10°C to 32.5°C during summer, autumn, winter and spring 2012. Values are mean ± SE, N = 6.

0.032) between  $C_{wet}$  and  $T_a$  during summer and when  $T_a = 32.5^\circ\text{C}$  was removed from the model a linear relationship was still present ( $P = 0.006$ ). Significant linear ( $P < 0.001$ ) and quadratic ( $P < 0.001$ ) relationships were present between  $C_{wet}$  and  $T_a$  in autumn, winter and spring. When  $T_a = 32.5^\circ\text{C}$  was taken out of the analysis both relationships still remained ( $P = 0.004$  and  $0.042$ , respectively) in autumn, but only a linear relationship remained ( $P < 0.001$ ) in winter and no significant relationships remained ( $P > 0.369$ ) in

Neither  $C_{wet}$  nor  $C_{dry}$  were significantly influenced by season ( $F_{3,120} = 2.1$ ,  $P = 0.104$  and  $F_{3,120} = 1.99$ ,  $P = 0.119$ , respectively; Figure 3.5) and while  $C_{wet}$  was significantly influenced by  $T_a$  ( $F_{5,120} = 2.84$ ,  $P = 0.019$ ),  $C_{dry}$  was not ( $F_{5,120} = 2.29$ ,  $P = 0.05$ ). There was, however, a significant interaction between season and  $T_a$  for both  $C_{wet}$  ( $P = 0.022$ ) and  $C_{dry}$  ( $0.039$ ).  $C_{wet}$  at  $T_a = 32.5^\circ\text{C}$  was significantly different from all other  $T_a$  ( $P < 0.04$ ), while  $C_{wet}$  at all other  $T_a$  did not differ significantly ( $P > 0.755$ ). However, *a priori* contrasts indicate a significant linear relationship ( $P =$



**Figure 3.7:** Body temperature ( $^\circ\text{C}$ ) and metabolic rate (ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>), of short-beaked echidnas at ambient temperatures from 10°C to 32.5°C during spring 2011 (white circles) and Spring 2012 (black circles). Values are mean ± SE, N = 6.



**Figure 3.8:** Wet and dry conductances ( $\text{J g}^{-1} \text{h}^{-1} \text{ } ^\circ\text{C}$ ), of short-beaked echidnas at ambient temperatures from  $10^\circ\text{C}$  to  $32.5^\circ\text{C}$  during spring 2011 (white circles) and Spring 2012 (black circles). Values are mean  $\pm$  SE,  $N=6$ .  $30^\circ\text{C}$  were both significantly higher than at all other  $T_a$  ( $P < 0.002$ ).

Season did not influence RWE ( $F_{3,120} = 1.99$ ,  $P = 0.119$ ; Figure 3.6), though  $T_a$  did ( $F_{5,120} = 22.2$ ,  $P < 0.001$ ). RWE at  $T_a = 10^\circ\text{C}$  and  $T_a = 15^\circ\text{C}$  was significantly higher than that at all other  $T_a$  ( $P < 0.009$ ). The PRWE was  $-26.9^\circ\text{C}$  in summer,  $5.21^\circ\text{C}$  in autumn,  $4.27^\circ\text{C}$  in winter and  $10.1^\circ\text{C}$  in spring.

spring.  $T_a = 32.5^\circ\text{C}$  was left out of all analyses as data at this temperature became unreliable at this temperature as the difference between  $T_b$  and  $T_a$  was negligible.

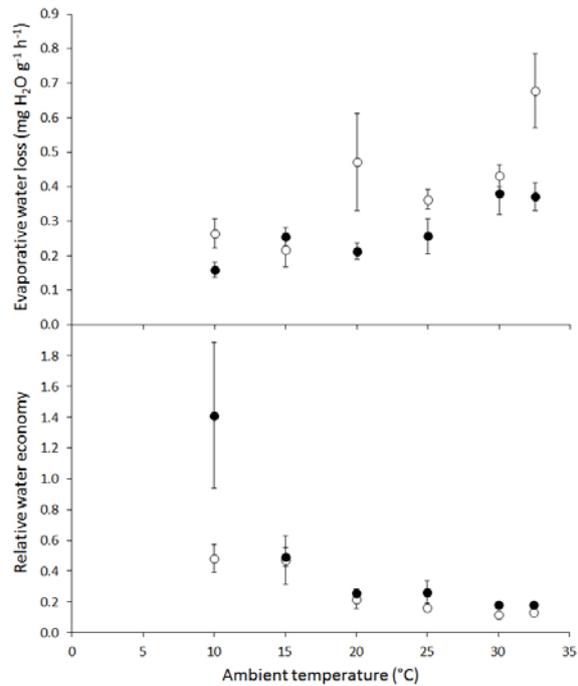
Evaporative water loss was significantly influenced by both season ( $F_{3,120} = 3.62$ ,  $P = 0.015$ ; Figure 3.6) and  $T_a$  ( $F_{5,120} = 28.66$ ,  $P < 0.001$ ). EWL in summer was significantly higher than that in autumn ( $P = 0.016$ ) and spring ( $P = 0.026$ ).

There was no significant difference in EWL between any other seasons ( $P > 0.057$ ). EWL at  $T_a = 32.5^\circ\text{C}$  and  $T_a =$

### Effects of captivity

There was no significant overall difference in the  $T_b$  ( $F_{5,1} = 0.066$ ,  $P = 0.799$ ),  $VO_2$  ( $F_{5,1} = 2.24$ ,  $P = 0.141$ ) or  $VCO_2$  ( $F_{5,1} = 1.71$ ,  $P = 0.198$ ) of short-beaked echidnas between spring 2011 and spring 2012 (Figure 3.7). The interaction between  $T_a$  and year was not significant for  $VO_2$  ( $P = 0.091$ ) nor  $VCO_2$  (0.718). Neither  $C_{wet}$  ( $F_{1,42} = 1.50$ ,  $P = 0.227$ ) nor  $C_{dry}$  ( $F_{1,42} = 2.02$ ,  $P = 0.162$ ) were significantly different between 2011 and 2012 (Figure 3.8).

EWL was significantly higher for short-beaked echidnas in 2011 than 2012 ( $F_{1,42} = 17.1$ ,  $P < 0.001$ ; Figure 3.9), and the relationship between  $T_a$  and EWL differed between the years ( $P = 0.031$ ). This is the only physiological parameter that significantly differed between the two years. RWE was not significantly different between the years ( $F_{1,42} = 2.77$ ,  $P = 0.103$ ; Figure 3.9).



**Figure 3.9:** Evaporative water loss ( $\text{mg H}_2\text{O g}^{-1} \text{h}^{-1}$ ) and relative water economy of short-beaked echidnas at ambient temperatures from  $10^\circ\text{C}$  to  $32.5^\circ\text{C}$  during Spring 2011 (white circles) and Spring 2012 (black circles). Values are mean  $\pm$  SE,  $N = 6$ .

## Discussion

Plasticity in terms of both geographic and seasonal variation is evident for short-beaked echidnas, presumably as adaptations to varying aspects of climate in their habitats. They had marked differences in physiology between two geographically distinct sub-species, as well as physiological differences between seasons. This indicates that echidnas are able to modify their physiological responses by acclimating to seasonal changes in their environment, and may also be subject to population based genetic and/or developmental

plasticity. Captivity had almost no effect on their physiology as they were housed in semi-natural conditions, experiencing natural variation in weather and photoperiod, consistent with previous studies on other small mammals.

### *Geographic variation*

Generally, mammals living in cooler areas are larger than those living in warmer areas (Bergmann's rule; Mayr 1963; Kendeigh 1969; Tracy and Walsberg 2001). This phenomenon is evident for the sister monotreme, the platypus (Furlan *et al.* 2011), but echidnas do not appear to conform to Bergmann's rule. Body mass of the echidnas I measured in W.A. and Tasmania did not differ significantly, nor did the body mass of echidnas from several locations around Australia (Augee and Ealey 1968; Dawson *et al.* 1979; Abensperg-Traun and De Boer 1992; Bech *et al.* 1992; Green *et al.* 1992; Frappell *et al.* 1994; Beard and Grigg 2000; Rismiller and McKelvey 2000; Brice *et al.* 2002; Nicol and Andersen 2007b). Many species do not conform to Bergmann's rule. Variation in body size may reflect resource availability with changing latitude as much as climate (McNab 2002), and for some species there is no pattern of changing resource availability. For example, jaguars (*Panthera onca*) show no obvious correlation between body size and latitude, while pumas (*Puma concolor*) show an increase in size at both lower and higher latitudes, where the larger jaguars are absent and therefore competition is reduced (McNab 2002). For echidnas, there may be no difference in resource availability between the W.A. and Tasmania sites, and the sub-species have adapted their diets to what is readily available in these habitats. Short-beaked echidnas in W.A. feed almost exclusively on ants and termites (Abensperg-Traun and De Boer 1992), while echidnas in Tasmania feed on ants and small invertebrates such as scarab larvae, with termites being almost absent from their diet (Nicol and Morrow 2012). However, while this may be an indication of minimal variation in body mass between sub-species, it may also be a result of a small sample size and lack of sex differentiation. Yom-Tov and Nix (1986) also found no significant difference in body mass between widely distributed echidnas, however they did find highly significant relationships between other measures of body size (skull

parameters) and climate, which were consistent with Bergmann's rule. As there was no difference in body mass between the representatives of the two sub-species, interpretation of the other physiological variables is not confounded by mass.

The Tasmanian short-beaked echidna sub-species had a higher  $T_b$ , MR and EWL than the W.A. sub-species. This was expected, consistent with the climatic differences between the two regions. The Tasmanian site was situated in the lowest rainfall area of Tasmania, however it was still cooler and more mesic than the semi-arid W.A. site. The  $T_b$  of Tasmanian echidnas was on average 2°C higher than W.A. echidnas. By maintaining a lower  $T_b$ , echidnas in W.A. not only conserve energy and water, but also have an enhanced heat storage capacity, particularly during activity at high  $T_a$ . This increases the time before lethal  $T_b$  levels are reached and is likely an adaptation to extend activity periods. They can begin foraging with a low  $T_b$  so foraging time is maximised before  $T_b$  becomes too high. An increasing  $T_b$  rather than a high  $T_b$  has been reported as an indicator of activity by previous studies (Augee *et al.* 1970; Brice *et al.* 2002), which supports this notion. Both sub-species allowed  $T_b$  to increase with increasing  $T_a$  at  $T_a \geq 25^\circ\text{C}$  and by maintaining an overall lower  $T_b$ , a larger buffer zone between  $T_b$  and lethal  $T_b$  is created by W.A. echidnas, providing greater scope for this adaptive hyperthermia (Cooper and Withers 2010). Few studies have examined intraspecific variation in  $T_b$ , but there have been some studies on interspecific variation for mammals. For example, Cooper and Withers (2010) studied four species of quoll (*Dasyurus maculatus*, *D. viverrinus*, *D. geoffrii* and *D. hallucatus*) occurring in different habitat types, and found that tropical northern quolls had a lower  $T_b$  than the mesic species, which is consistent with my findings for short-beaked echidnas.

It has been well documented that endotherms living in warmer, more arid environments have a lower MR than those living in cooler, more mesic regions (Lovegrove 2000; Mueller and Diamond 2001; Tracy and Walsberg 2001; Williams *et al.* 2001; Lovegrove 2003; Withers *et al.* 2006; Careau *et al.* 2007; Cooper and Withers 2010). Primary productivity, environmental variability and extreme  $T_a$  are the main factors attributed to this pattern of low MR in more arid regions (Dobson and Crawley 1987; Lovegrove 2000; Mueller and Diamond 2001; Lovegrove 2003). By maintaining a lower BMR, endogenous heat production is reduced, thus reducing the likelihood of heat stress, and

facilitating maintenance of a lower  $T_b$  with reduced EWL, thus conserving water (Kvist and Lindstrom 2001; Careau et al. 2007). By maintaining a higher BMR in colder regions, thermogenic capacity is increased to compensate for heat loss (Careau et al. 2007). Increasing BMR has the effect of broadening the thermoneutral zone, allowing endotherms to tolerate much lower temperatures (Careau et al. 2007). A lower BMR for individuals living in arid habitats has been documented for many endotherms, including quolls (genus level; Cooper and Withers 2010), canids (genus and species level; Careau et al. 2007) and larks (species level; Tieleman et al. 2003). The two sub-species of short-beaked echidnas studied here also conformed to this pattern; MR of echidnas from W.A. was significantly lower than those from Tasmania. Climatic variability is marked in W.A., especially in terms of rainfall (Abensperg-traun and De Boer 1992), and  $T_a$  is relatively high, while  $T_a$  is lower and less variable in Tasmania (Nicol and Morrow 2012), therefore Tasmanian echidnas are able to maintain a higher MR that allows them to benefit from enhanced thermogenic capacity in the cold.

Mammals from more arid environments typically have lower rates of EWL throughout the year than those from more mesic environments (Tracy and Walsberg 2001; Tieleman and Williams 2002). Few studies have examined this at an intraspecific level for mammals however, despite there often being environmental differences throughout the distribution range of a species (Tracy and Walsberg 2001). Tracy and Walsberg (2001) investigated water loss for Merriam's kangaroo rats (*Dipodomys merriami merriami*) from three differing environments. As expected from the earlier broad-scale studies, individuals from the more arid areas had a lower EWL than those from more mesic areas. More studies examining intraspecific variation in EWL have been carried out in birds than mammals, and they have arrived at similar conclusions; individuals from more arid areas have lower rates of EWL than their mesic counterparts *e.g.* five species of larks distributed along an aridity gradient were measured at constant temperatures, and those from more arid areas had a lower EWL than those from mesic habitats (Tieleman et al. 2003). The EWL measurements from echidnas in this study support previous findings, with Tasmanian echidnas having a higher rate of EWL than those from W.A. This is not surprising as Tasmanian echidnas have greater access to free water than W.A. echidnas as they live in a

cooler, wetter environment, which would require a less frugal approach to water conservation.

There is however, the confounding issue of captivity. EWL was the only variable to be influenced by captivity; EWL was lower in 2012 than 2011 which was most likely due to measurement protocol but captivity effect cannot be ruled out entirely. As W.A. echidnas were held in captivity for varying amounts of time, while Tasmanian echidnas were wild-caught and measured immediately, it may be possible that EWL of W.A. echidnas is reduced due to some captivity effect. In contrast, previous captivity studies on southern-brown bandicoots found that EWL increased as a result of captivity. However, the difference was only significant during autumn and summer (Larcombe and Withers 2007). As found in chapter two, EWL increases with increasing temperature, as is typical of most mammals. Many previous studies (Martin 1903; Robinson 1954; Griffiths 1968, 1978; Augee 1978) conclude that echidnas are poor thermoregulators that do not have any mechanisms to cool themselves in the heat, however, here I have shown in an additional sub-species that they indeed cool themselves by increasing EWL at high  $T_a$  just like most mammals.

While all other physiological variables differed for short-beaked echidnas between the two locations, thermal conductance did not. Based on physical appearance, conductance would be expected to differ between the two sub-species. Tasmanian echidnas have a dense covering of fur, with finely interspersed spines, while W.A. echidnas have a dense covering of spines, with finely interspersed fur. An early study by Augee (1978), examining acclimation of three sub-species, found that echidnas of the sub-species *T.a. acanthion*, from Queensland, had the highest C, while those of the sub-species *T.a. setosus*, from Tasmania, had the lowest (Table 3.1). I also find that if I calculate conductance values using the slope of the MR line *vs.*  $T_a$ , as did Augee (1978), *T.a. acanthion*, from W.A., has a higher C than *T.a. setosus*, from Tasmania. However, when C is calculated as  $C=MR/(T_b-T_a)$ , this pattern is inverse. This was due to the differing slopes of the lines, as the two sub-species respond differently to  $T_a$ . The former method, where C is calculated using the slope of MR presents potential problems as it assumes a Newtonian model of heat exchange, and echidnas are so heterothermic that they do not conform closely to this

model. Thus the latter method is used in preference, though further studies are required to resolve this paradox. Meaningful comparisons of  $C$  between the numerous echidna studies is difficult as different methods were used to obtain these values, and the methodology is unclear in some studies. Some studies have calculated  $C$  using the slope of the MR line (Augee 1978), others have calculated  $C$  at a particular  $T_a$  at the lower end of thermoneutrality (Schmidt-Nielsen et al. 1966), while others state minimal conductance values were used (McNab 1984). Further confounding issues include the considerable thermolability of short-beaked echidnas, seasonal differences, and variation in body fat content, which may be important for insulation.

When considering  $C$  at a  $T_a$  near the lower end of the echidnas thermoneutral zone ( $C=MR/(T_b-T_a)$ ), where proportional differences in MR and  $T_b$  mean that  $C$  remains constant, it seems likely that the pelage of echidnas may reflect adaptations to pressures other than thermoregulatory demands, possibly predation pressure. Spines may also provide a good insulative layer, with properties similar to the fur of the Tasmanian sub-species, however further investigation into heat flux through echidna pelts is required (although this may prove difficult as the spines are embedded in a large muscle layer). Examining the slopes of the MR lines for both sub-species, it also appears likely that the reason no significant differences are being detected is because the echidnas are not sufficiently thermally challenged at the temperatures measured, and if they were measured at lower  $T_a$ s, we would more likely see differences as insulation becomes more important. This may also be true for the higher temperatures, as at  $T_a = 32.5\text{ }^\circ\text{C}$  it appears that W.A. echidnas increase their  $C$  more than Tasmanian echidnas. It is of interest to measure short-beaked echidnas at higher  $T_a$  to more clearly see this pattern.

While W.A. echidnas increase their  $C$  more than Tasmanian echidnas at high  $T_a$ 's, Tasmanian echidnas increase their  $C$  earlier (from  $T_a=20^\circ\text{C}$ ) than W.A. echidnas, which don't begin to increase  $C$  until  $T_a = 30\text{ }^\circ\text{C}$ . Thus it appears Tasmanian echidnas are less tolerant of the heat and begin to activate heat loss mechanisms earlier than W.A. echidnas, which is as expected when considering the habitats of the two sub-species. Behavioural observations during experimentation also supports this, with Tasmanian echidnas sprawling out at much lower  $T_a$ 's than W.A. echidnas. Behavioural adaptations (*e.g.*

hibernation, torpor, shelter use) may also reduce the necessity to alter  $C$  in different climates, as there are generally a range of behavioural as well as physiological mechanisms employed to cope with different thermal challenges (Merritt et al. 2001). These differing responses to  $T_a$  in terms of  $C$  are further evidence that the two sub-species are employing different physiological adaptations to survive in differing environments, and are more than just anatomically different.

### *Seasonal variation*

Mammals typically use three different strategies to deal with low  $T_a$  and increased heat loss during winter. They increase their thermogenic capacity (often by increasing MR) or radiative heat gain (*e.g.* basking), decrease the rate of heat loss (by altering body mass and/or conductance), or reduce their  $T_b$  thus reducing the difference between  $T_b$  and  $T_a$  (Lovegrove 2005; Tattersall *et al.* 2012). There was no seasonal pattern in MR for short-beaked echidnas, which may be due to their low MR and hence low absolute thermoregulatory costs, which means they are less affected by seasonal food limitations (Abensperg-traun and De Boer 1992). Echidnas are also relatively large (>3kg) and have a compact spherical body shape and so have a small SA:V ratio, increasing cold tolerance (Larcombe and Withers 2008).

Many small mammals (<500g) reduce body mass in winter to reduce their overall energy expenditure when resources are more scarce. In contrast, larger mammals (500g-10kg) generally increase insulation and body mass during winter (Heldmaier 1989; Lovegrove 2005). Echidnas (3.7kg), show neither a winter reduction nor increase in body mass: there was no significant difference between summer and winter. However, body mass was significantly higher in autumn (Figure 3.4). This may be pre-winter fattening in preparation for torpor use during winter (Gieser *et al.* 1990), or in preparation for mating (McDevitt and Andrews 1995) which occurs in late winter (Ferguson and Turner 2012; Nicol and Morrow 2012). Wild echidnas increase foraging during spring and autumn (Abensperg-Traun and De Boer 1992) so this seasonal cue to increase foraging may explain some of the mass gain in these captive animals. As my study was carried out on

**Table 3.1:**  $T_b$ , BMR and  $C_{wet}$  of different sub-species of short-beaked echidnas from previous studies.

Location	Sub-species	$T_a$ (°C)	mass (kg)	$T_b$ (°C)	BMR (ml O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> )	$C_{wet}$ (ml O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> °C <sup>-1</sup> )	C Method	Source
NSW	<i>unknown</i>	25	3.40	31.3	0.132	-	-	Dawson <i>et al.</i> (1979)
NSW	<i>T.a. aculeatus</i>	25	3.50	30.2	0.150	-	-	Augee (1976)
VIC	<i>T.a. aculeatus</i>	25	-	30.2	0.210	0.028	MR slope	Augee (1978)
PNG	<i>T.a. lawesii</i>	25	2.14	30.4	0.131	0.021	Minimal C	McNab (1984)
QLD	<i>unknown</i>	23	3.12	31.2	0.155	-	-	Frappell <i>et al.</i> (1994)
QLD	<i>T.a. acanthion</i>	25	-	29.0	0.210	0.037	MR slope	Augee (1978)
WA	<i>T.a. acanthion</i>	25	3.45	29.8	0.090	0.023	MR slope	Current study
WA	<i>T.a. acanthion</i>	25	3.45	29.8	0.090	0.013	Minimal C	Current study
TAS	<i>T.a. setosus</i>	25	3.58	30.0	0.153	0.013	Minimal C	McNab (1984)
TAS	<i>T.a. setosus</i>	-	2.73	-	0.174	-	-	Bech <i>et al.</i> (1992)
TAS	<i>T.a. setosus</i>	25	-	30.4	0.200	0.022	MR slope	Augee (1978)
TAS	<i>T.a. setosus</i>	25	3.62	31.7	0.205	0.008	MR slope	Current study
TAS	<i>T.a. setosus</i>	25	3.62	31.7	0.205	0.016	Minimal C	Current study
unknown	<i>unknown</i>	20	3.00	30.9	0.217	0.025	$T_a=20^\circ\text{C}$	Scmidt-Nielsen <i>et al.</i> (1966)
unknown	<i>unknown</i>	19	3.80	28.4	-	-	-	Robinson (1954)
unknown	<i>unknown</i>	-	2.36	30.1	0.151	-	-	Martin (1902)

captive echidnas, maintained on the same diet and quantity of food all year round, we can conclude that the change in body mass between seasons is not a direct result of resource scarcity, but an environmentally or physiologically triggered response to seasonal changes. Nicol *et al.* (2012) found that free-ranging echidnas in Tasmania weigh the most in late summer, slightly earlier than those measured in W.A., but they enter hibernation just after this period in Tasmania.

Many small mammals also seasonally adjust their thermal conductance to aid in reducing heat loss to their environment in the colder months, maintaining a constant  $T_b$  at a lower energetic cost by increasing insulation (Lovegrove 2005; Larcombe and Withers 2008). This reduction in  $C$  reduces the lower critical temperature, which means the metabolic cost of maintaining  $T_b$  is reduced (Bartholomew 1982; Larcombe and Withers 2008). However, not all species show a seasonal change in  $C$ , such as southern brown bandicoots (*Isodon obesulus*; Larcombe and Withers 2008), Djungarian hamsters (*Phodopus sungorus*; Heldmaier and Steinlechner 1981), desert cottontails (*Sylvilagus audubonii*; Hinds 1973), and now short-beaked echidnas. Short-beaked echidnas did not alter their  $C$  seasonally, which may be due to behavioural adaptations that negate the necessity for an increase in insulation (*e.g.* basking), or more simply their size. An animal with a low conductance (*i.e.* it is well insulated) has a lower ability to gain heat from solar radiation through basking on cold sunny; a behavioural adaptation utilised by echidnas (Nicol *et al.* 2008). Solar heat gain may aid in thermoregulation, allowing a reduction in metabolism at low  $T_a$  (Cooper *et al.* 2003). This may be of particular importance for a myrmecophage such as the echidna, as their food source has a low energy density and is poorly digested. In many areas of their distribution, echidnas, like many mammals (McDevitt and Andrew 1995; Merritt *et al.* 2001), change from primarily nocturnal activity in summer to nocturnal/diurnal activity during winter to avoid extremes in temperature (Abenspergtraun and De Boer 1992; see also chapter five), which is a strategy used by many species (Tattersall *et al.* 2012) *e.g.* Arabian oryx (*Oryx leucoryx*; Williams *et al.* 2001). Larger mammals also have lower mass-specific thermal conductances than smaller mammals and thus it should be less energetically expensive for a larger mammal to survive under cold conditions than a smaller mammal, reducing the need for larger mammals to seasonally

alter C (Larcombe and Withers 2008). Further studies on large mammals are required to gain a better understanding of seasonal patterns of change in C, as many of the current studies are on small mammals <1000g.

As well as these behavioural adaptations, short-beaked echidnas adjusted their  $T_b$  seasonally; mean  $T_b$  was  $29.7 \pm 0.6^\circ\text{C}$  in winter compared to  $27.9 \pm 0.4^\circ\text{C}$  in summer. Echidnas also use hibernation and short-term torpor (Grigg *et al.* 1989; Rismiller and McKelvey 1996; Nicol and Morrow 2012; see also chapter five) as an energy conservation strategy during winter, rather than defending a high and constant  $T_b$ . Short-beaked echidnas feed on a low energy food source and lead a low energy lifestyle, thus it may be expected they reduce  $T_b$  rather than increase energy expenditure to maintain  $T_b$  constant in colder conditions. Echidnas did not use torpor in the laboratory during measurements (though they did appear torpid on several cold mornings when collected from their outdoor enclosure), which is common for wild-caught animals under measurement conditions (Gieser *et al.* 2000). Many mammals decrease their  $T_b$  during summer, including the myrmecophagous numbat (Cooper and Withers 2012). The summer reduction in  $T_b$  by echidnas may be an adaptation to reduce energy expenditure when termites are less abundant (Apensberg-Traun and De Boer 1990), as well as reducing water loss and allowing a greater 'buffer zone' before  $T_b$  reaches lethal levels as  $T_a$  rises (Cooper and Withers 2012), which also increases heat loss to the environment and reduces their dependence on evaporative cooling (Williams *et al.* 2001).

There is a lack of data for seasonal variation of EWL for mammals. From the few studies, the general pattern is that EWL is higher in summer than winter at low  $T_a$ , there is no difference between the two seasons at intermediate temperatures within the thermoneutral zone, and EWL is higher in summer than winter at high  $T_a$  (Hinds 1973; Larcombe and Withers 2008; Cooper and Withers 2012). Conforming to this general pattern, short-beaked echidnas had a higher EWL in summer, facilitating evaporative cooling and enabling them to dissipate heat more easily to cool themselves when  $T_a$  is high (Hulbert and Gordon 1972; Larcombe and Withers 2008). These data indicate that not only can echidnas use evaporative cooling as a mechanism to dissipate excess heat (as shown in

chapter two), but they can also modulate this and alter EWL seasonally to better meet thermoregulatory demands.

### *Effects of captivity*

Most physiological variables ( $T_b$ , MR, C or RWE) did not change over the two years of measurement in captivity for short-beaked echidnas. A previous study of myrmecophageous numbats also found that captivity had little effect on physiology (Cooper and Withers 2012). Larcombe and Withers (2007) studied captivity effects on southern-brown bandicoots and found that most physiological variables were unaffected by captivity, except those reflecting diet and nutrition. However, several studies of captivity effects on birds show significant captivity effects on BMR (Weathers *et al.* 1983). Maintaining individuals in an environment that is as close to natural conditions as possible may be a crucial factor in reducing physiological impacts of captivity. In this study, echidnas were housed in a large outdoor enclosure where they could move about freely, and experience natural photoperiod and weather variations, so it may be expected that there was little change in physiology. The one variable that was different between the years for echidnas was EWL, which was higher on initial measurement of newly captured individuals than when measured one year later after being held in captivity. This may be due to a change in experimental regime involving the timing of measurement; the first experiments were started between approximately 0600-0700 h, however water did not have enough time to wash out (become minimal and stable once the echidna became inactive, so resting EWL was measured rather than active EWL) and become minimal before the echidnas became active in the afternoon. This was identified and I started further experiments earlier in the morning (by 0500 h) so there was sufficient time for water loss to become stable and minimal before the echidnas became active; the data from 2011 was not used in any other analyses. However, the potential captivity effect for EWL means that seasonal and geographic comparisons for this variable should be interpreted with this in mind.

## *Conclusion*

Short-beaked echidnas have a great deal of physiological plasticity. This ability to physiologically adapt to changing environments may be a key factor in their abundance and wide distribution to date. Plasticity is likely to be an important feature in the future, as climate change alters habitats and resources; physiological plasticity in echidnas means they have the potential to continue to be successful in the future as the climate changes. Plasticity also has important implications for standardised measurements of physiological variables. Echidnas, as a model species, show that several physiological variables vary considerably between seasons and location. Therefore it is important to consider this when comparing physiological data for species from experiments conducted at different times of the year and at different locations.

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## Field metabolic rate and water flux of free-living short-beaked echidnas in Western Australia

### Abstract

The field metabolic rate ( $605.1 \pm 136.6 \text{ kJ day}^{-1}$ ) of West Australian short-beaked echidnas (*Tachyglossus aculeatus acanthion*) is within the expected range for a mammal of similar size, and is not low compared to other mammals, as might be expected from their very low basal metabolic rate. Their FMR:BMR ratio is higher than that expected for a generalised mammal, which reflects their basal metabolic rate being a relatively small portion (25%) of their field metabolic rate and other energetic costs (possibly locomotion and foraging) being high. There were no significant differences in the field metabolic rate of short-beaked echidnas from Western Australia., Tasmania nor Kangaroo Island, which is surprising given the differences in standard physiology between the sub-species. Total body water content of echidnas ( $72.1 \pm 4.1 \%$ ) was within the expected range for mammals, though slightly higher than previous measurements for echidnas in Tasmania, which may indicate that short-beaked echidnas in Western Australia had a poorer body condition (lower fat content). Water turnover rate was as expected ( $185.2 \pm 22.2 \text{ ml day}^{-1}$ ) for a generalised mammal, and similar to that for other echidna sub-species during summer. The water economy index (0.39) of Western Australian short-beaked echidnas was higher than for short-beaked echidnas from Kangaroo Island (0.19) and Tasmania (0.23), and another myrmecophage from Dryandra, the numbat (0.29), suggesting that diet differs significantly between locations.

## Introduction

Field metabolic rate (FMR) is the daily energy expenditure of a free-ranging animal, which includes basal metabolism (BMR) and energetic increments for thermoregulation, locomotion, feeding, predator avoidance, digestion, reproduction and growth. Any energetic savings from hypothermia and torpor are also included in FMR (Nagy 1987). FMR and field water turnover rate (FWTR) are commonly measured using the doubly labelled water technique, which uses isotopically labelled water ( $O^{18}$  and  $H^2$ ) to measure the rate of carbon dioxide production and water influx/efflux (Lifson and McClintock 1966; Speakman 1997). Loss of the oxygen isotope is faster than the loss of the hydrogen isotope, as oxygen is lost as both water and  $CO_2$ , while H is lost only as water, so  $CO_2$  production can be calculated from the difference between the washout rates of the two isotopes (Nagy 2005). Much insight into the physiology, ecology and evolution of an animal can be gained from knowledge of their energy budget (Nagy 1987). Although physiological studies on captive and domestic animals are valuable in terms of providing standardised, comparable data, these laboratory measurements can be difficult to apply to free-living animals in their natural environment, responding to a wide and varying range of environmental conditions (*e.g.* weather) and ecological pressures (*e.g.* predation and food patchiness) that captive animals may not experience (Nagy 1987). It is therefore important to measure energy expenditure of animals in their natural environments to gain a better understanding of their natural physiology.

The FMR of many non-reproductive endothermic mammals is generally two- to three-times higher than BMR (Cooper *et al.* 2003; Munn *et al.* 2013). However, despite the fact that BMR is commonly used as an index of FMR for free-living mammals (Koteja 1991; McNab 2002; Cooper *et al.* 2003), there is no consistent relationship between BMR and FMR for all mammals (Koteja 1991; Nagy *et al.* 1995; McNab 2002; Cooper *et al.* 2003; Nagy 2005). As with BMR, much of the variation in mammalian FMR is accounted for by body mass (Nagy 2005; Riek 2008), though FMR and BMR generally scale differently. Smaller, more active species have a higher FMR to BMR ratio, while larger species have a lower FMR to BMR ratio (Koteja 1991; Cooper *et al.* 2003; Munn *et al.* 2013), since BMR comprises a greater proportion of FMR for larger species than it

does for smaller species. This is presumably due to the increased costs for thermoregulation and higher activity levels of smaller species (Cooper *et al.* 2003). Reproduction and lactation will also increase this ratio (Cooper *et al.* 2003; Munn *et al.* 2013). The ratios will also differ depending on lifestyle; active versus sluggish lifestyles will have a great impact on FMR, and the cost of activity cannot be assumed to be a constant multiple of BMR (McNab 2002). It might be expected that echidnas would have a similar BMR/FMR ratio to other myrmecophages, as this specialist diet has been related to a low BMR. Numbats (*Myrmecobius fasciatus*) have an FMR three times that of their BMR (Cooper *et al.* 2003), and aardwolves (*Proteles cristatus*) have an FMR 2.7 times that of their BMR (Williams *et al.* 1997), which are within the expected range for an endotherm. Green *et al.* (2002) reported that FMR of short-beaked echidnas on Kangaroo Island was about three times higher than SMR estimates of Schmidt-Nielsen *et al.* (1966), and five times higher than SMR estimated from Dawson *et al.* (1979) and McNab (1984). These previous FMR:BMR comparisons were made between studies using different animals from different locations and during different times of the year, so more direct comparisons between the same population would be beneficial in determining a more accurate ratio for this species.

Many factors influence FMR, though these are difficult to isolate and analyse for free-living animals. As FMR is generally only measured over a short period, and represents a small part of a year, values include daily, seasonal and individual variation, which may mask the general effects of taxon, diet and habitat (Nagy 1994). At the particular time of measurement it is just as likely that an animal may be influenced more by its local circumstances as its genetics (Nagy 1994) and this is one of the disadvantages of FMR measurement as compared to standardised laboratory measurements. However, researchers have been able to make some broad conclusions about the influence of single factors, such as habitat and diet, on FMR. Mammals from arid habitats generally have lower (by approximately 30%) FMRs than their non-arid counterparts (Speakman 2000; Cooper *et al.* 2003; Nagy 2005; Munn *et al.* 2012), which is consistent with patterns for BMR. Strong correlations that exist between diet and BMR are also evident for FMR (McNab 2002; Munn *et al.* 2012). Dietary classes such as grazing, insectivory, carnivory,

and nectivory/frugivory are associated with high FMR, while folivory, granivory and myrmecophagy are associated with low FMR (Speakman 2000; Cooper *et al.* 2003). Previous studies of echidnas in other parts of Australia have concluded that echidnas have an FMR lower than predicted from their body size (Green *et al.* 2002; Schmid *et al.* 2003), consistent with a low  $T_b$  and BMR (Chapter 2) that is typical of other myrmecophagous mammals (McNab 1984, 1986; Cooper *et al.* 2003). It is of interest to compare the FMR of W.A. short-beaked echidnas to these different sub-species (from Kangaroo Island and Tasmania), as W.A. echidnas inhabit a much hotter and drier environment, and have considerable differences in standard physiology from the other sub-species (Chapter 3).

The importance of the cost of locomotion as a contributor of daily energy expenditure (DEE) has been largely debated. Many argue that the contribution of locomotion to DEE is minimal in most small mammals; however, for large mammals (>1000kg), especially carnivores with larger home ranges, this contribution may be higher (Garland 1983; Chappell *et al.* 2013). For example, wildebeest (*Connochaetes taurinus*; 180kg) spend an average of 8% of their energy budget on transport costs (Pennycuik 1979), howler monkeys (*Alouatta palliata*; 6.5kg) spend 2.3% (Nagy and Milton 1979), and antelope ground squirrels (*Ammospermophilus leucurus*; 85g) spend 2.2-4.4% depending on season (Karasov 1981; Garland 1983). However, myrmecophagous aardwolves (*Proteles cristatus*; 7.8kg) spend 36.3% of their FMR on locomotion, which is considerably higher than expected (Williams *et al.* 1997). While body mass has a substantial impact on locomotor costs, mode of locomotion (*e.g.* hopping) may also influence the proportion of energy spent on locomotion (Munn *et al.* 2013). Thus, some animals will have higher BMR:FMR ratios and expend a larger percent of their total energy budget on locomotion, due to an expensive mode of locomotion. Though echidnas have primitive limb girdles and distally heavy limbs, the energy requirement for locomotion is lower than for many mammals (Edmeades and Baudinette 1975), and mode of locomotion may not be as primitive as once thought (Gambaryan and Kuznetsov 2013).

Total body water content (TBW) can be interpreted as an index of body fat content and therefore body condition, with higher values indicating poorer body condition. Schmid

*et al.* (2003) reported a median body water content for Tasmanian echidnas of 62% (in spring), which is similar to that measured by Green *et al.* (1992) for Kangaroo Island echidnas (58%; in summer). Here I investigate the TBW of W.A. echidnas to determine if they have a similar body condition in a hot, dry habitat.

To compensate for water loss (urinary, faecal and evaporative), animals intake water by drinking, as preformed water in food, and by metabolic water production (Schmidt-Nielsen and Schmidt-Nielsen 1952). Mammals that ingest a large portion of soil as they feed, such as myrmecophages like echidnas, can have a significant water intake from 'free water' if the soil is damp (Cooper and Withers 2004a). FWTR was similar for Tasmanian (about 170 ml day<sup>-1</sup>) and Kangaroo Island (167 ml day<sup>-1</sup>) short-beaked echidnas (Green *et al.* 1992; Schmid *et al.* 2003), and Green *et al.* (1992) interpreted their FWTR as being one of the lowest recorded for a mammal, comparable to that of sloths (*Bradypus variegatus*) and koalas (*Phascolarctos cinereus*; Table). Echidnas have been seen to drink on occasion (McKelvey 1987); however by estimating food intake, Green *et al.* (1992) calculated that drinking does not represent an important avenue of water influx for echidnas on Kangaroo Island. Here I determine if short-beaked echidnas from a semi-arid habitat in W.A. have similar body water content and water flux rates as those of other sub-species and calculate if they require access to free water to balance their water budget.

I use the doubly labelled water technique to measure FMR and FWTR for short-beaked echidnas free-ranging in a semi-arid habitat, Dryandra woodland, in W.A. This is combined with GPS tracking to characterise daily movements, to gain a better understanding of the field energetics of this wide-spread mammal. This is the first study on short-beaked echidnas that correlates field physiology with daily movements and activity. By comparison with published data for Tasmanian and Kangaroo Island echidnas, I aim to provide a better understanding of the physiological adaptations between sub-species that have allowed echidnas to be so successful in a wide range of environments.

## Materials and Methods

### *Data collection*

Six free-ranging echidnas were captured by hand at Dryandra Woodland (31° 46' S 117° 1' E), 170 km south-east of Perth, Western Australia, during March 2013. Free water from a dam was available in the home range of one echidna but there was no known standing free water within the home ranges of any of the other echidnas during the study period. There was a negligible amount of rain (0.2mm over one day) in the middle of the two weeks of experimentation. During the study period the mean day time  $T_a$  was 21.7 °C, mean night time temperature was 17.9 °C, minimum  $T_a$  was 7.3 °C and maximum  $T_a$  was 32.9 °C (data from local BOM weather station). The echidnas were fitted with small radio-transmitters (approximately 6 g; Holohil RI-2C) to allow re-capture, and five echidnas were also fitted with CatTrack 1 global positioning system (GPS) loggers (see Chapter 6).

Echidnas were captured and weighed, before a background blood sample was taken. Blood samples and injections were conducted under general anaesthesia (3% isoflurane in O<sub>2</sub>), and blood was sampled from the venous sinus at the tip of the beak (Schmid *et al.* 2003). Each echidna was injected intraperitoneally with approximately 1.2 ml doubly labelled water from a mixture of 9ml of 98% enriched O<sup>18</sup> and 5ml of H<sup>2</sup> (Table 4.1); injection mass was determined by weighing the syringe to 0.0001 g before and after injection. Echidnas were held for an equilibration period of 4 h (based on previous FMR studies for

**Table 4.1:** d<sup>18</sup>O and d<sup>2</sup>H for the injection solution, both before and after distillation.

<b>Injection solution</b>	<b>Distillation</b>	<b>d18O</b>	<b>d2H</b>
1	Not distilled	145.4	1023.9
2	Not distilled	152.6	1083.9
3	Not distilled	142.8	1016.1
4	Not distilled	153.1	1067.5
1	Distilled	113.6	769.7
2	Distilled	111.1	747.0
3	Distilled	98.1	649.4
4	Distilled	105.2	696.5

echidnas; Schmid *et al.* 2003); a second blood sample was then taken. The echidna was then released at the point of capture. The echidnas ranged freely, uninterrupted, until re-captured five days later. A recapture blood sample was taken and they were again released at the point of capture. Seven days later the echidnas were re-captured once again

and a final blood sample was taken. After collection, blood was transferred to capillary tubes and flame-sealed on-site. Upon return to the laboratory, blood samples were vacuum distilled into glass pipettes which were then flame sealed and stored at 4 °C until analysis (after Speakman 1997).

### *Water flux and field metabolic rates*

The injectate enrichment was calculated by diluting a weighed sample of the isotope solution (same quantity as injected into echidnas, approximately 1.2 ml) into a weighed quantity of a background sample of water (approximately 2.6 kg; collected from a dam

**Table 4.2:**  $d^{18}\text{O}$  and  $d^2\text{H}$  for six echidnas free-ranging at Dryandra Woodland, Western Australia.

<b>Echidna</b>			
<b>I.D.</b>	<b>Sample type</b>	<b>d18O</b>	<b>d2H</b>
1	Background	5.4	7.6
	Equilibration	173.5	1231.3
	Days 1-5	119.0	812.4
	Days 6-12	82.1	609.7
2	Background 1	10.4	32.6
	Background 2	20.4	76.2
	Equilibration	171.7	1150.9
	Days 1-5	115.5	797.8
3	Days 6-12	42.9	294.9
	Background	8.1	18.2
	Equilibration	190.8	1327.3
4	Days 6-12	49.4	434.5
	Background	3.9	6.3
	Equilibration	187.4	1281.2
	Days 1-5	121.6	932.4
5	Days 1-5 replicate	150.4	1150.2
	Days 6-12	76.4	572.9
	Background	3.9	-1.1
	Equilibration	169.3	1303.5
6	Days 1-5	96.0	847.6
	Days 6-12	40.3	220.4
	Background	16.4	35.7
	Equilibration	222.7	1837.3
	Days 1-5	119.9	905.5
	Days 6-12	46.2	401.3

within the study site). The solution was thoroughly mixed and a sample was taken for analysis. The stable isotope composition was analysed for all samples (background water samples and four blood samples per echidna) using a Picarro L1115-I isotopic liquid water analyser (Picarro, Santa Clara, California, USA) by the West Australian Geochemistry Centre, University of Western Australia (Skrzypek and Ford 2014). Prior to analyses, all samples were isotopically diluted with deionised water of a known isotope composition. The  $d^2\text{H}$  and  $d^{18}\text{O}$  raw values of the samples were normalised to the Vienne

Standard Mean Ocean Water (VSMOW) scale, following the principals of the three-point normalisation (Skrzypek 2013), and were replicated twice.

Total body water content, water flux and CO<sub>2</sub> production were then calculated from the raw values (Table 4.2), after Speakman (1997), using Speakman's revision (addressing the overestimation of evaporative water loss; EWL) of the Lifson and McClintock (1966) method. FMR was converted from ml CO<sub>2</sub> day<sup>-1</sup> to kJ day<sup>-1</sup>, assuming 25 kJ CO<sub>2</sub> l<sup>-1</sup> (mixed protein/fat diet; Nagy *et al.* 1999). Total body water content was calculated from the hydrogen dilution.

Data from days 1-12 were used to calculate FMR for each echidna (as a longer time period results in a better estimate of FMR) except for echidna 5, where data from days 1-5 were used as a distillation error meant data from the second recapture could not be used.

### *Allometry*

Echidnas were compared to the mammalian allometric relationship for FMR and FWTR using conventional linear regression (StatistiXL V1.1) and autoregression (using custom written VB software, P. Withers), based on the mammal super-tree of Bininda-Emonds *et al.* (2007). The data set for mammalian FWTR (73 species) and FMR (90 species) was collated from Cooper *et al.* (2003) and Munn *et al.* (2012). Conformation of echidnas to the respective datasets was determined using the 95% prediction limits after Cooper and Withers (2006). ANOVA was used to compare my data with previously published data for echidnas (Green *et al.* 1992; Schmid *et al.* 2003).

### *Dietary water and energy intake*

Calculations for digestibility and termite/ant consumption requirements were based on data from Cooper and Withers (2004) and Withers and Dickman (1995) for termite and ant mass, digestible energy and water contents. I assumed a water content of 77% for termites and 62% for ants and an energy content of 0.02 kJ individual<sup>-1</sup> for termites and 0.006 kJ individual<sup>-1</sup> for ants, with a digestibility of 72% for termites and 59% for ants.

The number of termites/ants ingested per day based on FMR was calculated as FMR/digestible energy per termite or ant. The number of termites/ants ingested based on FWTR was calculated as FWTR-MWP/water per termite or ant. Water economy index (WEI) was calculated as FWTR/FMR.

### *Distance travelled and activity*

Movement and activity data (time spent inactive and distance travelled per day) of short-beaked echidnas were manually calculated using Garmin MapSource Version 6.16.3 to view and edit the locations obtained from the GPS units strapped to a metal cradle glued to the echidnas back (see Chapter 6). GPS units were calibrated by placing them in known locations, both in the open and under cover, to determine error when stationary, and by walking and driving along a known route of a known distance to calculate error when moving. When moving, GPS coordinates were accurate to within approximately  $\pm 20\text{m}$ , but when stationary the accuracy decreased considerably, to within approximately  $\pm 100\text{m}$ . Echidnas were considered inactive when these 'noisy' consecutive GPS points were non-directional, 'star-shaped' and centred around an area  $\leq 50\text{m}$  radius. Echidnas were considered active when data points moved directionally. Any singular points  $> 30\text{m}$  from the obvious track were considered 'noise' and deleted based on calibration data. StatistiXL V1.1 was used to perform simple correlations between the two activity parameters (time inactive and distance travelled daily) and FMR and FWTR to determine if activity was related to FMR and FWTR.

## **Results**

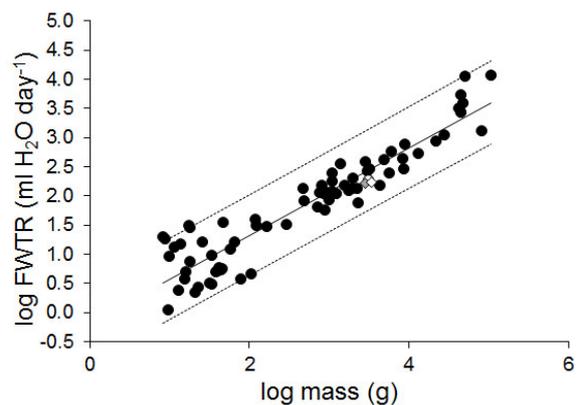
Mean body mass of the six short-beaked echidnas was  $3.15 \pm 0.07$  kg (N = 6, n = 18). There was a significant increase in body mass between initial ( $3.1 \pm 0.06$  kg) and week two recapture ( $3.3 \pm 0.08$  kg) body masses ( $t_5 = 5.432$ ,  $P = 0.003$ ; Table 4.3).

### *Water flux and field metabolic rate*

Mean of TBW of short-beaked echidnas was  $72 \pm 4\%$  ( $N = 6$ ), ranging from 55% to 85%. There was no correlation between TBW and body mass ( $t = 0.391$ ,  $P = 0.716$ ). Mean FWTR for short-beaked echidnas was  $185 \pm 22$  ml day<sup>-1</sup> over the duration of the FMR measurement, ranging from 126 ml day<sup>-1</sup> to 280 ml day<sup>-1</sup> ( $N = 6$ ). The mean FMR for echidnas was  $605.1 \pm 136.6$  kJ day<sup>-1</sup>, ranging from 193.4 kJ day<sup>-1</sup> to 1056 kJ day<sup>-1</sup> (Table 4.3). WEI of myrmecophagous echidnas was 0.39 mg H<sub>2</sub>O mg O<sub>2</sub><sup>-1</sup>.

### *Allometry*

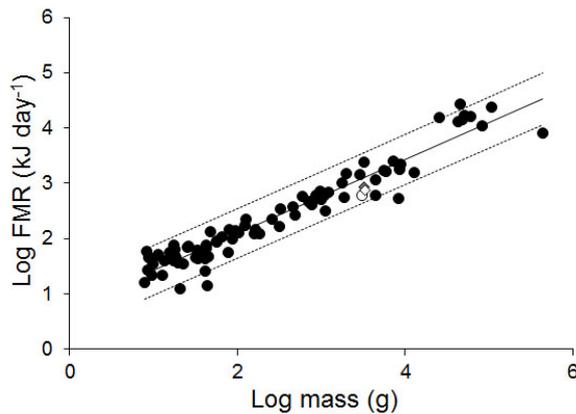
There was a positive linear relationship (Figure 4.1) between log(body mass) and log(FWTR) for terrestrial mammals ( $n = 73$ ;  $y = 0.7497x - 0.1699$ ;  $R^2 = 0.874$ ,  $F_{1,70} = 487$ ,  $P < 0.001$ ). W.A. short-beaked echidnas fell within the 95% prediction limits for this regression, as did the other two estimates of FMR for short-beaked echidnas (Green *et al.* 1992; Schmid *et al.* 2003). This relationship remained after the data were rendered phylogenetically independent by autocorrelation ( $0.668x + 0.024$ ;  $R^2 = 0.878$ ,  $F_{1,87} = 624$ ,  $P < 0.001$ ), and short-beaked echidnas still remained within the prediction limits. There was also a strong positive linear relationship between body mass and FMR (Figure 4.2) of terrestrial mammals ( $n = 90$ ;  $y = 0.6696x + 0.7553$ ;  $R^2 = 0.928$ ,  $F_{1,87} = 1119.5$ ,  $P < 0.001$ ). W.A. short-beaked echidnas conformed to this relationship, falling within the 95% prediction limits, as did short-beaked echidnas from Kangaroo Island and Tasmania. After phylogenetic (autoregression) correction, the significant



**Figure 4.1:** Field water turnover rate (ml H<sub>2</sub>O day<sup>-1</sup>) of mammals (black circles; Cooper *et al.* 2004 and Munn *et al.* 2012) and short-beaked echidnas from W.A (this study; white circle), Tasmania (Schmid *et al.* 2003; white diamond) and Kangaroo Island (Green *et al.* 1992; grey diamond), as a function of body mass, with the regression line (solid) and 95% prediction limit bands (dashed).

relationship remained ( $y = 0.6327x + 0.0142$ ;  $R^2 = 0.878$ ,  $F_{1,87} = 624$ ,  $P < 0.001$ ) and short-beaked echidnas still fell within the prediction limits.

#### *Dietary water and energy intake*



**Figure 4.2:** Field metabolic rate ( $\text{kJ day}^{-1}$ ) of mammals (black circles; Cooper *et al.* 2004 and Munn *et al.* 2012) and short-beaked echidnas from W.A (this study; white circle), Tasmania (Schmid *et al.* 2003; white diamond) and Kangaroo Island (Green *et al.* 1992; grey diamond), as a function of body mass, with the regression line (solid) and 95% prediction limit bands (dashed).

than their food, or the ratio of ants/termites eaten accounts for this difference, or echidnas have a lower digestibility. Echidnas had a water turnover rate of  $185 \pm 22 \text{ ml day}^{-1}$ , of which (if feeding exclusively on termites) 125 ml was preformed dietary water and 17 ml metabolic water production (MWP), leaving 38 ml water unaccounted for, presumably from other sources such as moist soil and drinking. If ants were assumed to be the primary food source, then 56 ml of their daily FWTR would come from dietary water, and 17 ml from MWP, leaving 108 ml water from other sources. Assuming a diet composed of 70/30 mix of termites and ants (Griffiths 1978), echidnas would need to consume 49684 ants/termites to sustain FMR and 85977 ants/termites to sustain FWTR. Dietary water would be  $95 \text{ ml H}_2\text{O day}^{-1}$ , MWP would be 17 ml, leaving  $69 \text{ ml H}_2\text{O day}^{-1}$  from other unaccounted sources. Abensperg-Traun and De Boer (1992) found that diet composition varied seasonally, and termites comprised  $>90\%$  of prey ingested during January to March. Using a diet composition of 90% termites or above results in the smallest amount

The number of termites/ants calculated to be required to sustain FWTR (51389 termites  $\text{day}^{-1}$  or 501777 ants  $\text{day}^{-1}$ ), was considerably higher than that calculated using FMR (39251 termites or 170933 ants), assuming 72% digestibility for termites (Cooper and Withers 2004) and 59% digestibility for ants (Withers and Dickman 1995). This suggests that echidnas are obtaining water from additional sources other

of unaccounted water ( $50 \text{ ml H}_2\text{O day}^{-1}$  for 90/10 and  $45 \text{ ml H}_2\text{O day}^{-1}$  for 95/5). Preliminary analyses from scats collected from my field site suggest that the dietary composition of echidnas at Dryandra was quite different to those suggested above, with an average of 24% termites and 70% ants (with the rest being cockchafer larvae). Some individuals consumed up to 30% cockchafer larvae (Nicol, *unpublished*). However, this data was collected from June to October and we know diet composition varies seasonally. Assuming a diet composition of 30% termites and 70% ants results in  $74 \text{ ml H}_2\text{O day}^{-1}$  of unaccounted water, which suggests that these echidnas must be drinking. There is evidence that echidnas do drink when free water is available, so this is not entirely unexpected, however the fact that there was no known free water in their home ranges does conflict with this. The only conclusion we can draw from this is that perhaps during the cooler months these echidnas ingest considerably more ants than termites, however during the warmer months this may not be the case and the proportion of ants/termites may drastically change to balance water.

#### *Distance travelled and activity*

Mean distance travelled during the study period was  $1894 \pm 298 \text{ m day}^{-1}$ , ranging from  $1327 \text{ m day}^{-1}$  to  $2951 \text{ m day}^{-1}$ . Mean time spent inactive was  $17:43 \pm 0:28 \text{ h day}^{-1}$ , ranging from  $16:05 \text{ h day}^{-1}$  to  $18:46 \text{ h day}^{-1}$ . There were no significant correlations between FMR or FWTR with these activity variables (Table 4.4).

**Table 4.3:** Body mass (initial and recapture), water turnover rate, field metabolic rate, time spent inactive and distance travelled for six free-living short-beaked echidnas at Dryandra Woodland, Western Australia

<b>Time period</b>	<b>Echidna I.D.</b>	<b>Body mass (initial; kg)</b>	<b>Body mass (recapture; kg)</b>	<b>FWTR (ml H<sub>2</sub>O day<sup>-1</sup>)</b>	<b>FMR (kJ day<sup>-1</sup>)</b>	<b>TBW (%)</b>	<b>WEI (FWTR/FMR)</b>	<b>Inactive time (hours)</b>	<b>Distance travelled (m per day)</b>
Days 1-12	1	3.20	3.35	126	193	73.33	0.65	18:46:38	1333
Days 1-12	2	2.92	2.99	280	521	85.27	0.54	17:38:08	1838
Days 1-12	3	3.07	3.39	194	875	72.10	0.22	16:05:19	2951
Days 1-12	4	3.08	3.27	144	291	76.77	0.50	17:43:30	2020
Days 1-5	5	3.29	3.27	170	1056	69.32	0.16	18:25:01	1327
Days 1-12	6	2.95	3.14	197	695	55.06	0.28	-	-
	<b>Mean</b>	<b>3.08</b>	<b>3.24</b>	<b>185</b>	<b>605</b>	<b>71.97</b>	<b>0.39</b>	<b>17:43:43</b>	<b>1894</b>
	<b>SE</b>	<b>0.06</b>	<b>0.06</b>	<b>22</b>	<b>136</b>	<b>4.06</b>	<b>0.08</b>	<b>00:27:44</b>	<b>298</b>

**Table 4.4:** R values (above the diagonal) and P values (below the diagonal) for the correlations between time spent inactive, field metabolic rate, water turnover rate, and distance travelled, for six free-living short-beaked echidnas at Dryandra Woodland, W.A.

	Inactive time	Distance travelled	FMR (kJ/day)	WTR (ml/day)
Inactive time	-	-0.994	-0.358	-0.294
Distance travelled	0.001	-	0.419	0.237
FMR (kJ/day)	0.554	0.483	-	0.275
WTR (ml/day)	0.631	0.701	0.654	-

## Discussion

Short-beaked echidnas have an FMR and FWTR as predicted for a mammal of similar size, which is particularly interesting as their BMR and  $T_b$  are significantly lower than expected (Chapter 2). This suggests that other aspects of their energetics have a higher contribution to FMR than BMR, such as cost of locomotion and their digging foraging behaviour. Compared to other echidna sub-species, neither FMR nor FWTR vary between the three geographic locations (W.A., Kangaroo Island and Tasmania).

### *Field metabolic rate*

The FMR of W.A. short-beaked echidnas in my study ( $605.1 \pm 136.6$  kJ day<sup>-1</sup>) did not significantly differ from the previous two measurements for echidnas. Green *et al.* (1992) measured an FMR of  $911.5 \pm 116.4$  kJ day<sup>-1</sup> for short-beaked echidnas on Kangaroo Island, and Schmid *et al.* (2003) measured an FMR of  $755 \pm 8.8$  kJ day<sup>-1</sup> for short-beaked echidnas in Tasmania. It is somewhat surprising that there is no geographical variation in the FMR of echidnas, as my laboratory measurements showed significant physiological plasticity in BMR and other standard physiological variables between W.A. and Tasmanian echidnas (Chapter 3). However, the large individual variation seen for FMR in most field studies makes it difficult to detect any minor differences; the individual variation in FMR seen here for short-beaked echidnas is of the magnitude common amongst mammals (Riek 2008). Further field studies with larger sample sizes (to enable more power) may be beneficial to further examine

potential for geographic variation in the field physiology of echidnas, but sample sizes are limited by the combined cost of transmitters, isotope and sample analyses.

Generally, arid-zone mammals have an FMR approximately 30% lower than species from non-arid habitats (Nagy 1987; Gibson and Hume 2000), so it might be expected that W.A. short-beaked echidnas would have a lower FMR than those from cooler areas such as Tasmania. However, all three sub-species fell within the prediction limits for both arid and non-arid habitats, indicating that their FMRs are not particularly specialised for different habitats. As an FMR measurement is only a short snapshot in time, it is also possible that the values seen here are a result of the animals responding to particular local environmental conditions at the time of measurement, rather than an overall geographic pattern (Nagy 1994); this is one of the issues with interpreting FMR/FWTR data. Abensperg-Traun and De Boer (1992) indicated that in the wheat belt of W.A. short-beaked echidnas are much more active in March than the preceding summer months, as well as showing reduced activity in winter. Echidnas from different areas of Australia may have different seasonal responses, and the time at which echidnas in W.A. were measured may be a time of particularly high energy expenditure.

That the FMR of short-beaked echidnas falls within the prediction limits (90% of predicted) for a mammal of similar size (both before and after accounting for phylogenetic history; Figure 4.2), is particularly interesting as their BMR is considerably lower than expected. It is often thought that a low BMR translates into a low FMR, but the contribution of BMR to daily energy expenditure is quite variable, from 27% to 45%, so this is not always the case (Nagy 1987; Williams *et al.* 1997). For example, the marmot (*Marmota flaviventris*) has a relatively low BMR, but an expected FMR (Salsbury and Armitage 1994) as seen here for the short-beaked echidna. BMR comprises 25% of FMR for W.A. echidnas, and their BMR:FMR ratio of four is higher than the expected ratio of two- to three- times for most endothermic mammals (Degen and Kam 1995; Cooper *et al.* 2003), but agrees with the previous ratio for echidnas suggested by Green *et al.* (1992) of three- to five- times higher than previously published laboratory measured BMR (Schmidt-Nielsen *et al.* 1966; Augee 1969; Dawson *et al.* 1979; McNab 1984). However, the BMR measurements were for different sub-species, making direct comparisons difficult. My comparison of BMR and

FMR are from echidnas of the same sub-species and sourced from the same study site at a similar time of the year, allowing for more direct comparisons.

The higher than expected FMR:BMR ratio of short-beaked echidnas suggests that they have a high energy expenditure. It may be assumed that one of the most likely causes of this would be a high cost of locomotion. Echidnas have a primitive reptilian-like pectoral girdle and appendicular skeleton, with distally heavy limbs (specialised for digging) and a unique locomotory movement which involves rotation of the humeral rather than anteroposterior protraction (Edmeades and Baudinette 1975; Griffiths 1992). Despite this, Edmeades and Baudinette (1975) found that 'cost of transport' for short-beaked echidnas is similar to other mammals. Gambaryan and Kuznetsov (2013) also suggested that locomotion for long-beaked echidnas is perhaps not as primitive as first thought. The lack of any significant relationship between distance moved and FMR supports their conclusion that locomotion is not particularly 'expensive' for echidnas, so a high cost of locomotion probably does not account for the high BMR:FMR ratio. Further studies into the energetic cost of locomotion for echidnas would be beneficial, however, as Edmeades and Baudinette (1975) had a very small sample size and Gambaryan and Kuznetsov (2013) examined long-beaked echidnas. Rather than locomotion, a high energetic cost of burrowing and digging for food might be associated with a higher than expected FMR (Gibson and Hume 2000). Bilbies make feeding scrapes up to 20cm deep, and this energetically demanding activity (Morgan and Price 1989; Wallis *et al.* 1997) has been attributed as a cause for their higher than expected FMR (Gibson and Hume 2000). Echidnas spend a large amount of time digging and foraging for food (Griffiths 1992; Clemente *et al.* 2016) during which they move significant amounts of soil, so this may be a contributing factor to their high BMR:FMR ratio.

Nagy (1987) and Speakman (2000) suggested that there is no clear relationship between diet and FMR, as there is between diet and BMR, though other studies have found that some of the dietary patterns seen for BMR are also evident for FMR (Munn *et al.* 2012). It has also been suggested that food availability, rather than food composition, accounts for some of the variation in FMR. While termitivorous aardwolves and myrmecophagous silky anteaters have significantly lower (59% and 62% respectively) than expected FMRs (Williams *et al.* 1997; Nagy 1987), the termitivorous numbat has a lower FMR (76%) than predicted, though not

significantly different from that predicted for a similar size marsupial (Cooper *et al.* 2003). The myrmecophagous echidna follows more closely the pattern of numbats, and also have an FMR that is arithmetically lower than (90%), though not significantly different from, that predicted for a similar size mammal. All of these myrmecophages have other factors that reduce FMR, such as arid-zone distribution, phylogeny, and a reduced  $T_b$ , so it is unclear if these low FMRs are a result of diet or if the other factors have pre-adapted these species to a low energy niche.

#### *Field water turnover*

Total body water content of mammals is inversely related to body fat content, and is not correlated with hydration state, so it can be used as an indicator of body condition (Gibson and Hume 2000). TBW may vary seasonally, reflecting changes in environmental conditions and thus fat content, and as such has a large range (55-85%; Hulbert and Gordon 1972; Bradshaw *et al.* 1994; Nagy *et al.* 1991; Gibson and Hume 2000; Cooper *et al.* 2003). Tasmanian short-beaked echidnas have shown a substantial increase in TBW at the end of mating season; 69% before mating season, and 80% after (Nicol, *unpublished*). This shows how much seasonal variation there is in body condition throughout the year. TBW of W.A. short-beaked echidnas was  $72.1 \pm 4.1$  %; higher than previous TBW measurements for short-beaked echidnas from Kangaroo Island ( $57.5 \pm 7.7$  %; Green *et al.* 1992) and Tasmania ( $62.2 \pm 1.8$  %; Schmid *et al.* 2003). This may indicate that short-beaked echidnas in W.A. had a poorer body condition (lower fat content), possibly reflecting dietary differences and the lower productivity of a semi-arid habitat. Personal observations of short-beaked echidnas from Tasmania having better body condition than W.A. echidnas (Chapter 3) supports this idea. There are distinct dietary differences between echidnas living in the two locations, which may affect body condition. During summer, when this study was carried out, termites can comprise >90% of the diet of W.A. short-beaked echidnas (Abensperg-Traun and De Boer 1992), while the diet of short-beaked echidnas at the Tasmanian study site comprised of approximately 66% ants, and the remainder being larvae of cockchafer beetles and pasture grubs (Nicol and Morrow 2012). W.A. echidnas would also be expected to have the poorest

body condition in summer, when termites are least abundant in the soil (Abensperg-Traun and De Boer 1990). Captive short-beaked echidnas showed a seasonal variation in body mass, being the heaviest in autumn despite consistent food availability year round (Chapter 3). Further studies on seasonal variation of body condition of free-ranging short-beaked echidnas would be interesting to determine limitations on echidna energetics.

The mean FWTR of W.A. short-beaked echidnas ( $185.2 \pm 22.2$  ml day<sup>-1</sup>; Table 4.1), did not significantly differ from previous values for echidnas, ( $167.2 \pm 27.7$  ml day<sup>-1</sup>;  $F_{2,12} = 0.179$ ,  $P = 0.840$ ; Green *et al.* 1992 and  $172.7 \pm 15.8$  ml day<sup>-1</sup>;  $F_{2,12} = 0.179$ ,  $P = 0.683$ ; Schmid *et al.* 2003). Short-beaked echidnas (this study, Kangaroo Island and Tasmania) conformed closely to the allometric predictions for FWTR, and I find no evidence to suggest that short-beaked echidnas have a low FWTR (Figure 4.2). This is in contrast to Green *et al.* (1992) who concluded that the FWTR of Kangaroo Island echidnas was amongst the lowest reported for free-ranging mammals. It is unclear how Green *et al.* (1992) could interpret their measured FWTR as being so low, perhaps due to the minimal data available for comparison at the time, but this highlights the need to test differences from allometric predictions with a rigorous statistical approach (Cooper and Withers 2006).

The FWTR of another monotreme, the platypus, is approximately eight times higher than that of echidnas (Hulbert and Grant 1983), presumably reflecting their semi-aquatic lifestyle. There are no FWTR data available for the only other extant monotremes, the long-beaked echidna. The contrast in lifestyle between the two monotreme groups makes general comments about the physiology of this distinct order difficult, as the values seen here appear to be a response to lifestyle rather than a phylogenetically constructed feature of the group. This is, however, evidence that monotremes are capable of considerable adaptive variation in physiology.

#### *Dietary water and energy intake*

An animal is in water balance when water gain is equal to water loss. There are three avenues for water loss: urinary, faecal and evaporative. To compensate for water loss, animals gain water by drinking, as preformed water in food, and by metabolic water production (Schmidt-

Nielsen and Schmidt-Nielsen 1952). Echidnas and other myrmecophages may also ingest damp soil as a by-product of feeding. The WEI (FWTR/FMR) evaluates water turnover in comparison to energy turnover (Cooper and Withers 2004a). Different diets have characteristic WEIs, with values of 0.15-0.27 ml H<sub>2</sub>O kJ<sup>-1</sup> for fresh vegetation, 0.07-0.17 ml H<sub>2</sub>O kJ<sup>-1</sup> for animal materials, and 0.05 ml H<sub>2</sub>O kJ<sup>-1</sup> for seeds (Nagy and Peterson 1988), based on the energy, water and digestibility of these foods. A myrmecophagous diet has a higher WEI than other animal-based diets, as ants and termites have a low digestibility and energy content, and a moderate to high water content (Cooper and Withers 2004a). The WEI of W.A. short-beaked echidnas at Dryandra was 0.39 ml H<sub>2</sub>O kJ<sup>-1</sup>, which is higher than the 0.30 ml H<sub>2</sub>O kJ<sup>-1</sup> for the termitivorous numbat at the same study site, also measured in summer (Cooper and Withers 2004a). However, if we remove the echidna that appeared to be drinking from a dam in its home range, then WEI=0.31, and more closely resembles that of the numbat. As WEI is strongly influenced by diet, we would expect these two species to have a similar WEI. Compared to previous studies of short-beaked echidnas, the WEI of short-beaked echidnas in W.A. is higher than those on Kangaroo Island (0.19 ml H<sub>2</sub>O kJ<sup>-1</sup>; Green *et al.* 1992) and Tasmania (0.23 ml H<sub>2</sub>O kJ<sup>-1</sup>; Schmid *et al.* 2003). The WEI of W.A. short-beaked echidnas is also higher than aardwolves eating termites (Williams *et al.* 1997) and thorny devils eating ants (Withers and Bradshaw 1995). As the individual with free-water in their home range, that appeared to be drinking, was removed from the average, it is unlikely that this higher WEI is due to drinking. It may be because their diet had a higher water content or lower digestibility than those from other areas. The diet of short-beaked echidnas in Tasmania is quite different from those living in W.A., so both of these explanations are possible. Tasmanian short-beaked echidnas eat few termites, and most of their diet is comprised of ants and scarab larvae (Nicol and Morrow 2012). However, Green *et al.* (1992) assumed the same dietary composition as assumed here, so it is unclear why the WEI is different between these two sub-species. The WEI of short-beaked echidnas on Kangaroo Island more closely approximates aardwolves, feeding exclusively on termites, so it would be of interest to determine what these echidnas are feeding on. Further studies into dietary composition of short-beaked echidnas in different areas of Australia would be of benefit to obtain a clearer picture on variation between the sub-species.

On examining the composition of W.A. echidna FWTR, 22 ml H<sub>2</sub>O day<sup>-1</sup> was unaccounted for, assuming a mixed diet of 95% termites and 5% ants (Abensperg-Traun and De Boer 1992), and excluding the individual with access to a dam. This suggests that echidnas are obtaining water from sources other than their diet and metabolic water production (MWP), which is most likely to be from ingesting damp soil while feeding as there was no known free water available in any of the other home ranges. Numbats living in the same area also obtained water from an external source during the same time of year (Cooper *et al.* 2003), so it is likely that this is due to damp soil consumption as a result of diet/feeding mode.

### *Movement and activity*

On average, short-beaked echidnas spent 6:15 h day<sup>-1</sup> active during the summer study period, which was higher than that observed in a recent study on short-beaked echidna locomotion (Clemente *et al.* 2016), which found short-beaked echidnas to be active for 13.7% during summer compared to the 21.4% I observed here. This may be due to the use of accelerometers as well as GPS in the previous study, compared to only GPS in this study. My estimate of echidna activity duration was similar to that of the termitivorous numbat (5 h day<sup>-1</sup>; Cooper and Withers 2004b), while another, larger myrmecophage, the aardwolf, spends 8.77 h day<sup>-1</sup> active during summer (Williams *et al.* 1997). It is not surprising that aardwolves are active for the longest time of these myrmecophages, given the size difference (aardwolves are 7 – 10 kg while short-beaked echidnas are 3 – 4 kg and numbats are only 550g) and thus larger food requirement (222455 termites day<sup>-1</sup>; Williams *et al.* 1997). Numbats may be active for the same amount of time as echidnas, despite being considerably smaller and thus having a smaller food requirement, due to their inability to break and dig into termite mounds and feed on a more concentrated food source, as echidnas do. In addition to size, activity patterns may also be influenced by diet. Animals feeding on a high-energy diet, such as insects (except ants and termites which have a low energy density), probably have shorter activity periods than frugivores and granivores which eat foods containing a high proportion of indigestible material, as they require less foraging time to meet their daily energy requirements (Vieira and Baumgarten 1995). There were no

significant correlations when comparing FMR to active/inactive time. This is likely due to the large individual variation and small sample size, thus limited power.

The daily distance moved by mammals varies widely according to differences in lifestyle. Foraging mode and diet has a considerable influence on daily movement; carnivores search over a large area, herbivores move more slowly over a smaller area, and omnivores are intermediate (Kenagy and Hoyt 1989; Covell *et al.* 1996). Distances range from 0.44 km day<sup>-1</sup> for folivores (Nagy and Milton 1979), 0.79 km day<sup>-1</sup> for frugivores (Galdikas 1988), and 18.5 km day<sup>-1</sup> for carnivores (Covell *et al.* 1996). Echidnas travelled an average of 1.9 km day<sup>-1</sup>, ranging from 1.3 to 2.9 km day<sup>-1</sup>, which is intermediate as you would expect from a myrmecophagous mammal, as they do not have to cover large distances and hunt and chase prey as do most carnivores, but there is still a considerable amount of foraging required to locate ant and termite galleries. Distance travelled does however, vary greatly between seasons so this must be taken into consideration when analysing any movement data. FMR does not easily correlate with distance travelled as although animals may be large and thus have a higher FMR, they may be less active than smaller species due to diet and foraging strategy, and thus do not travel as far. Daily movement distance has been positively correlated with body size and diet (Covell *et al.* 1996), however correlations between daily movements and FMR or FWTR are scarce. One study that looked at this for kangaroos and sheep (Munn *et al.* 2013), also concluded that there were no significant correlations between FMR and activity for either of these two species.

### *Conclusion*

Short-beaked echidnas living in W.A. have an FMR and FWTR as expected for a mammal of similar size. This is particularly interesting as their BMR (and  $T_b$ ) is significantly lower than expected, which has been attributed to diet, lifestyle and phylogeny. This indicates that BMR only contributes a relatively small proportion of FMR, and other factors such as locomotory costs and foraging mode (digging) may have a considerable impact on this species, despite previous studies finding that locomotory costs are not high. Further studies into locomotory costs of short-beaked echidnas would be of great benefit and shed more light

on their energy budget and survival strategies in the wild. Echidnas in W.A. appeared to be drinking/uptaking water from another source other than food or MWP. Echidnas are well adapted to survive in hot, dry environments with an ability to gain a lot of water from their diet, so that they are obtaining additional water is of interest. Further studies examining FMR and FWTR of a larger number of echidnas throughout all seasons and from different geographic locations would be beneficial to clarify this behaviour.

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## Thermoregulation of free-ranging short-beaked echidnas in Western Australia

### Abstract

Free-living short-beaked echidnas (*Tachyglossus aculeatus acanthion*) in South-West Western Australia maintain a lower mean normothermic body temperature (28.7 °C) than those living in cooler areas of Australia (30-32 °C), which is most likely a result of differing degrees of heterothermia as an adaptation to an environment characterised by lower primary productivity. Despite differing mean body temperature, all sub-species are thermolabile and body temperature seemingly rises during activity and falls during inactivity. It was however, well-regulated and does not fluctuate uncontrollably with ambient temperature. Like short-beaked echidnas living in other areas of Australia, W.A. echidnas utilised hibernation and daily torpor as energy conservation strategies, despite previous research indicating that they do not hibernate. However, the duration and depth of heterothermic bouts were reduced compared to those observed for short-beaked echidnas in cooler areas. It would be of interest to further examine the hibernation strategies of short-beaked echidnas from different areas of Australia, and determine if echidnas living in the hotter and drier areas in northern W.A. also hibernate. Seasonally, mean body temperature is significantly higher in summer (30.9 °C) than in winter (27.4 °C), and daily heterothermy is utilised much more in winter (amplitude of 2.6°C) than in summer (amplitude of 1.2 °C). The thermoregulatory strategies of echidnas show a great deal of adaptation to conserve energy and support their low energy lifestyle, and also indicate that they are in fact effective thermoregulators who are well adapted to living in a wide range of habitats.

## Introduction

There has been much debate surrounding the nature of the observed thermolability of short-beaked echidnas (*Tachyglossus aculeatus*), with some studies claiming they tightly regulate body temperature ( $T_b$ ) at a specific temperature, albeit considerably lower than a 'typical' mammal, while others have found that  $T_b$  is quite labile, rising during activity and declining during inactivity (Brice 2009). There have been few studies on free-ranging echidnas that examine thermoregulatory strategies in the wild (there are several studies that look at reproductive strategies of free ranging echidnas, but not normothermic thermoregulation; Beard *et al.* 1992; Beard and Grigg 2000; Rismiller and McKelvey 2000; Nicol and Andersen 2006b), and while laboratory studies provide an excellent foundation for understanding the physiology of animals, in a standardised and comparable manner, their physiology under laboratory conditions may not be an accurate representation of what occurs in the wild. While there are distinct morphological differences between the sub-species of short-beaked echidnas, there is a lack of information on their physiology and in particular the thermoregulation of Western Australian (W.A.) echidnas. To determine if there are physiological and behavioural differences accompanying the morphological differences between the sub-species, here I examine short-beaked echidnas free-ranging in south-west W.A. and compare these to existing published data for short-beaked echidnas living in other areas of Australia (such as Queensland, the Australian Alps and Tasmania).

Despite occupying all habitats throughout Australia, including hot arid zones, some laboratory studies have concluded that echidnas do not have any physiological adaptations to survive ambient temperatures ( $T_a$ ) above 35 °C, and a  $T_b$  of 38 °C is fatal (Robinson 1954; Augee 1976). As such, it has been suggested that echidnas must rely on behavioural thermoregulation, becoming nocturnal during hot weather and sheltering in areas with cooler microclimates during the day (Brice *et al.* 2002b). Activity is restricted to times when  $T_a < 33^\circ\text{C}$  on Kangaroo Island (Augee *et al.* 1975), in the wheatbelt of W.A. (Abensperg-Traun and De Boer 1992), and in south-west Queensland (Brice *et al.* 2002a). While it is clear that behavioural thermoregulation is important for echidnas living in hot environments, their ability to tolerate high temperatures and survive in hot microclimates

has been previously underestimated, as they are now known to survive extended periods of time where temperatures in their shelters are in the high 30° (Brice *et al.* 2002a), exceeding the lethal  $T_a$  estimated from laboratory studies. However, measures of  $T_b$  did not accompany these measures of high  $T_a$  so it is still not clear exactly what mechanisms echidnas employ to survive these temperatures; although the laboratory studies I present in Chapter 2 suggested that echidnas can use evaporative heat loss when  $T_a$  exceeds  $T_b$ , with a similar efficiency to other mammals.

At low  $T_a$ , there is a considerable energetic cost of maintaining a high  $T_b$ , especially for smaller mammals (Stone and Purvis 1992; Geiser 2004b). One way this energetic cost may be reduced is by utilising daily torpor, where  $T_b$  during the rest-phase is reduced and regulated below normothermic  $T_b$  to reduce the energy used compared to maintaining a high  $T_b$  (Stone and Purvis 1992; McKechnie and Lovegrove 2002; Cooper *et al.* 2008; Geiser 2004b). Heterothermic strategies include daily torpor and hibernation, which are generally classified as two distinct states (Geiser and Ruf 1995; Geiser 2004b), characterised by a precisely controlled lowering of  $T_b$  and the ability to rewarm without access to external heat, unlike obligate hypothermia which is usually a result of an inability to thermoregulate (Barclay *et al.* 2001; McKechnie and Lovegrove 2002). By utilising torpor, daily energy expenditure can be reduced by up to 95% compared to active animals (Warnecke and Geiser 2009), which is important for species such as the echidna which feed on a very low energy myrmecophagous diet (Griffiths 1989).

Hibernation or multi-day torpor is utilised by many species and has been observed for marsupials, placentals and monotremes (Geiser 1994). Echidnas living in eastern Australia have been documented utilising deep hibernation, where  $T_b$  is reduced to below 10°C, and the duration is up to several months (Grigg *et al.* 1992; Nicol and Andersen 1996; Falkenstein *et al.* 1999; Beard and Grigg 2000). Echidnas show a 'classical' mammalian pattern of hibernation, with their long hibernation season interrupted by periodic arousals (Augee and Ealey 1968; Augee *et al.* 1970; Grigg *et al.* 1989; Nicol and Andersen 2002). Tasmanian echidnas enter hibernation around March and remain there until June (Nicol and Andersen 2002). Augee and Ealey (1968) noted that echidnas in Victoria also appeared hibernate, based on laboratory studies where they went torpid for prolonged

periods when exposed to cold temperatures and food was withheld. They then confirmed this by tracking an echidna in the wild, which remained underground for weeks and entered deep hibernation. Echidnas in the Australian Alps also hibernate during winter, dropping  $T_b$  to almost  $T_a$ . While they did not feed during their periodic arousals from hibernation, they did sometimes move to another location. Most echidnas in the Alps ended hibernation in July – August; the coldest time of the year, and mated shortly afterwards, similar to the timing of hibernation arousal and mating in Tasmanian echidnas (Grigg and Beard 2000). Abensperg-Traun and De Boer (1992) state that echidnas living in the wheatbelt of Western Australia do not hibernate based on their observations of animals facilitated by radio-tracking, so I will further investigate this using implanted temperature loggers, to determine if short-beaked echidnas living in south-west W.A. use hibernation as an energy conservation strategy, and if so, if the depth and duration vary compared to published data for echidnas from other geographic locations.

Previous studies have found that the rate of rewarming from torpor is similar for marsupials, placentals and monotremes, despite the apparent lack of brown fat in marsupials and monotremes (Geiser and Baudinette 1990; Stone and Purvis 1992). Many aspects of torpor are quite similar between these subclasses, and even birds, and the differences between daily torpor and hibernation in species within mammalian groups are often greater than the differences between the subclasses (Geiser 1994). For example, minimum  $T_b$  and MR during torpor are quite similar for eastern states short-beaked echidnas (Augee and Ealey 1968; Grigg *et al.* 1989; Nicol *et al.* 1992), pygmy possums (Geiser 1994), hedgehogs (Thati 1978; Fowler and Racey 1990), rodents (Heller and Hammel 1972; Florant and Heller 1977; Geiser *et al.* 1990) and even some insectivorous bats (Hock 1951; Geiser 1988). However, species within the same mammalian group may show more of a difference in these variables and it is therefore of interest to examine torpor and hibernation in W.A. short-beaked echidnas, to determine if this geographically distinct group varies from their eastern states counterparts.

As well as daily torpor and hibernation, basking is also an important energy-conservation strategy for many mammals in arid areas of Australia (Geiser 2004a). Rewarming from torpor is usually the most energetically expensive phase of torpor, and by basking to aid

in rewarming, energetic costs can be reduced by up to 85% (Geiser and Drury 2003). As echidnas live a low energy lifestyle and employ many energy conservation strategies, it is of interest to determine if they utilise basking to rewarm from torpor. This would be likely for echidnas living in south-west W.A. as there is often a considerable amount of sunshine during winter which would allow this, coupled with low overnight  $T_a$ . Cooling and rewarming rate of echidnas living in W.A. has not yet been measured, so it is also of interest to compare this to echidnas living in other areas of Australia, which have different metabolic capacities, insulation and conductance which may influence the rate at which they can cool and enter and also rewarm and arouse from hibernation.

Here I investigated  $T_b$  and thermoregulatory strategies of free-ranging short-beaked echidnas in south-west W.A. I determined if the thermolability observed in the laboratory translated to free-living echidnas from the same location and examined if patterns in  $T_b$  co-inside with circadian rhythm and activity or if they were more related to  $T_a$ . I explored seasonal changes in  $T_b$  regulation and determined if short-beaked echidnas in south-west W.A. do indeed use daily torpor and/or hibernation; these traits were compared to echidnas living in different geographic locations to determine if these are plastic over their distribution.

## **Materials and Methods**

### *Animal capture and data collection*

Six free-ranging short-beaked echidnas were captured by hand at Dryandra Woodland (31° 46' S 117° 1' E), 170km south-east of Perth, Western Australia, and were fitted with a radio transmitter (Holohil RI-2C, weighing <20g, and thus <1% body mass, Holohil Systems Ltd.), glued to the spines on their back, and with a small aluminium 'backpack' into which a global positioning system logger (CatTrack 1, weighing 22g) and aniButton ( $\pm 0.5^\circ\text{C}$ , iButton thermochron DS1922L, Maxim Integrated Products) were placed (wrapped in black heat shrink to measure an approximate of black bulb temperature) to continuously record location and external temperature (total weight of backpack and contents <1.5% body weight). This temperature data is 'back temperature' ( $T_{\text{back}}$ ) and is

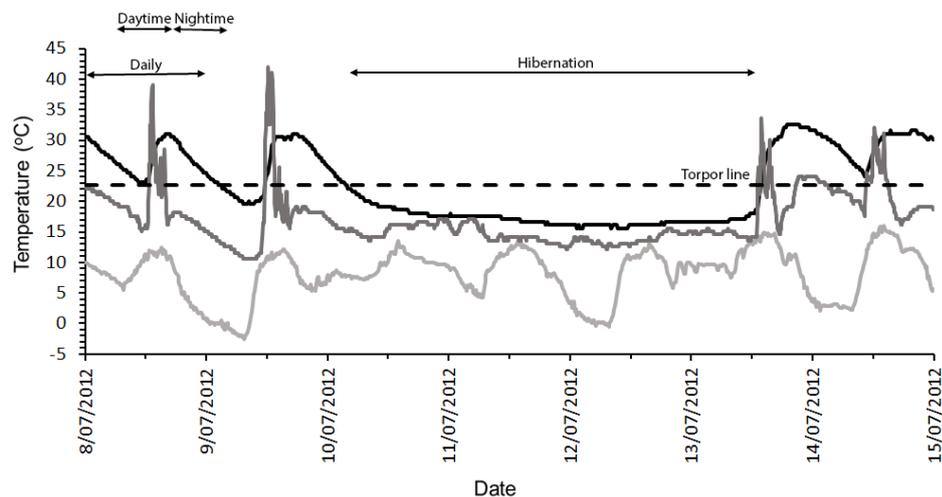
the temperature echidnas experience in their immediate microclimate.  $T_a$  was obtained from the Bureau of Meteorology (BOM) website from a local weather station.

When first captured, echidnas was transported to Curtin University to implant an internal temperature logger. Loggers were calibrated to the nearest 0.1 °C in a water bath with a thermometer traceable to a national standard (5 – 45 °C, 5 °C increments) and coated in biologically inert wax (Elvax, Du-point) before implantation. Surgeries were conducted under general oxygen/isoflurane anaesthesia (3-4 % induction, 2-3 % maintenance), using a custom made face mask and a Stinger portable anaesthetic machine. Once the echidnas were anaesthetised, a small incision was made in the ventral abdominal wall, lateral to the pouch, and an iButton was inserted into the interperitoneal cavity. The iButton was tethered to the abdominal tissue using silk suture (to enable removal at the end of the study) and then the wound was sutured closed using dissolvable suture on both the muscle and skin layer. Analgesia was administered via a subcutaneous dose (0.2 mg kg<sup>-1</sup>) of Meloxicam (Boehringer Ingelheim, Georgia, United States of America). Echidnas were held in an outdoor enclosure for one to two days to enable post-surgical monitoring before being released at point of capture.

The radio transmitters were used to locate the echidnas every fortnight; they were weighed (if they could be removed from their burrow/log) and transmitter attachment was checked. The echidnas were tracked for up to 12 months to obtain data for each season to test for seasonal effects. The internal iButtons recorded  $T_b$  every 20 min, and additional surgeries were carried out on each echidna every three months to retrieve the data and implant a new iButton. This timeframe was chosen to minimise the number of invasive surgeries required, while still allowing a relatively frequent sampling rate. Surgeries were carried out either at Curtin University or at the field laboratory on site. The external iButtons were changed and the data were downloaded every month using 1-wire iButton reader software (Maxim).

## Data analyses

Various  $T_b$ ,  $T_{back}$  and  $T_a$  variables (Figure 5.1) were determined using a custom-written Visual Basic (VB) programme (P. Withers). Such variables included daily  $T_b$  (internal temperature taken over 24 h, midnight to midnight),  $T_{back}$  (back temperature taken over 24 h, midnight to midnight) and  $T_a$  (ambient temperature taken over 24 h, midnight to midnight), daytime  $T_b$  (internal temperature taken from sunrise to sunset),  $T_{back}$  (back temperature taken from sunrise to sunset) and  $T_a$  (ambient temperature taken from sunrise to sunset), and nighttime  $T_b$  (internal temperature taken from sunset to sunrise),  $T_{back}$  (back temperature taken from sunset to sunrise) and  $T_a$  (ambient temperature taken from sunset to sunrise).



**Figure 5.1:** Temperature trace describing the measured variables (Daytime  $T_b$ ,  $T_{back}$  and  $T_a$ ; Nighttime  $T_b$ ,  $T_{back}$  and  $T_a$ ; Daily  $T_b$ ,  $T_{back}$  and  $T_a$ ), and showing a hibernation bout. The dotted line shows the cut-off with which torpor was defined. Black line is  $T_b$ , dark grey line is  $T_{back}$  and light grey line is  $T_a$ .

Linear mixed effect models (GLMM) were used to examine temperature and seasonal effects, while accounting for repeated measurements of individual echidnas precluding a traditional multivariate repeated measures approach (Withers and Cooper 2011). Not all individuals were monitored every season due to differences in initial capture time and the limited battery life of the radio transmitters. Lmer (Bates *et al.* 2014) and lmerTest (Kuznetsova *et al.* 2014) libraries were used in R (R Core Team 2014) for all of these temperature variables. I examined the physiological response to  $T_a$ , with season as a fixed factor and individual as a random factor. I also examined the physiology of hibernation

(duration, minimum  $T_b$ , entry rate and arousal rate), with  $T_a$  as a fixed factor and individual as a random factor.

To quantify daily heterothermy a cosinor analysis was used after Maloney *et al.* (2013), characterising the parameters of the rhythm (mesor, amplitude, acrophase and minimum and maximum temperature) of  $T_b$  for each animal in each season. The mesor and amplitude were compared between seasons to determine if the level of heterothermy varied between seasons. Linear mixed effects models were used to examine these variables, while taking into consideration any effects of individuals. I defined hibernation as bouts of torpor (drop in  $T_b$  more than 6 °C from normothermic values; mean normothermic  $T_b$  is  $28.7 \pm 0.08$  °C) lasting >24h, and daily heterothermy of bouts of torpor lasting <24h.

## Results

Of the six short-beaked echidnas monitored, two were male and four were female. The mean body mass was  $3.1 \pm 0.01$  kg, ranging from 2.4kg to 3.8kg. Mass significantly differed between seasons ( $N = 6$ ,  $n = 18$ ;  $F_{3,1086} = 211$ ,  $P < 0.001$ ), being highest in summer ( $3.5 \pm 0.01$  kg) and lowest in winter ( $2.8 \pm 0.01$  kg). There were significant mass differences between individuals ( $\chi^2_1 = 1411$ ,  $P < 0.001$ ).

### *Ambient temperature*

$T_a$  varied significantly between seasons ( $n=1094$ ;  $F_{3,1076}=283$ ,  $P<0.001$ ), and as such it would be expected that behaviour and  $T_b$  of short-beaked echidnas would also vary between seasons. Mean daily  $T_a$  (00:00 – 24:00) was highest during summer and lowest in winter. Comparatively,  $T_{back}$  had a much smaller variation than  $T_b$ , though mean daily  $T_{back}$  was also highest in summer and lowest in winter ( $N = 6$ ,  $n = 738$ ; Table 1). During summer, mean minimum  $T_a$  was  $13.8 \pm 0.33$  °C while mean minimum  $T_{back}$  was  $17.5 \pm 0.28$  °C, and in winter mean minimum  $T_a$  was  $3.3 \pm 0.20$  °C while mean minimum  $T_{back}$  was  $12.9 \pm 0.19$  °C. Absolute minimum  $T_a$  was lowest in autumn at  $-3.3$  °C, while absolute minimum  $T_{back}$  was lowest at  $5.1$  °C in both winter and spring. This demonstrates

that short-beaked echidnas were sheltering from the extremes in cold temperature. Mean maximum  $T_a$  was highest during summer, at  $31.2 \pm 0.39$  °C, while mean maximum  $T_{back}$  was  $36.4 \pm 0.71$  °C. Absolute maximum  $T_a$  was 41.2 °C in summer, with an absolute maximum  $T_{back}$  of 59.5 °C in summer. This indicates that echidnas were exposed to high temperatures and their immediate microclimate is often hotter than  $T_a$ , most likely due to the added heat load of solar radiation.

**Table 5.1:** Mean temperature variables ( $T_b$ ,  $T_{back}$  and  $T_a$ ) during each season for short-beaked echidnas from Dryandra Woodland, W.A.

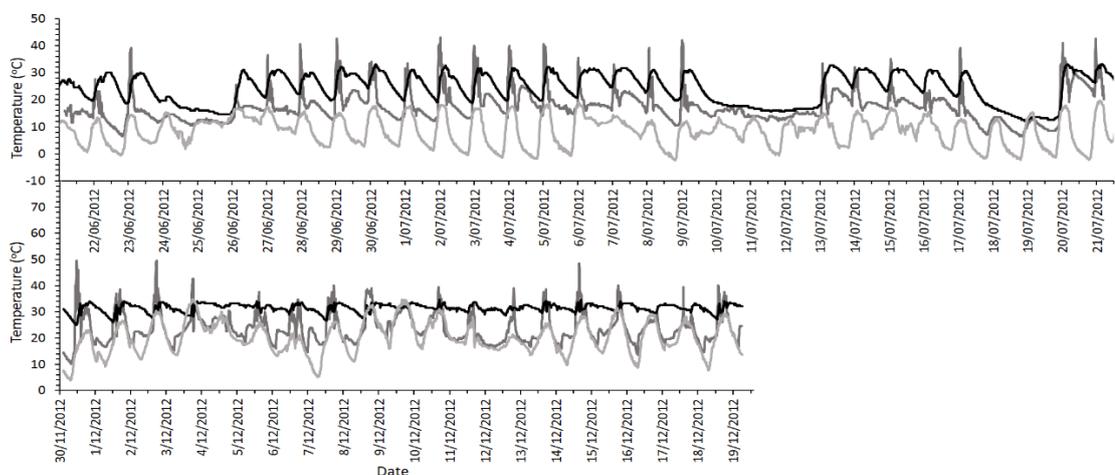
	summer	autumn	winter	spring
<b>Daily <math>T_a</math></b>	$22.7 \pm 0.31$ °C	$15 \pm <0.001$ °C	$9.6 \pm <0.001$ °C	$14.4 \pm 0.21$ °C
<b>Daily <math>T_{back}</math></b>	$24.6 \pm 0.24$ °C	$22.2 \pm 0.18$ °C	$18.1 \pm 0.21$ °C	$21.3 \pm 0.16$ °C
<b>Daily <math>T_b</math></b>	$31.1 \pm 0.08$ °C	$28.3 \pm 0.21$ °C	$24.7 \pm 0.36$ °C	$28.3 \pm 0.18$ °C
<b>Minimum <math>T_a</math></b>	$13.8 \pm 0.33$ °C	$8.6 \pm 0.42$ °C	$3.3 \pm 0.20$ °C	$6.5 \pm 0.20$ °C
<b>Minimum <math>T_{back}</math></b>	$17.5 \pm 0.28$ °C	$17.2 \pm 0.20$ °C	$12.9 \pm 0.19$ °C	$14.9 \pm 0.16$ °C
<b>Minimum <math>T_b</math></b>	$28.2 \pm 0.31$ °C	$25 \pm 0.26$ °C	$22.8 \pm 2.6$ °C	$23.9 \pm 0.22$ °C
<b>Maximum <math>T_a</math></b>	$31.2 \pm 0.39$ °C	$22.3 \pm 0.38$ °C	$16.4 \pm 0.12$ °C	$22 \pm 0.26$ °C
<b>Maximum <math>T_{back}</math></b>	$36.4 \pm 0.71$ °C	$33.4 \pm 0.75$ °C	$9.8 \pm 0.71$ °C	$35.2 \pm 0.46$ °C
<b>Maximum <math>T_b</math></b>	$33.3 \pm 0.08$ °C	$31.6 \pm 0.21$ °C	$28.4 \pm 0.08$ °C	$31.8 \pm 0.08$ °C

### *Field body temperature*

The mean daily  $T_b$  (0000 h – 2400 h) of short-beaked echidnas was  $28.7 \pm 0.08$  °C ( $n = 6$ ,  $N = 916$ ), and there was a significant difference between seasons ( $F_{3,901} = 40$ ,  $P < 0.001$ ; Table 5.1). Mean winter  $T_b = 24.7 \pm 0.36$  °C, summer  $T_b = 31.1 \pm 0.08$  °C, autumn  $T_b = 28.3 \pm 0.21$  °C and spring  $T_b = 28.3 \pm 0.18$  °C. Mean daytime  $T_b$  (sunrise to sunset) was  $28.2 \pm 0.09$  °C, and nighttime  $T_b$  was  $29.2 \pm 0.10$  °C. There was a significant difference between winter and summer for both daytime  $T_b$  ( $F_{3,379} = 21.94$ ,  $P < 0.001$ ) and nighttime  $T_b$  ( $F_{3,355} = 28.91$ ,  $P < 0.001$ ). Individual had a significant effect on all variables ( $\chi^2_1 > 17.1$ ,  $P < 0.001$ ).

### *Daily torpor*

Absolute minimum daily  $T_b$  (excluding multi-day torpor bouts) recorded for a short-beaked echidna was 11.1 °C, which occurred during spring at 09:34. The lowest  $T_b$  measured for a short-beaked echidna during summer was 20.1 °C and occurred at 11:09, compared with that measured during autumn (16.1 °C at 06:04) and winter (13.1 °C at 09:35). The highest daily  $T_b$  recorded occurred during summer and was 35.6 °C. This is only slightly higher than the maximum recorded during spring (35.1 °C), followed by autumn (34.1 °C) and winter (34.1 °C) which had the same maximum. The mesor



**Figure 5.2:** Temperature trace during winter (top) showing clear multi-day torpor bouts and daily torpor, and summer (bottom), showing considerably less variation in  $T_b$  and an absence of torpor, for one short-beaked echidna at Dryandra Woodland. Black line is  $T_b$ , dark grey line is  $T_{back}$  and light grey line is  $T_a$ .

differed significantly between seasons ( $F_{3,10} = 6.134$ ,  $P = 0.012$ ), with the lowest  $T_b$  occurring during winter (27.4 °C) and the highest minimum occurring during summer (30.9 °C). There was also a significant difference in amplitude between winter (2.6 °C) and summer (1.2 °C), indicating that daily torpor is utilised much more in the colder months than the warmer ones (Figure 5.2). In winter echidnas entered shallow torpor regularly (almost daily), though there is quite a lot of variation between individuals, whereas in summer they do not use daily torpor. In winter daily torpor lasted an average of 7h 23min  $\pm$  30min, and mean  $T_b$  during torpor was 21.2  $\pm$  0.04 °C.

### *Multi-day torpor*

Four short-beaked echidnas (females) entered multi-day torpor several times throughout winter as well as in early spring. One echidna underwent one short bout of multi-day torpor at the start of spring but not during winter (male), and the sixth one (male) did not enter multi-day torpor during the time I was tracking it but this animal was not monitored during winter. Mean multi-day torpor frequency was eight times throughout the year (winter and spring) for the females, and the one male that entered multi-day torpor only did so once. Mean duration between torpor bouts was  $6.9 \pm 1.06$  days, with the minimum time between bouts being one day and the maximum being 21 days. Mean minimum  $T_b$  for all short-beaked echidnas during multi-day torpor was  $13.7 \pm 0.19$  °C and the lowest (absolute) minimum  $T_b$  recorded for an echidna was 10.57 °C. Earliest entry into multi-day torpor was at 14:20 and latest was 08:15, with the majority of the bouts beginning between 19:00 and 02:30. Earliest arousal was 01:07 and latest was 17:03, with the majority of the bouts ending between 11:30 and 15:00.

Mean duration of the multi-day torpor bouts was  $4.5 \pm 0.2$  days, with the minimum duration being 1.6 days and the maximum being 10.7 days. There was a significant negative relationship between duration and minimum  $T_b$  ( $F_{1,146} = 65$ ,  $P < 0.001$ ). However, there was no significant relationship between duration and body mass ( $F_{1,11.1} = 0.036$ ,  $P = 0.85$ ). Season significantly influenced duration ( $F_{1,151.9} = 5.849$ ,  $P = 0.017$ ) and there was also a significant difference in minimum  $T_b$  between the seasons ( $F_{1,148.7} = 14.67$ ,  $P < 0.01$ ), with multi-day torpor bouts being longer and deeper in winter than spring. Neither torpor entry rate ( $-0.67 \pm 0.02$  °C h<sup>-1</sup>;  $F_{1,144.8} = 0.484$ ,  $P = 0.487$ ) nor arousal rate ( $3.17 \pm 0.23$  °C h<sup>-1</sup>;  $F_{1,145.7} = 0.048$ ,  $P = 0.826$ ) were influenced by season. Entry rate into multi-day torpor was not significantly influenced by  $T_a$  ( $F_{1,152.6} = 0.894$ ,  $P = 0.346$ ), body mass ( $F_{1,5.9} = 2.87$ ,  $P = 0.1417$ ), nor minimum  $T_b$  ( $F_{1,147.8} = 0.168$ ,  $P = 0.68$ ). Arousal rate was also not influenced by  $T_a$  ( $F_{1,152.4} = 0.615$ ,  $P = 0.424$ ),  $T_{back}$  ( $F_{1,89} = 0.886$ ,  $P = 0.3489$ ) nor body mass ( $F_{1,11.7} = 1.36$ ,  $P = 0.267$ ), however there was a significant positive relationship with minimum  $T_b$  ( $F_{1,154.6} = 4.47$ ,  $P = 0.036$ ).

## Discussion

The daily range in  $T_b$  measured here for free-ranging short-beaked echidnas in south-west W.A. demonstrated the extent of their heterothermy, and seasonal influences on  $T_b$ . I provide unequivocal evidence that echidnas in W.A. utilise multi-day torpor in winter, while also utilising daily torpor, reducing  $T_b$  during their rest phase to further conserve energy in the colder months. Here I demonstrated that short-beaked echidnas living in Western Australia showed a great deal of thermolability in the wild, as do the other subspecies, but they also appear to utilise multi-day torpor in a different manner to the other subspecies, and were physiologically well adapted to survive a wide range of temperatures throughout the year.

### *Normothermic physiology*

The mean daily  $T_b$  of free-ranging short-beaked echidnas in this study was 28.7 °C; considerably lower than most other mammals. Monotremes have the lowest  $T_b$ , followed by marsupials ( $T_b = 35.5$  °C), then placentals ( $T_b = 38$  °C), non-passerine birds ( $T_b = 39.5$  °C) and passerine birds ( $T_b = 40.5$  °C; Dawson and Hulbert 1970). This low  $T_b$  has been documented in numerous studies for short-beaked echidnas in an array of habitats, as well as for other monotremes (platypus and long-beaked echidna; De Miklouho Maclay 1883; Martin 1903; Wardlaw 1915; Schmidt-Nielsen *et al.* 1966; Beard *et al.* 1992; Beard and Grigg 2000; Brice 2009). The value reported in this study was slightly lower than previously measured for short-beaked echidnas living in other areas of Australia (30 °C – 32 °C), which may be due to geographic variation in physiology, and differing  $T_b$  set points as a result of differing local climatic conditions and differing degrees of heterothermia. For example, conditions are generally cooler and more mesic in Tasmania (with a mean maximum  $T_a$  of 17.7 °C and a minimum of 8.7 °C), than in semi-arid W.A. (which has a mean maximum  $T_a$  of 25.7 °C and a mean minimum of 13.3 °C; Australian Bureau of Meteorology 2016). Consequently, echidnas living in W.A. maintain a lower  $T_b$  than those living in cooler climates. This is consistent with my

findings in chapter 3, where in a laboratory environment short-beaked echidnas from W.A. had a lower  $T_b$  than those from Tasmania.

Presumably a lower  $T_b$  is maintained by W.A. short-beaked echidnas to not only conserve water and energy, but to also enhance heat storage capacity, which is of particular importance during activity at high  $T_a$ . By maintaining a lower  $T_b$ , the time before lethal  $T_b$  levels are reached during activity is increased, and is likely an adaptation that allows echidnas living in hot climates to extend their activity periods and thus increase foraging bouts. By maintaining a lower  $T_b$ , echidnas in W.A. also create a larger buffer zone and greater scope for adaptive hyperthermia (Cooper and Withers 2010). A low  $T_b$  is also potentially important to conserve energy in a semi-arid habitat with low primary productivity. Generally,  $Q_{10}$  is about 2.5 (Guppy and Withers 1999) so BMR increases 10 % for every 1 °C increase in  $T_b$ . Therefore by maintaining an approximately 2 °C lower  $T_b$ , echidnas in W.A. are saving approximately 20 % energy.

Robinson (1954) found that echidnas' ability to thermoregulate deteriorated at  $T_a > 35$  °C, and a  $T_b$  of 38 °C is fatal. Studies since then have also found that echidnas are unable to defend their  $T_b$  at high  $T_a$  and Augee (1976) observed that one died when exposed to  $T_a = 40$  °C. However, the echidnas ability to tolerate  $T_a$  in excess of 35 °C has been debated, and more recently Brice *et al.* (2002) found that temperatures of 40 °C regularly occur in hollow logs that echidnas use for shelter (amongst other types of shelters) during summer. The maximum environmental temperature recorded by an echidna's external iButton ( $T_{back}$ ) in this study was 59.52 °C, which is considerably higher than the reported lethal  $T_a$  of 40°. This value does include radiative heat (as the iButton is covered in black heat shrink and stuck to the echidnas back), and so approximates black bulb temperature and is a good indicator of environmental temperature experienced by the animals. Echidnas were regularly exposed to  $T_a > 35$ °C for up to an hour at a time, with the maximum recorded duration being 4h 45min.  $T_{back}$  also showed that echidnas regularly experienced a microclimate of  $> 35$  °C for up to an hour at a time, with the maximum recorded duration being 9h. While many previous studies (Martin 1903; Robinson 1954; Augee 1976) have concluded that echidnas have a poor tolerance for high  $T_a$ , and they do not pant, sweat or lick to cool themselves and often become hyperthermic, this finding

somewhat dispels that idea that echidnas are poor thermoregulators who rely solely on behaviour to regulate  $T_b$ , and cannot survive any substantial period of time at high  $T_a$ . However, my data demonstrated conclusively that they can clearly survive extended periods of time at very high  $T_a$ , while maintaining their  $T_b$  within a tolerable range. Maximum daily  $T_b$  recorded was 35.64 °C, which occurred during summer, while  $T_b$  regularly reached 34 °C throughout the season. The ability to survive extended periods of time at high  $T_a$  also suggests that echidnas do in fact physiologically control their  $T_b$ , and it is not just a reflection of environmental conditions. Interestingly, echidnas in Tasmania have a meanmaximum  $T_b$  of 35.3 °C (Nicol and Andersen 2006a), compared to a meanmaximum  $T_b$  of  $30.75 \pm 0.14$  °C for my echidnas, which is somewhat unexpected as ambient temperature is considerably milder in Tasmania. However, the basal normothermic  $T_b$  of echidnas in Tasmania is higher (Chapter 3), which is likely to affect the maximum  $T_b$ .

#### *Daily torpor*

Homeotherms maintain a high and stable  $T_b$  by means of endogenous heat production, which has a considerable energetic cost, especially at low  $T_a$ 's (Withers and Cooper 2010). A strategy used by many mammals and some birds to reduce this energetic cost is torpor. Torpor is often defined as a drop in  $T_b$  below some arbitrary value, most often <30°C (Geiser and Baudinette 1988; Nagel 1989; Schmid *et al.* 2000; Geiser and Brigham 2000; Barclay *et al.* 2001). However, this arbitrary value does not take into consideration the variation in normothermic  $T_b$  between taxa (Barclay *et al.* 2001; Withers and Cooper 2010). As such, many other definitions have been used over the years, including  $T_b$  at or slightly above  $T_a$  (Audet and Fenton 1988; Hickey 1993; Arlettaz *et al.* 2000),  $T_b$  < lowest normothermic  $T_b$  (Audet and Thomas 1997; Lovegrove and Raman 1998; Lovegrove *et al.* 1999a) and MR < 75% resting MR (RMR; Geiser 1988; Geiser and Baudinette 1988), to name a few. These definitions become problematic for species whose  $T_b$  is considerably different than a 'typical mammal' (such as monotremes), as well as those that regulate  $T_b$  below normothermic levels but significantly above  $T_a$  (Barclay *et al.* 2001). Therefore a

more appropriate definition is a drop in  $T_b$  more than 5°C below normothermic  $T_b$  (Hickey and Fenton 1996), as this takes into consideration. It is this definition that I adopt here, as the normothermic  $T_b$  of monotremes is almost below the common 'torpid' value (<30°C) used for many other mammal groups.

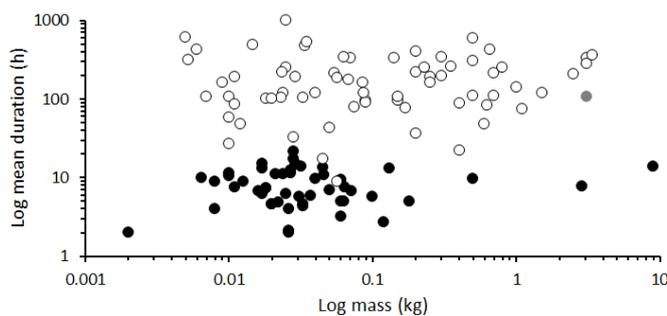
Torpor is generally divided into two categories; daily torpor which is shallow and lasts less than 24h, with minimum  $T_b$  of 10-25°C, and hibernation, which is categorised by  $T_b$  of <10°C and lasts several days to weeks (Geiser and Ruf 1995). The issue with these classifications is that there is often overlapping  $T_b$  between the two groups.  $T_b$  below 10°C have been reported in several species that use daily torpor (Carpenter 1974; Reinertsen 1983; Withers *et al.* 1990), while  $T_b$  of well above 10°C have been reported for some hibernators (Scholl 1974; Watts *et al.* 1981; Fowler and Racey 1988). The only variable that appears to have no overlap between the two groups is maximum duration, and as such is the best indicator of torpor strategy (Geiser and Ruf 1995). This provides some support to the view that hibernation and torpor are just a continuum of metabolic and thermoregulatory states, rather than two distinct states (Withers and Cooper 2010). The  $T_b$  of short-beaked echidnas from south west W.A. did not fall below 10°C at any time, however they utilised both daily and multi-day torpor.

Many mammals have strong nycthemeral  $T_b$  cycles and heterothermy, with a marked variation in  $T_b$  over a 24h period, reducing  $T_b$  during inactivity to reduce thermoregulatory costs (Bligh and Hawthorn 1965; Bartholomew and Rainy 1971; Fowler and Racey 1990; Cooper and Withers 2004; Seguy and Perret 2005). Some species further reduce energetic costs by reducing  $T_b$  even further and utilising daily heterothermy/torpor (Warnecke *et al.* 2007). Daily torpor has been well documented for echidnas living in different areas of Australia. Not only do they utilise hibernation during winter, but they also show marked variation in  $T_b$  throughout a 24h period. This variation in  $T_b$  is independent of  $T_a$ , rather it correlates to activity;  $T_b$  drops during inactive phases, and rises when active (Grigg *et al.* 1989, 1992a; Brice *et al.* 2002; Nicol *et al.* 2004). This diurnal variation in  $T_b$  has also been noted for short-beaked echidnas in captivity in Melbourne (Augee *et al.* 1970), where  $T_b$  dropped to a minimum of 28.7°C and did not rise until the animal became active, and maximum  $T_b$  (31.5°C) was recorded at the end

of each period of activity. Short-beaked echidnas free ranging in the Southern Alps during late summer had an active  $T_b$  of 31-33°C and a resting  $T_b$  of 27-29°C, depending on the length of inactivity and ambient temperature (Grigg *et al.* 1989). These values are higher than those of short-beaked echidnas living in Western Australia, where mean maximum (active)  $T_b$  was approximately 31°C and mean minimum (resting) was approximately 24°C. Short-beaked echidnas living in Queensland had an active  $T_b$  of 30-35°C and a resting  $T_b$  of 28-32°C (Brice *et al.* 2002). Hedgehogs (*Erinaceus europaeus*), another armoured insectivorous mammal, also show strong circadian cycles in  $T_b$  ranging from 31.4 – 37°C, with maximum  $T_b$  coinciding with activity (Fowler and Racey 1990). These daily torpor bouts during the echidnas' rest phase (in winter) have a large variation in start time (mean 11:16 ± 7h 45min), ranging from 12:07 to 11:22. Bouts lasted an average of 7h 23min ± 30min, and frequency had a large variation between individuals, ranging from daily to less than weekly occurrence. The duration of torpor for echidnas is similar to that of many Australian marsupials, which lasts up to 19.5h but is more commonly 2-8h (Geiser 1994).

### *Multi-day torpor*

Hibernation and torpor are used as an effective energy conservation strategy and a means to avoid extremes in environmental temperatures by many mammals spanning a wide range of environments (Geiser 2004b). Previous studies have noted that short-beaked echidnas do not hibernate in response to extreme cold or food shortages, and entry can



**Figure 5.3:** Mean duration (h) of torpor for 120 species of mammals (Data from Ruf and Geiser 2014). Black circles are daily torpor and white circles are hibernation. Short-beaked echidnas from this study are the grey circle.

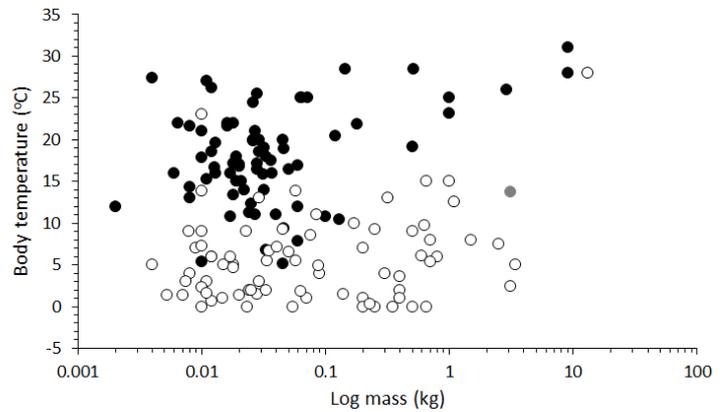
often be during the warmest part of the year (Nicol and Andersen 1996). In Tasmania, short-beaked echidnas enter hibernation during late summer and remain in a deep state of hibernation for several

months, with males arousing in early June and females arousing between July and September dependant on if they have young or not (Nicol and Andersen 2002). During winter echidnas living in W.A. utilise multi-day torpor, rather than true hibernation, as those living in cooler climates such as Tasmania. The duration of torpor/hibernation bouts generally shorten with increasing  $T_a$ , so it is not surprising that short-beaked echidnas living in W.A. have a shorter duration than those living in Tasmania. Most of the echidnas measured here entered multi-day torpor several times throughout winter (with first torpor bouts beginning in June), as well as at the start of spring, before  $T_a$  increased to a level that would make prolonged torpor unviable. Short-braked echidnas from northern New South Wales (NSW; Falkenstein *et al.* 1999), southern NSW (Beard *et al.* 1992) and southeast Queensland (Beard and Grigg 2000) display a similar pattern of hibernation to those in Tasmania, so it appears that the W.A. sub-species is somewhat unique in this aspect. Eastern states short-beaked echidnas seemed to show a more seasonal pattern of hibernation, where seasonal cues trigger entry into hibernation where they remain for several months. Comparatively, W.A. short-beaked echidnas appear to use torpor more opportunistically, without such strong seasonal cues, and perhaps more a reflection of environmental requirements. When rainfall in the area was compared to individual mutli-day torpor bouts, there did not appear to be a strong relationship between rainfall and use of torpor. When the  $T_b$  traces for all individuals monitored in this study were plotted on the same graph, the timing of daily torpor was very similar between individuals, however multi-day torpor was more variable in timing and duration. This suggests that they are responding more to immediate environmental conditions rather than seasonal cues.

Compared to other mammals, the duration of multi-day torpor for W.A. echidnas was shorter than other hibernating mammals of a similar size, and more comparable to daily heterotherms. This is where classification of, and differentiation between, daily heterothermy and hibernation becomes unclear, as the echidnas in this study use torpor that lasts several days (as may be expected for mammalian hibernation), as well as a daily  $T_b$  cycle indicating daily heterothermy, throughout a large portion of the year. Instinctively, these two distinct strategies may be categorised as daily torpor and

hibernation, however this depends on what definition is used. My data show no real physiological basis to discriminate between daily torpor and hibernation, so I refer to daily torpor as lasting less than 24 h, and multi-day or prolonged torpor as lasting more than 24 h.

Abensperg-Traun and De Boer (1992) stated that short-beaked echidnas in the wheatbelt of W.A. showed no evidence of hibernation, only short-term torpor as all study animals were active on most days throughout the year. However, continuous



**Figure 5.4:** Minimum  $T_b$  during heterothermia for 150 species of mammal (Data from Ruf and Geiser 2014). Black circles are daily torpor and white circles are hibernation; short-beaked echidnas from this study (N=6) indicated with a grey circle.

$T_b$  records demonstrate that while it may not be classified as true hibernation, and although not as regular and deep as hibernation for other Australian short-beaked echidnas, W.A. echidnas clearly utilise multi-day torpor, albeit for comparatively short periods. The lowest minimum  $T_b$  during multi-day torpor was 10.57°C, and the mean was 13.7°C, which is higher than those utilising hibernating on the New England Tablelands (5.9°C – 8.8°C; Falkenstein *et al.* 1999), and in Tasmania (4.7°C; Nicol and Andersen 2007), though similar to those hibernating in semi-arid southwestern Queensland (14°C; Brice *et al.* 2002). A minimum  $T_b$  of 3.7°C was recorded at Mount Kosciuszko, however this was taken using the pulse rate of a  $T_b$  transmitter, which may not be as accurate as implanted temperature loggers (Grigg *et al.* 1992a). That short-beaked echidnas living in the cooler climates of Tasmania and NSW reduce their  $T_b$  to a lower value than those living in W.A. and Queensland is not surprising given that they undergo a much longer torpor duration and duration is correlated with minimum  $T_b$  (Geiser 1994). However, Nicol and Andersen (2007) found that during the coldest month of the year short-beaked echidnas in Tasmania prefer to maintain  $T_b$  at 8-10°C, rather than allowing  $T_b$  to drop close to  $T_a$  as during other times of the year. This is closer to the

$T_b$  maintained during multi-day torpor for short-beaked echidnas in W.A. The minimum  $T_b$  seen here for echidnas living in W.A. is as expected for a hibernating mammal of similar size, and falls in the upper values of hibernating mammals (Figure 5.4; Ruf and Geiser 2014), which is as expected. The location of this study (and potentially a large portion of W.A.) may not drop to a low enough  $T_a$  for a long enough period to maintain a lower  $T_b$  and enter true hibernation, and this is most likely a major determinant for minimum  $T_b$  during hibernation, and why echidnas fall in the upper values of hibernating mammals. When taking into consideration the interbout arousals of hibernating echidnas in Tasmania (eight times in a four month hibernation bout; Nicol and Andersen 2007), the continuous bout length is still greater than that for W.A. echidnas, further supporting the notion that Tasmanian echidnas enter true hibernation, while W.A. echidnas are more so classified as multi-day torpor.

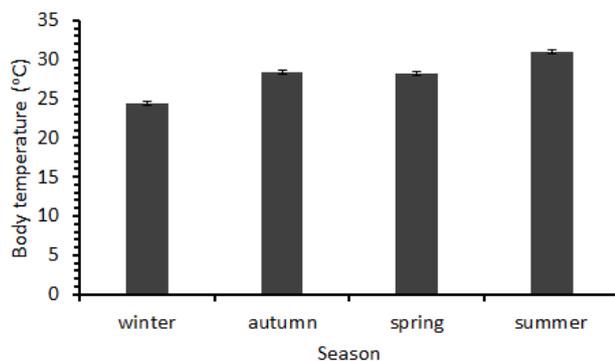
Echidnas in this study had a multi-day torpor cooling rate of  $-0.669^{\circ}\text{C h}^{-1}$ , compared to Tasmanian short-beaked echidnas which cooled at a much slower rate of  $-0.079^{\circ}\text{C h}^{-1}$ . From  $T_a$  alone it may be expected that echidnas living in a cooler climate such as Tasmania would enter hibernation at a faster rate than those living in a warmer climate as  $T_a$  is lower and thus there is a greater thermal gradient. However, this was not the case for echidnas. One possible explanation is differences in insulation between the two sub-species, however laboratory experiments revealed no difference in thermal conductance between these two sub-species at thermoneutrality and low  $T_a$  (see Chapter 3). Instead of the cooling rates reflecting insulation in the sub-species, it is perhaps a reflection of how well insulated the 'hibernaculum' used is. Tasmanian echidnas do not appear to dig or construct a hibernaculum as such, but bury themselves in loose sand or hibernate under sandstone or grass tussocks, or in decaying logs (Nicol and Andersen 2007). The principal means of heat loss is most likely conduction to the substrate, as they protect themselves reasonably well from convective and radiant heat exchange (Nicol and Andersen 2007). While there have been no studies on hibernacula of echidnas in W.A., Abensperg-Traun (1991) found that echidnas in the wheatbelt of W.A. always preferred subterranean shelters in heath. Personal observations of echidnas during this study showed use of a wide range of shelters throughout the year, such as under granite slabs, in decaying logs, under soil, in burrows

and in ironstone rock caves. However, I did not study which shelter types were used specifically for prolonged torpor so this is an area that warrants further investigation. Another factor that may influence cooling rate is depth and duration of torpor/hibernation. As W.A. short-beaked echidnas enter prolonged torpor for a much shorter period of time, and often at a higher  $T_b$ , than echidnas hibernating in Tasmania, it is important that they reach minimum  $T_b$  in a shorter time to maximise energy savings. Body mass is one of the major factors influencing rewarming rates, and echidnas lie at the extreme end of the hibernation weight range. Geiser and Baudinette (1990) derived a regression for predicting rewarming rates of hibernators, which predicts an animal of an echidna's size would rewarm at a rate of  $7.0^{\circ}\text{C h}^{-1}$ . However, echidnas warm at a much slower rate; mean rewarming rate of W.A. echidnas was  $2.7^{\circ}\text{C h}^{-1}$ , while Tasmanian echidnas rewarmed at a mean rate of  $1.9^{\circ}\text{C h}^{-1}$  (Nicol and Andersen 2006a). Similar to rewarming rate, cooling rate was also much faster for W.A. short-beaked echidnas than Tasmanian echidnas. Higher burrow temperature and higher initial  $T_b$  may contribute to the faster arousal rate for short-beaked echidnas in W.A. Within the W.A. sub-species there was a significant negative relationship between arousal rate and minimum  $T_b$ , so it is reasonable to assume that this relationship holds true between sub-species as well. Low  $T_a$  considerably slows the arousal rate from hibernation in marsupials (Geiser and Baudinette 1990), so this is most likely also influencing the difference seen here between the two sub-species. Despite Geiser and Baudinette (1990) finding a correlation between BMR and maximum heat production during rewarming from torpor (which is not surprising given they are both related to the thermogenic capacity of the tissues), short-beaked echidnas in Tasmania had a higher BMR, though a slower rate of re-warming than those in W.A.. This may be due to the use of passive re-warming by W.A. echidnas. As seen in Figure 5.2, the back temperature of echidnas shows a sharp spike simultaneous to re-warming from torpor, which indicates that they are utilising basking to re-warm to reduce the energetic cost of this energetically expensive phase of torpor. Previous studies have shown that radiant heat significantly reduces energy expenditure during arousal from torpor (Geiser and Drury 2003; Warnecke and Geiser 2009). A rise in  $T_a$  can reduce the energetic cost of rewarming from torpor to approximately 36% of that during active

rewarming at a constant  $T_a$ , though active rewarming was initiated once  $T_b$  reached about 25°C (Lovegrove *et al.* 1999b). In contrast, when basking is utilised arousal costs can be reduced to approximately 15% of that during active rewarming and during the whole arousal process active rewarming is not initiated when exposed to radiant heat (Geiser and Drury 2003). The energetic cost of rewarming actively is approximately 340% of BMR, whereas if passive rewarming is utilised the approximate energetic cost is only 70% of BMR (Geiser and Drury 2003). By utilising passive rewarming and basking during arousal from hibernation, echidnas save a considerable amount of energy and the overall savings from torpor and hibernation are greater, thus warranting the use of multi-day torpor by echidnas in W.A.

#### *Seasonal variation*

Seasonal variation in  $T_b$  for short-beaked echidnas was first suggested by Wardlaw (1915) who found  $T_b$  to be higher in spring than autumn (NSW), and later supported by Nicol *et al.* (2004) who found maximal  $T_b$  to occur in early summer (Tasmania). Short-beaked echidnas living in W.A. showed significant seasonal variation in mean  $T_b$  (Figure 5.5), displaying their thermolability and adaptability of thermoregulatory strategies. It appears that food availability between the seasons would not affect  $T_b$  as most studies report that echidnas do not suffer from food shortages, although termites are less abundant in summer (Abensperg-Traun 1991) so diet composition would most likely change during this time. The lower mean  $T_b$  during the day than during the night is presumably due to activity



**Figure 5.5:**  $T_b$  of six echidnas free-ranging at Dryandra Woodland, W.A. during each season.

patterns and echidnas being more active after sunset and before sunrise. This has previously been documented for free-living echidnas in both Tasmania (Nicol *et al.* 2004) and Queensland (Brice *et al.* 2002b); a labile  $T_b$  that rises as a

result of activity and declines during inactivity. It is only during incubation that echidnas tightly maintain  $T_b$  and display typical mammalian thermoregulation, presumably to enhance development of the embryo (Nicol *et al.* 2004; Brice 2009). Minimum  $T_b$  occurred at an average of 10:45 in summer and 23:37 in winter, which agrees with previous studies where echidnas are more nocturnal in summer and diurnal in winter (Brice *et al.* 2002b).

Other mammals also show seasonal changes in  $T_b$ , for example, hedgehogs (*Erinaceus europaeus*) have a  $T_b$  approximately 1°C higher in summer (July) compared to winter (September), as well as a strong circadian  $T_b$  cycle that correlates closely with photoperiod (Fowler and Racey 1990). These seasonal patterns of  $T_b$  are a result of food shortages and low temperatures during winter. While it appears that echidnas do not suffer from seasonal food shortages, the seasonal changes in  $T_b$  for echidnas is likely to be largely a reflection of changing  $T_a$  and environmental conditions. Djungarian hamsters (*Phodopus sungorus*) also have strong seasonal variation in physiology as well as morphology, with normothermic  $T_b$  being 1.2 – 1.4°C higher in summer than in winter, as well as the use of torpor in winter but not summer (Heldmaier and Steinlechner 1981). See chapter 3 for seasonal effects on physiology of captive echidnas.

W.A. short-beaked echidnas are far more heterothermic during winter than summer, not only in terms of use of prolonged torpor but also daily torpor. During winter echidnas allow their  $T_b$  to vary by an average of 2.6°C throughout their daily cycles of inactivity and activity, whereas in summer  $T_b$  only varies by 1.2°C. Daily torpor is more utilised in the colder months as more energy is required to maintain  $T_b$  when  $T_a$  is low and therefore it is of greater importance during the colder months. Additionally,  $T_a$  also has considerably more variation in winter than summer, so perhaps the variation in  $T_b$  is somewhat reflecting this variation in  $T_a$ . The variation in  $T_a$  during summer is however, much larger than the variation in  $T_b$  so the echidnas are still regulating to maintain a more stable  $T_b$ , which they do not do in winter. Presumably this is due to the energetic cost of maintaining a higher and more stable  $T_b$  when  $T_a$  is lower. This pattern of heterothermy appears to be in part an adaptation to their environment and low energy lifestyle rather than a characteristic monotreme trait, as there is no strong evidence to suggest that platypus'

hibernate or use torpor, and they maintain  $T_b$  close to 32°C without any significant fluctuations (Grant 1983; Grigg *et al.* 1992b).

### *Conclusions*

Free-living short-beaked echidnas in south-west W.A. have a low and labile  $T_b$ , like their counterparts in other areas of Australia. Their 'average' normothermic  $T_b$  is however, slightly lower than those living in the cooler and more mesic areas of Australia. This is presumably an adaptation to survive the higher ambient temperatures experienced in W.A., by increasing the 'buffer zone' between normothermic  $T_b$  and hyperthermia to reduce the risk of overheating. Echidnas living in W.A. also utilise daily torpor and multi-day torpor like the other sub-species, however they do not enter 'seasonal hibernation' like some other sub-species, presumably because of the warmer climate. In contrast to the subspecies living in the cooler parts of Australia, short-beaked echidnas in W.A. utilise basking to rewarm from torpor, further reducing energy expenditure. Short-beaked echidnas in south-west W.A. also show strong seasonal variation in thermoregulation. Mean  $T_b$  is considerably lower in winter than summer, and daily torpor is utilised to a greater extent. Combined with the use of multi-day torpor in winter, these thermoregulatory strategies all reduce energy expenditure during a time of year that would normally be energetically expensive for endotherms. All of these thermoregulatory adaptations indicate that short-beaked echidnas living in W.A. have a wide range of adaptations to reduce energy expenditure, in line with the low energy lifestyle of echidnas, and other mammals living in a semi-arid climate such as Western Australia.

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## General Discussion

The overall aim of my thesis was to obtain a comprehensive standardised physiological data set for the little studied (southwest) Western Australian (W.A.) short-beaked echidna (*Tachyglossus aculeatus acanthion*) to determine how plastic their physiology is, and to further investigate the question of ‘primitive or specialised’ in regards to the physiology of echidnas. I addressed a number of still-controversial questions concerning echidna physiology to gain a better understanding of their metabolic, thermal and hygric physiology in an ecological and phylogenetic context. There are five sub-species of short-beaked echidnas, categorised primarily by morphology as well as geographic location, so it was of interest to determine if these sub-species are physiologically distinct, which is a central theme to my study. Here I provide data for the most widely distributed sub-species and interpret it with respect to both previously published data for other echidna sub-species/populations and to data I collected for Tasmanian echidnas (*Tachyglossus aculeatus setosus*).

### *Metabolic physiology*

Echidnas have a low metabolic rate (MR; Dawson and Hulbert 1970; Augee 1976; Dawson *et al.* 1979; Frappell *et al.* 1994), which I confirm here for south-west W.A. echidnas (Chapter 2). This low energy physiology is seemingly more associated with lifestyle and diet than phylogeny (being monotremes). Long-beaked echidnas have a similarly low MR to short-beaked echidnas, however platypus have a considerably higher MR (similar to that of marsupials). The higher MR of platypus has been attributed to their semi-aquatic lifestyle, which is generally associated with a higher MR (for mammals) due to the thermally demanding environment (Dawson *et al.* 1979; Fish 1979; Dawson and Fanning 1981; Lavigne *et al.* 1986; McNab 2008), which may suggest that a low MR

is an adaptation to a low energy lifestyle rather than being a primitive monotreme feature. Both long- and short-beaked echidnas have low energy lifestyles; short-beaked echidnas are myrmecophagous whereas long-beaked echidnas are more generally insectivorous. A myrmecophagous diet is particularly energetically poor and has a low digestibility (McNab 1984; Redford and Dorea 1984; Cooper and Withers 2004; McNab 2008), which is linked to a low MR. It is difficult to differentiate whether a low MR is a primitive phylogenetic trait or a derived trait resulting from a myrmecophagous diet, or other factors such as armoured morphology, use of torpor, low  $T_b$  and semi-fossorial lifestyle. I suggest that it is perhaps a combination of all these factors, and that 'primitive' monotremes are predisposed to a low MR (and low  $T_b$ ) due to their early divergence; however adaptations to lifestyle mean the semi-aquatic platypus has an increased MR compared to echidnas.

Many mammals have a seasonal variation in MR, which is a reflection of food availability and climatic conditions altering thermoregulatory demand (McDevitt and Speakman 1996; Merritt *et al.* 2001). Generally, myrmecophagous mammals have a lower MR during summer, when termites are less abundant in the soil (Cooper and Withers 2004). Mammals such as the numbat rely on sub-surface soil galleries of termites for feeding as they are unable to extract termites from mounds where they shelter during high ambient temperatures ( $T_a$ ; Cooper and Withers 2004). In contrast, echidnas appear not to suffer any considerable food shortage as they have strong limbs that are able to dig into termite mounds to locate food year-round. It is perhaps for this reason that echidnas do not have any significant seasonal variation in basal metabolic rate (BMR; Chapter 3). In addition to not experiencing substantial seasonal food shortages, echidnas also use behavioural mechanisms throughout the year to conserve energy, and are quite plastic in this respect. While most mammals are strictly diurnal, nocturnal or crepuscular, echidnas are more opportunistic. They alter their activity patterns throughout the year as climatic conditions change, to avoid extremes in temperature. During winter they become more diurnal, while during summer they are more nocturnal; this means that they are reducing the thermal challenges of each season, thus minimising the effect on MR and avoiding seasonal changes.

While BMR did not vary seasonally, it is significantly different between sub-species from different geographic locations (W.A. and Tasmania as these are two of the most morphologically distinct sub-species; Chapter 3). Generally, endotherms that live in hotter, arid environments have a lower MR than those living in cooler, mesic environments (Lovegrove 2000; Mueller and Diamond 2001; Tracy and Walsberg 2001; Williams et al. 2001; Lovegrove 2003; Withers et al. 2006; Careau et al. 2007; Cooper and Withers 2010). Lower primary productivity and higher  $T_a$  contribute to the lower MR of animals inhabiting more arid regions, as by doing this they reduce the likelihood of heat stress (due to reduced endogenous heat production) and facilitate a lower  $T_b$ , which reduces EWL and conserves water in a dry environment (Mueller and Diamond 2001; Kvist and Lindstrom 2001; Lovegrove 2003; Careau *et al.* 2007). A higher MR associated with cooler regions is often a result of increased thermogenic capacity to compensate for heat loss, and allows a larger thermoneutral zone so they are able to tolerate lower temperatures (Careau *et al.* 2007).

This pattern of geographic variation in MR holds true for short-beaked echidnas, with W.A. echidnas having a significantly lower BMR than those from Tasmania (Chapter 3). W.A. has a large variation in climate and  $T_a$  is relatively high (Abensperg-Traun and De Boer 1992) compared to Tasmania, which has less variation and a lower mean  $T_a$  (Nicol and Morrow 2012). This allows Tasmanian echidnas to maintain a higher BMR than those in W.A., which allows them to better deal with the cold. This marked difference in BMR between echidnas from different geographic locations shows the species' plasticity, and their ability to adapt to local conditions. This plasticity has allowed them to not only survive, but thrive in a wide range of climatic conditions, and be one of the most widespread mammals in Australia, inhabiting almost every habitat type.

Despite having such a distinct difference in BMR, the field metabolic rate (FMR) of short-beaked echidnas from W.A. and Tasmania are not significantly different (nor is that of echidnas from Kangaroo Island; Chapter 4). There is a large amount of individual variation for FMR for most species, which makes it difficult to determine any minor differences between populations. Based on laboratory studies and the different habitats of echidna sub-species', I expected that the FMR of south-west W.A. echidnas would be

lower than that of Tasmanian echidnas. The lack of a difference may be due to the fact that FMR is measured for a short period of time and provides only a snapshot of free-living energetics, so it is possible that no differences were detected between the sub-species as the values obtained were a reflection of particular local environmental conditions at the time of measurement, rather than showing an overall geographic or annual pattern (Nagy 1994). While FMR is a good measure of field energetics as it takes into account daily pressures and environmental conditions faced by free-ranging mammals, it is difficult to use as a comparative measure between different populations due to the large number of uncontrollable variables.

### *Thermal physiology*

Several studies have documented the comparatively low body temperature ( $T_b$ ) of short-beaked echidnas at thermoneutrality, generally agreeing that  $T_b$  is approximately 30-31°C (Martin 1903; Robinson 1954; Schmidt-Nielsen *et al.* 1966; Augee 1976; Dawson *et al.* 1978; Frappell *et al.* 1994; Nicol and Andersen 2007). I confirmed this low  $T_b$ , for W.A. echidnas which had an even lower thermoneutral  $T_b$  of 29.5°C (Chapter 2), possibly reflecting stringent measurement conditions and/or between-population plasticity (see later).

At 29.5°C (short-beaked echidnas; this study), 31.7°C (long-beaked echidnas; Dawson *et al.* 1978) and 32°C (platypus; Grant and Dawson 1978), the thermoneutral  $T_b$ 's of monotremes are remarkably similar despite their long phylogenetic separation, and are considerably lower than that of other mammal lineages. The general consensus is that this low  $T_b$  is a primitive trait, shared by all species of monotreme. However, early studies concluded that echidnas' overall thermoregulatory strategy is quite primitive, as they have an inability to tightly regulate  $T_b$  and attempts at homeothermy largely fail, allowing  $T_b$  to increase and decrease with  $T_a$  (De Miklouho Maclay 1883; Martin 1903). While the low  $T_b$  of short-beaked echidnas might be a primitive feature, I argue that the thermoregulatory strategies of short-beaked echidnas are not primitive. While they are very thermolabile over a range of  $T_a$ , they are still able to tightly regulate  $T_b$  at any  $T_a$  in

that range, and their labile  $T_b$  is apparently not an inability to thermoregulate but a strategy to conserve energy at low  $T_a$  in accord with their low energy lifestyle.

Through laboratory and field studies I found that not only do short-beaked echidnas have a lower  $T_b$  than other mammals, but south-west W.A. echidnas have a lower  $T_b$  than those in Tasmania and the eastern states of Australia. On average,  $T_b$  of Tasmanian echidnas was 2°C higher than those from W.A. (Chapter 3). The lower  $T_b$  of W.A. echidnas allows for energy and water conservation, but also enhanced their heat storage capacity during activity when  $T_a$  is high. This further demonstrates the thermal plasticity of echidnas; that those inhabiting different areas of Australia and experiencing different climates, have adapted to have significantly different thermal physiology.

Echidnas from all areas of Australia show considerable thermolability, and a strong nycthemeral rhythm throughout the year. This is in contrast to the platypus, which has a low but quite constant  $T_b$  without the characteristic thermolability of short-beaked echidnas. This has lead researchers to conclude that platypus are more 'advanced' than echidnas (Martin 1903; Robinson 1954; Grant and Dawson 1978), but I interpret these differences as adaptations to environmental niches. Reducing  $T_b$  during inactivity significantly reduces thermoregulatory costs (Bligh and Hawthorn 1965; Bartholomew and Rainy 1971; Fowler and Racey 1990; Cooper and Withers 2004; Seguy and Perret 2005), which is important for a mammal such as an echidna as they lead a low energy lifestyle. Many aspects of echidna's lifestyle necessitate the frugal use of energy; they are armoured, myrmecophagous and semi-fossorial which are traits commonly associated with low energy lifestyles.

Previous studies on echidnas both in captivity and free-ranging have documented that  $T_b$  is allowed to rise with activity and fall with inactivity (Augee *et al.* 1970; Brice *et al.* 2002). Both sub-species studied here showed this variation in  $T_b$  for laboratory studies (Chapter 3), and my field studies of short-beaked echidnas in W.A. also showed  $T_b$  varied with activity (Chapter 5). The nycthemeral rhythm of  $T_b$  for echidnas is another adaptation to conserve energy, and occurs throughout the year though to differing degrees. During winter, echidnas have a considerable variation in  $T_b$  over a 24h cycle, with an average  $T_b$

fluctuation of 2.6°C, compared to summer where  $T_b$  only varies by a mean of 1.2°C over a 24h cycle (Chapter 5). More energy is required to maintain a high and stable  $T_b$  during the colder months when  $T_a$  is low, therefore daily torpor and multi-day torpor are utilised in winter to conserve energy during an energetically expensive time of year. The use of daily torpor by short-beaked echidnas appears to be an adaptive trait resulting from environmental pressures and a low energy lifestyle, rather than a characteristic monotreme trait and a reflection of their 'primitive' phylogenetic position. There is no evidence to suggest that platypus use torpor or have strong daily fluctuations in  $T_b$ , as they maintain their  $T_b$  close to 32°C (Grant 1983; Grigg *et al.* 1992b). Consequently, these aspects of physiology (torpor, nycthemeral  $T_b$  variability) may not be attributed to being a 'primitive' monotreme.

In addition to a thermolabile  $T_b$  and the use of daily torpor during winter, echidnas also utilise prolonged torpor and hibernation to avoid unfavourable weather conditions and conserve energy. Previous studies have shown that echidnas from eastern Australia and Tasmania have a typical mammalian pattern of hibernation, whereby they enter deep, prolonged torpor for several months, with periodic arousals to normothermia throughout the duration (Beard *et al.* 1992; Falkenstein *et al.* 1999; Beard and Grigg 2000; Nicol and Andersen 2002). While echidnas in south-west W.A. utilise multi-day torpor, they do not show a typical pattern of hibernation, and do not remain torpid as deeply or for as long as many of their eastern states counterparts (mean multi-day torpor  $T_b = 13.7^\circ\text{C}$  for W.A. echidnas,  $7.4^\circ\text{C}$  for New England Tableland echidnas and  $4.7^\circ\text{C}$  for Tasmanian echidnas; Falkenstein *et al.* 1999; Nicol and Andersen 2007). Tasmanian echidnas hibernate for several months, and there appears to be strong seasonal cues for the timing and duration of hibernation, with entry consistently occurring during late summer and arousal in June-September depending on breeding condition (Nicol and Andersen 2002). Comparatively, W.A. echidnas enter prolonged torpor for an average of only  $4.5 \pm 0.2$  days, and timing is more opportunistic dependent on local conditions (Chapter 5). Echidnas from other areas of Australia have a similar pattern of hibernation as Tasmanian echidnas (Beard *et al.* 1992; Falkenstein *et al.* 1999; Beard and Grigg 2000), and to date it appears that only echidnas from south-west W.A. differ in this aspect of physiology. However, it will be of

interest to determine if echidnas living in northern W.A. and hotter areas of Australia show a similar pattern of torpor to those in south-west W.A. and determine just how plastic this aspect of their physiology is.

### *Hygric physiology*

Many previous studies have reported that echidnas appear to lack sweat glands and the ability to make vasomotor adjustments, and do not enhance evaporative cooling at high  $T_a$ , further indicating that they have a 'primitive' physiology (Martin 1903; Schmidt-Nielsen *et al.* 1966; Augee 1976). It has been suggested that this primitive capacity for thermoregulation in the heat necessitates behavioural thermoregulation as the only mechanism to avoid overheating. While echidnas do behaviourally thermoregulate and avoid extremes in  $T_a$  by sheltering in burrows and caves, and becoming largely nocturnal during summer, I have demonstrated that they also have physiological mechanisms to survive hot environmental conditions, such as a labile  $T_b$  and enhanced evaporative heat loss (Chapter 2). Evaporative water loss (EWL) increases significantly at high  $T_a$ , just as it does for most mammals, so echidnas have an evaporative mechanism to dissipate excess heat. As well as evaporative cooling, echidnas also utilise postural adjustments to thermoregulate. At low  $T_a$  echidnas rest tightly curled to reduce heat loss, while at high  $T_a$  they sprawl out to increase the surface area from which heat can be lost. I also noted that at high  $T_a$  echidnas blow bubbles from their noses, which presumably provides evaporative cooling. At the highest laboratory  $T_a$ 's that I measured (30°C and 32.5°C), nasal mucous formed bubbles on expiration, which broke on their highly vascular snout (Chapter 2). This is presumably a cooling mechanism that functions in a similar manner to saliva spreading by rodents (Collins 1973), and kangaroos licking their vascular forearms (Dawson *et al.* 1974) to cool themselves while resting at high  $T_a$ .

Supporting the laboratory data, field studies of free-ranging echidnas (Brice *et al.* 2002; this study) show that they regularly tolerate high  $T_a$ , well above temperatures previously thought lethal (40°C). The maximum recorded environmental temperature (recorded from an iButton on an echidna's back) in this study was 59.53°C; considerably higher

than this supposedly lethal temperature (Chapter 5). Not only are they able to tolerate extremes in  $T_a$ , but they are regularly exposed to temperatures above 35°C for extended periods of time (up to 9h). This indicates that enhanced physiological heat loss is necessary to avoid lethal  $T_b$  at these high  $T_a$ 's, and suggests that echidnas do in fact have several mechanisms (both evaporative and behavioural), to cope with the heat and maintain their  $T_b$  within a tolerable range, and are perhaps less primitive in this aspect than previously thought.

Similar to FMR, field water turnover rate (FWTR) did not differ significantly between sub-species and was also not significantly different to that predicted for a mammal of similar size (Chapter 4). This is again most likely due to the measurement of this trait being over such a short period and perhaps reflecting responses to immediate environmental conditions rather than general characteristics of populations. Platypus have a FWTR approximately eight times higher than echidnas (Hulbert and Grant 1983), which reflects their semi-aquatic lifestyle and diet. This makes it difficult to comment on this characteristic in relation to phylogeny, however it appears to be more a response to lifestyle, and since it is not significantly different from most other mammals, it appears not to fall into the 'primitive' category of physiology and rather is an adaptation to lifestyle.

### *Conclusions*

It has long been thought that the short-beaked echidna (like other monotremes) is a very primitive mammal, as monotremes diverged long before modern-day therian mammals. While echidnas retain many primitive morphological features, not all aspects of their physiology are primitive. Their low and labile  $T_b$  is physiologically 'primitive', but is also a reflection of their low energy lifestyle (being myrmecophagous, armoured, semi-fossorial heterotherms). However, other aspects of their thermal, metabolic and hygric physiology indicate sophisticated, typically mammalian, regulatory systems. They are effective thermoregulators that have the ability to tightly control  $T_b$  when necessary, however they also utilise daily torpor and multi-day torpor to reduce thermoregulatory costs during

energetically expensive periods. Echidnas also show considerable physiological plasticity to accommodate varying daily, seasonal and geographical environmental demands. As a consequence of this plasticity, there are significant differences in the metabolic, hygric and thermal physiology of two of the most distinct sub-species of short-beaked echidna (*T.a. acanthion* and *T.a. setosus*).

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