Heat tolerance and cold indifference in the short-beaked echidna, *Tachyglossus aculeatus*:

body temperature management in a mammalian constitutional eurytherm

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Dedicated to the memory of
Jo Brice
(1931 – 2004)

Statement of Originality

I declare that the work presented in this thesis is, to the best of my knowledge and belief, original and my own work, except as acknowledged in the text, and that no material included in this thesis has been submitted, either in whole or in part, for a degree at this or any other university.

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Abstract

Short-beaked echidnas, *Tachyglossus aculeatus*, are heterothermic, prototherian mammals whose distribution extends across Australia and parts of New Guinea. They are reputed to be poor physiological thermoregulators in the heat as they lack the evaporative cooling mechanisms associated with other mammals and can tolerate ambient temperatures in excess of 35 ^{o}C to 37 ^{o}C for only short periods in laboratory experiments. However, in the field in western Queensland, they routinely tolerate ambient temperatures in some of their daytime retreats that exceed 35 ^{o}C for several hours and peak at 42 ^{o}C. In other, and colder, situations, except when incubating, body temperatures of echidnas vary on a daily basis, increasing with activity and decreasing with inactivity. They appear to be fully coordinated even with body temperatures as low as 20 ^{o}C. This suggests that the classical model of mammalian thermoregulation, assuming as it does, the maintenance of high and stable body temperatures, is of little relevance to non-incubating echidnas.

The primary aim of this project was to investigate physiological adjustments that allow echidnas to tolerate ambient temperatures in the field that would be lethally high in laboratories. The secondary aim of this project was to develop simple heat budget modelling that could be used to analyse physiological adjustments and responses in animals with significant thermal inertia, endothermy and heterothermy in complex, dynamically fluctuating (field) thermal environments. The third aim of the project was to investigate the relevance of traditional concepts about mammalian thermoregulation to echidnas within the context of their heterothermy.

The first aim was addressed by experiments conducted under field conditions where metabolic rate, evaporative heat loss, thermal conductance and body temperature were determined in response to ambient temperature fluctuations similar to those experienced by echidnas in the wild. It was found that echidnas in these conditions utilised the low ambient temperatures of the early morning to lose body heat passively to their environment which then gave them scope to absorb heat as ambient temperatures increased during the day. In this way, resting echidnas tolerated ambient temperatures that peaked in the range 30 ^{o}C to 49 ^{o}C as long as their body temperatures remained below 33 ^{o}C: slightly higher than the modal body temperatures of foraging echidnas. Because body temperatures rose only slowly in these

relatively large animals, echidnas were frequently unperturbed by experimental conditions until after ambient temperatures had reached their daytime peak.

The second aim of the project was addressed by developing integrated parameter, *a priori* heat budget modelling to predict body temperature responses of animals in general to combined variations of metabolic heat production, thermal conductance and other physiological factors in dynamically fluctuating (field) thermal environments. This modelling was found to predict well the body temperature fluctuations in a wide range of ectotherms and endotherms of various sizes in various environments. When applied to data collected from echidnas under field experimental conditions, this modelling highlighted the importance of maintaining low thermal conductance and low metabolic heat production in the ability of echidnas to tolerate high ambient temperatures. It also showed that there was no evidence of aestivation or metabolic suppression in echidnas exposed to a high heat load while sheltering inside artificial retreats.

The third aim of the project was addressed by experiments conducted under laboratory conditions where thermoregulatory responses of echidnas to constant ambient temperatures in the range 13 ^{o}C to 31 ^{o}C were determined. In these, similarities and differences between echidna and other mammalian thermoregulation were found. In common with many mammals, echidnas varied their thermal conductance from low levels in low ambient temperatures to higher levels in warmer conditions. Thermoneutrality was difficult to identify precisely but metabolic rate was minimal at ambient temperatures of between 25 ^{o}C and 28 ^{o}C and increased at higher temperatures. Below ambient temperatures of 25 ^{o}C, echidnas sometimes demonstrated shivering thermogenesis and sometimes torpor, both common responses in other mammals. However, unusually for an endotherm, echidnas also demonstrated varying metabolic rates intermediate to these two extremes. This resulted in variable or labile body temperatures with this variability increasing at lower ambient temperatures. Also unusual for an endotherm, echidnas continue to show apparently normal activity and behaviour with body temperatures as low as 20 ^{o}C without becoming torpid.

These results suggest that echidnas have alternative thermoregulatory strategies to many mammals. Thermogenesis and evaporative cooling are usually associated with mammals as physiological mechanisms resulting in the maintenance of high and stable body temperatures. However echidnas are not only capable of tolerating fluctuations in body temperature, they use them to advantage. On the one hand, they use cyclic storage of heat to tolerate short term exposure to high ambient temperatures in some day time retreats without the need for cooling mechanisms which require energy. On the other hand, in cooler conditions, they frequently dispense with energetically expensive shivering thermogenesis, allowing their body temperature to fall as a result, apparently without compromise to their foraging activities. This energy economy likely gives to echidnas advantages unavailable to many mammals and may allow them to persist in relatively resource poor environments.

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Abbreviation	Units	Parameter
a		algebraic parameter converting exponential increase in M into an S-shaped curve approaching an asymptote in modelling.
A_{ed}	m^2	emitting surface area of dorsal element of animal (Appendix 2)
BMR	W	basal metabolic rate: the metabolic rate of a resting, post-absorptive, unstressed endotherm in the TNZ.
C	J.^{o}C^{-1}	whole animal heat capacitance.
$CEWL$	g.min^{-1}	rate of cutaneous evaporative water loss by the animal.
CT		constant temperature as in CT room.
d	hr:min	time of day
dT_b/dt	^{o}C.min^{-1}	instantaneous rate of change of T_b used in modelling.
E	W	rate of evaporative heat loss by the animal.
E_{BMR}	W	evaporative cooling when $M = BMR$.
E_{eq}	W	evaporative heat loss of animal in equilibrium with its thermal environment.
$E_{eq.t}$	W	E_{eq} calculated according to conditions pertaining at time t.
E_t	W	E at time t.
EWL	g.min^{-1}	rate of evaporative water loss by the animal.
f		a proportionality factor to vary the ratio V_i to M as M varies.
$F_{E.H2O}$	prop	fractional concentration of H_2O in the excurrent air from a metabolic chamber.
$F_{E.O2}$	prop	fractional concentration of O_2 in the excurrent air from a metabolic chamber.
G	W.^{o}C^{-1}	conductive conductance
h_a	g.l^{-1}	absolute humidity of air (excurrent from a metabolic chamber).
H_d	W.^{o}C^{-1}	convective conductance.
h_{Ta}	prop	relative humidity of air at temperature T_a.
i	min	interval between concurrent calculations of equilibria in dynamic modelling.
K	W.^{o}C^{-1}	thermal conductance in a generalised sense.
K_{dry}	W.^{o}C^{-1}	dry thermal conductance of the animal.
k_n		algebraic constants used in modelling input where n = 0,1,2 ...
K_o	W.^{o}C^{-1}	overall thermal conductance of the animal in its environment: an integrated parameter accounting for radiative, conductive and convective conductances.
$K_{o.av}$	W.^{o}C^{-1}	mean overall thermal conductance.
$K_{o.eq}$	W.^{o}C^{-1}	overall thermal conductance of animal in equilibrium with its thermal environment.

Abbreviation	Units	Parameter
$K_{o.eq.t}$	W.^{o}C^{-1}	$K_{o.eq}$ calculated according to conditions pertaining at time t.
$K_{o.\max}$	W.^{o}C^{-1}	maximum value K_o may take for an animal in its thermal environment.
$K_{o.\min}$	W.^{o}C^{-1}	minimum value K_o may take for an animal in its thermal environment.
$K_{o.t}$	W.^{o}C^{-1}	K_o at time t.
K_{od}	W.^{o}C^{-1}	K_o for dorsal element of animal (Appendix 2)
K_{sfd}	W.^{o}C^{-1}	thermal conductance of shell (subcutaneous tissues and pelage) of dorsal element of animal (Appendix 2)
K_{wet}	W.^{o}C^{-1}	wet thermal conductance of the animal.
l_n		algebraic constants used in modelling input where n = 0,1,2 ...
M	W	metabolic rate or metabolic heat production of the animal in question.
m		algebraic constant used in modelling input.
M_{av}	W	mean metabolic rate (e.g. in the TNZ).
$M_{edge.l}$	W	minimum metabolic rate as a function of T_a determined by edge detection in Chapter 2.
$M_{edge.u}$	W	maximum metabolic rate as a function of T_a determined by edge detection in Chapter 2.
M_{eq}	W	metabolic rate of animal in equilibrium with its thermal environment.
$M_{eq.t}$	W	M_{eq} calculated according to conditions pertaining at time t.
$M_{\max}$	W	maximum metabolic rate (e.g. to imply an aerobic scope)
$M_{\min}$	W	minimum metabolic rate (e.g. in the TNZ).
M_{ref}	W	an arbitrary reference metabolic rate used in the modelling to predict the time course of T_b in animals with different *BMR*, *M*, metabolic depression etc. for different dynamic environments.
M_t	W	*M* at time *t*.
p		initial proportion of V_i to *M* defined for any *M* (and possibly varied by *f*).
Q_{10}		quantification of van't Hoff temperature effect of T_b on *M*.
Q_a	W	radiation absorbed by dorsal element of animal (Appendix 2)
r		rate of increase in *M* (e.g. in an arousal from torpor where *M* increases exponentially in modelling).
R_d	W.^{o}C^{-1}	radiative conductance
REWL	g.min^{-1}	rate of respiratory evaporative water loss by the animal.
RMR	W	metabolic rate of animal at rest (may include thermogenesis).
RQ		respiratory quotient: ratio of V_{O2} to V_{CO2}.
R_w	J.kg^{-1}.K^{-1}	ideal gas constant.
S	W	rate of heat exchanged with the heat stored in the tissues of the animal.

Abbreviation	Units	Parameter
SMR	W	standard metabolic rate of a resting, post-absorptive, unstressed animal (especially an ectotherm).
S_t	W	S at time t.
t	min	time of simulation from its start.
T_a	^oC	temperature of the air (stream) immediately surrounding the animal.
T_b	^oC	mean core body temperature of the animal in question.
$T_{b.eq}$	^oC	body temperature of animal in equilibrium with its thermal environment.
$T_{b.eq.t}$	^oC	$T_{b.eq}$ calculated according to conditions pertaining at time t.
$T_{b.lsp}$	^oC	lower set point T_b.
$T_{b.swap}$	^oC	T_b threshold at which some parameter (e.g. K_o) or algorithm changes from one value or function to another; sometimes similar to T_{set}.
$T_{b.t}$	^oC	T_b at time t.
$T_{b.usp}$	^oC	upper set point T_b.
T_{cp}	^oC	cheek pouch temperature.
T_{CT}	^oC	the temperature to which a CT room is set.
T_e	^oC	operative environmental temperature of the animal *sensu* Bakken *et al.* (1985): the temperature of an isothermal blackbody enclosure with the same convection (wind) conditions as the actual environment, which would result in the same net sensible heat flow to or from the same animal with the T_b; an integrated parameter accounting for radiation, conduction, convection and animal surface conditions.
$T_{e.amp}$	^oC	amplitude of T_e fluctuations in modelling.
$T_{e.av}$	^oC	mean T_e.
$T_{e.eq}$	^oC	operative temperature for animal in equilibrium with its thermal environment.
$T_{e.eq.t}$	^oC	T_{eq} at time t (= $T_{e.t}$).
$T_{e.exd.amp}$	^oC	T_e amplitude in “exposed” environment in modelling.
$T_{e.exd.\max}$	^oC	max T_e in “exposed” environment in modelling.
$T_{e.exd.\min}$	^oC	min T_e in “exposed” environment in modelling.
$T_{e.exd.t}$	^oC	T_e in “exposed” environment at time t.
$T_{e.\max}$	^oC	max T_e.
$T_{e.\min}$	^oC	min T_e.
$T_{e.shel.amp}$	^oC	T_e amplitude in “sheltered” environment in modelling.
$T_{e.shel.\max}$	^oC	max T_e in “sheltered” environment in modelling.
$T_{e.shel.\min}$	^oC	min T_e in “sheltered” environment in modelling.
$T_{e.shel.t}$	^oC	T_e in “sheltered” environment at time t.
$T_{e.t}$	^oC	T_e at time t.
T_{ed}	^oC	T_e for dorsal element of animal (Appendix 2)

Abbreviation	Units	Parameter
T_{es}	^{o}C	standard operative temperature of the animal in its environment *sensu* Bakken *et al.* (1985): the temperature of an isothermal black body enclosure with a fixed, standard convection condition which would allow the same net sensible heat flow to or from the same animal with the same T_b; differs from T_e when windspeed > 0.
TEWL	$g.min^{-1}$	total rate of evaporative water loss by animal (= *CEWR* + *REWL*).
$TEWL_t$	$g.min^{-1}$	*TEWL* at time *t*
T_{ex}	^{o}C	temperature of air expired by animal.
T_g	^{o}C	ground temperature
T_{lc}	^{o}C	lower critical temperature: a measure of T_a or T_e defining the lower limit of the TNZ.
t_{max}	min	length of simulation in modelling.
TNZ		thermal neutral zone: (conventionally) the range of T_a or T_e where *M* is basal in resting, post-absorptive, unstressed endotherms and (not so conventionally) where heat exchange is regulated via variations in *K*.
T_{rect}	^{o}C	rectal temperature.
T_{set}	^{o}C	set point or preferred T_b.
T_{uc}	^{o}C	upper critical temperature: a measure of T_a or T_e defining the upper limit of the TNZ.
T_{ue}	^{o}C	reduction in temperature of expired air due to nasal turbinate scrolls in the animal (= $T_b - T_{ex}$).
V_{CO2}	$ml.g^{-1}.hr^{-1}$	rate of carbon dioxide produced by the animal.
$V_{E(1)}$	$ml.min^{-1}$	flow rate of air excurrent from a metabolic chamber measured before being scrubbed of CO_2 and H_2O.
$V_{E(3)}$	$ml.min^{-1}$	flow rate of air excurrent from a metabolic chamber measured after being scrubbed of CO_2 and H_2O.
V_i	$ml.min^{-1}$	volume of air entering (and exiting) the respiratory tract of the animal per unit time.
$V_{i.t}$	$ml.min^{-1}$	V_i at time *t*.
V_{O2}	$ml.g^{-1}.hr^{-1}$	rate of oxygen consumption by the animal.
WVP	Pa	water vapour pressure in air.
τ	min	thermal time constant of animal: the time it takes for T_b to change by $(1 - 1/e)(T_{b.eq.t} - T_{b.t})$ or by 63% of the difference between current T_b and current $T_{b.eq}$.
χ_T	$g\ H_2O.ml^{-1}$	water vapour content of air saturated at temperature *T*.
$\delta T_b/\delta t$	$^{o}C.s^{-1}$	mean rate of change of T_b over a sample time.
ε		emissivity.
ϕ	^{o}C	a correction factor to convert T_a to T_e in the field metabolic chamber (only) as described in Chapter 4 and Appendix 2.
λ_{Tb}	$J.g^{-1}$	latent heat of evaporation at temperature T_b.
σ	$W.m^{-2}.K^{-4}$	Stefan-Boltzmann constant: 5.67×10^{-8}.

Chapter 1: General Introduction

Aims

This project was initiated primarily to investigate the heat tolerance of the short-beaked echidna, *Tachyglossus aculeatus*, and was prompted by an apparent paradox in its thermal biology. On the one hand, short-beaked echidnas lack the common mammalian cooling mechanisms of panting, sweating and saliva spreading (see review below). Therefore, they are often considered poor physiological thermoregulators in the heat and it is presumed that they rely on behavioural mechanisms, such as the seeking of cooler microenvironments, when temperatures are high. Consequently, it is assumed that the ambient temperatures of their daytime retreats never challenge their heat tolerance. On the other hand, recent data show that in the field, echidnas tolerate (without ill effect) temperatures in some of their daytime retreats that are both higher than those shown to be lethal in laboratory studies and higher than the body temperature of echidnas using those retreats. Therefore, this study was designed to determine the mechanisms whereby a short-beaked echidna might tolerate such heat loads in its daytime retreat without significant evaporative cooling.

A secondary aim developed from the need to analyse the heat budget of echidnas in hot retreats. Given their mammalian thermal conductance, their size, their thermal inertia, their endothermy, probable interactions between metabolic heat production and body temperature and the dynamics of their thermal environments in the field (again for all the details, see below), it was necessary to use heat budget modelling to quantify these various interactions, and to determine significance in physiological variations that might explain echidna thermoregulation in the heat. As no available modelling seemed to meet these needs without being overly complex, the secondary aim of this project was to develop relatively simple, yet versatile heat budget modelling *ab initio*. This modelling was developed *a priori* from simple principles of heat exchange. This enabled the modelling to be applied to a wide range of taxa in a wide range of thermal environments. Therefore, it was found to be useful for a variety of animals exhibiting a variety of physiologies which may be characterised by different levels of metabolic heat production, thermal conductance, and so on. Further, by using an integrated parameter approach (once again see below), this modelling retained conceptual simplicity, required relatively few parameter inputs and remained tractable and relatively easily compiled in a simple computer program.

Finally, in order to utilise the modelling for echidnas, various reference values for metabolic rate, variability of thermal conductance and other physiological parameters were required. Although studies defining these values were published almost four decades ago, they pre-date key insights into the thermal ecology of echidnas and conclusions based on assumptions now known to be false were drawn. These conclusions were inconsistent between studies and, in one case at least, were inconsistent with what is now known about echidna thermal biology. Therefore, a third aim of this project was to re-examine the thermal biology of echidnas (in laboratories) both to re-quantify basic physiological parameters (values for basal metabolic rate, thermal conductance etc. that might then be utilised in modelling) and to re-investigate conventional concepts of thermoregulation as they may or may not apply to what can now be regarded as a very unconventional mammal.

Design

The project was designed in three parts according to the stated aims. With respect to the first aim to investigate mechanisms of heat tolerance in echidnas in the field, experiments were designed and conducted in the open using equipment that allowed natural variations of temperature in daytime retreats in the wild to be mimicked. This equipment allowed direct determination of oxygen consumption, evaporative heat loss (if any), ambient and body temperatures (and therefore metabolic rate, thermal conductance and other physiological parameters) from echidnas habituated to life and handling in a large semi-natural enclosure situated at Pinjarra Hills on the western outskirts of Brisbane in south east Queensland.

With respect to the second aim, modelling was programmed in the QBASIC (Microsoft) language. This programming as well as novel aspects of the modelling were tested against data published for a range of endotherms and ectotherms in various situations. Because of the need to incorporate temperature fluctuations into the modelling, and because of their unusual thermal biology, some modelled situations could only be tested against data collected from echidnas experiencing controlled variations in ambient temperature. In these cases, suitable experiments were conducted on echidnas in controlled laboratory situations independently of the other experiments described above or below.

With respect to the third aim of the project, which was to investigate afresh aspects of the thermoregulation of echidnas, conventional thermoregulatory experiments were conducted on echidnas in controlled (stable) temperatures. In these, the responses of echidnas to various constant ambient temperatures were determined. These experimentally derived physiological parameters were then used to apply the modelling in analyses of data collected from heat tolerance trials.

Because data from the first set of experiments could only be analysed using modelling and because reference values of parameters were required for the modelling, the three components of the project are reported in reverse order. Chapter 2 reports the responses of echidnas to conventional thermoregulatory experiments. Chapter 3 reports the modelling. Chapter 4 reports the heat tolerance experiments conducted in the open.

Short-beaked Echidnas: a Background

As an “alternative mammal” the short-beaked echidna warrants special consideration. The short-beaked echidna is the only recognised species in the genus *Tachyglossus*, one of only three extant genera of the Prototheria. The other prototherian genera comprise *Zaglossus*, encompassing the long-beaked echidna(s) of New Guinea, and *Ornithorhynchus*, encompassing the platypus of eastern Australia. Once considered primitive, these highly specialised mammals are in fact intriguing combinations of plesiomorphies (e.g. elements in their pectoral girdles) and apomorphies (e.g. edentate jaws at least in adults). These combinations of ancestral and derived characters are explained by the ancient heritage of the Prototheria, arising as they probably did before the Cretaceous (Musser, 2003) and evolving separately from, but in sympatry with, the Theria; the marsupial Metatheria and the “placental” Eutheria. Of the extant Prototheria, the short-beaked echidna is the most widespread; the long-beaked echidna being restricted to New Guinea and the platypus being restricted to streams and rivers more or less east of the Great Divide in Australia. In contrast, the short-beaked echidna successfully reproduces in a wide variety of habitats from warm, moist rainforests to cold, seasonally snow bound mountains and to hot, dry deserts. Its range includes all of Australia, including the islands of Tasmania and Kangaroo Island and parts of New Guinea (Strahan, 1983).

Possibly related to its success in such varied environments is the distinct physiology of the short-beaked echidna (aside from the oviparity and low metabolic rate it shares with other Prototheria). It is a heterotherm, as mammalian heterotherms go, a fairly large one with adults ranging from about two and a half to five kilograms (Rismiller & McKelvey, 2000), sometimes as much as seven kilograms in cold environments (Grigg & Beard, pers. com.). Other aspects of its heterothermy seem unusual. Body temperature cycles of 5 ^{o}C or more occur over time frames ranging from seasonal (i.e. hibernation) to daily without any apparent distinction (Kuchel, 2003). Daily body temperature cycles (of as much as 5 ^{o}C to 10 ^{o}C) correlate with daily cycles of activity and rest. Body temperatures rise during, not necessarily before, bouts of foraging (Grigg*, et al.*, 1989; Grigg*, et al.*, 1992b; Brice*, et al.*, 2002b; Nicol*, et al.*, 2004) and short-beaked echidnas are known to be capable of coordinated activity with body temperatures as low as 20 ^{o}C (Brice*, et al.*, 2002b; Kuchel, 2003; Grigg*, et al.*, 2004). Thus, the lethargy associated with many (though probably not all: Geiser & Warnecke, pers. com.) daily heterothermic therians is not necessarily implied and short-beaked echidnas can be classified as constitutional eurytherms *sensu* Eisentraut (1960) as they have this ability to remain active over a wide range of body temperatures (Grigg*, et al.*, 2004). It is not certain that their constitutional eurythermy or their heterothermy is plesiomorphic given that (i) the platypus is homeothermic (Grant & Dawson, 1978; Grigg*, et al.*, 1992a), conforming much more closely to the classical concept of mammalian thermoregulation and (ii) Ornithorhynchidae apparently have a more ancient evolutionary heritage than Tachyglossidae (Musser, 2003). However, an animal combining mammalian insulation and thermogenesis with heterothermy and constitutional eurythermy may have represented a stage between ectothermic basal synapsids and endothermic mammals and the short-beaked echidna has been used as a model for the evolution of endothermy (Grigg*, et al.*, 2004). Whatever the case may be, their constitutional eurythermy implies for short-beaked echidnas a flexibility in physiology that may be unavailable to many therians.

Certainly, short-beaked echidnas are resilient to broad scale environmental changes. Unlike so many similar sized therians, short-beaked echidnas persist in the arid-zone of Australia (Strahan, 1983; Burbidge & McKenzie, 1989). The decline in range of their formally sympatric and similarly sized native Australian therians in the past 220 years has been attributed largely to anthropogenic ecological factors. These include the introduction of domestic and feral competitors and predators, unsustainable reduction in primary productivity (in the biological as well as the agricultural sense) and changes in fire patterns since European settlement (Burbidge & McKenzie, 1989; Morton, 1990). The persistence of short-beaked echidnas is usually attributed to their low energy requirements correlating with their low tachyglossid metabolic rate, their ubiquitous food supply of ants and termites and the formidable anti-predator defences of their ability to roll into a tight ball of long, stout spines or to dig rapidly and powerfully into the substrate (Friend, 1990; Abensperg-Traun, 1991b). Consideration of possible physiological factors in their persistence has not had so much attention. This project focuses on some of these physiological processes. In particular physiological responses to temperature fluctuation, especially to high temperatures are considered. Temperature fluctuation in terrestrial environments is marked, especially in the continental climates of much of Australia (Linacre & Hobbs, 1977), and poses challenges upon the survival of organisms. Animals may need to seek shelter from the sun, especially on hot days, and broad anthropogenic changes to the landscape may have interfered with the availability of suitable shelter for animals of some size classes. To explain the lack of niche for medium sized macropodoids away from rocky outcrops, Fox (1989) and Norbury *et al.* (1989) speculated on the thermoregulatory constraints of other habitats in the arid zone.

This project includes the first study to quantify aspects of short-beaked echidna physiology when thermoregulating in environments similar to those experienced in the wild. It shows how short-beaked echidnas (henceforth simply "echidnas") survive high ambient temperatures despite the widely held belief that they are physiologically incompetent in the heat (Chapter 4). This project also highlights flexibility in echidna physiological responses to low ambient temperatures and shows for the first time how optional conventional mammalian thermoregulation (thermogenesis and/or torpor) is for this unique mammal. This is in addition to a new and simple approach to heat budget modelling which proves to be versatile and widely applicable beyond the scope of just one mammal.

Review of Body Temperature and Heat Tolerance in Echidnas

De Miklouho Maclay (1883) measured the body temperature, T_b, of some echidnas he had been keeping for other experiments. Apparently expecting temperatures typical of other mammals (around 38 ^{o}C), he at first doubted his surprisingly low readings until he repeated them. Then, in case others should also doubt the readings, he had the Government Analytical Chemist in Brisbane repeat the measurements independently. Overall the average T_b of the echidnas was found to be 28 ^{o}C (de Miklouho Maclay, 1883).

By the early twentieth century, echidnas were considered to be "the lowest in the scale of warm-blooded animals" as their "attempts at homeothermism fail to the extent of 10 ^{o}C when the environment varies from 5 ^{o}C to 35 ^{o}C" (Martin, 1903). Martin compared the thermoregulation of Prototheria and Metatheria to that of a poikilothermic (to use the terminology of the time) lizard on the one hand and a homeothermic rabbit and cat on the other. He varied the air temperature, T_a, within a metabolic chamber over a range from about 4 ^{o}C to 40 ^{o}C and measured the animals' CO_2 production and T_b in response. The Metatheria and the Prototheria, particularly the echidna, exhibited T_b that fluctuated more than in the "homeotherms", but not as much as in the "poikilotherm".

Additionally, Martin found an echidna exposed to 37 ^{o}C for 71 minutes died and questioned their ability to tolerate heat. Along with many other investigators (see Griffiths, 1968), he was unable to find any sweat glands on the echidna. Nor, by submerging echidna feet in both cold (0 ^{o}C) and hot (40 ^{o}C) water and looking for colour changes in the skin, could he find any evidence of vasomotor control of the peripheral blood flow. As CO_2 production varied with different (particularly low) T_a, Martin (1903) concluded that in echidnas, temperature regulation must rely on regulation of heat production alone.

Wardlaw (1915) found that the T_b of echidnas kept in outside sheds varied on a daily basis. He measured their T_b twice a day between April and December and found mid-morning values to be typically around 30 ^{o}C and mid-afternoon values of about 33 ^{o}C over all the seasons. Although these fluctuations appeared to be strongly influenced by T_a, in that they varied (usually) in the same sense, there were enough discrepancies for Wardlaw (1915) to consider that other factors may also be influencing T_b. Wardlaw (1915) classified the echidna as a "true" homeotherm and queried the use of temperature and homeothermism as a basis for ranking vertebrates on an evolutionary scale. He pointed out that many of the "higher" mammals that hibernate show seasonal T_b variations far greater than those seen in echidnas.

Nevertheless, the view that echidnas were primitive with respect to their thermoregulation persisted for many years (Griffiths, 1968). For example, Robinson (1954) further investigated the heat tolerance of Australian Prototheria and Metatheria by subjecting them to various high temperatures and humidities, this time for seven hour periods. She noted that in the Prototheria, T_b responded rapidly, rising to 38 ^{o}C when T_a was set at 35 ^{o}C, though they responded less to varying humidities. There was no change in respiration rate with increased T_a (Robinson, 1954). She concluded (i) from this and from weight loss observations that echidnas in particular could not prevent overheating by using evaporative water loss, (ii) from Martin's crude experiments and from a lack of response of pulse rate to high T_a that monotremes show no vasomotor adjustments to regulate T_b, (iii) that Martin was correct in that only by varying metabolic heat production could echidnas hope to regulate their T_b and that (iv) therefore, echidnas were indeed the most primitive of the mammals with respect to their thermoregulation.

Schmidt-Nielsen *et al.* (1966) challenged the notion that echidnas were primitive pointing out that, at least in some respects, they are competent physiological thermoregulators. From their respirometry studies they showed that in addition to becoming hypothermic on occasion, echidnas were able to maintain stable, if low, T_b (~30 ^{o}C) in the face of 12 hours exposure to a T_a of 5 ^{o}C through an increase in their metabolic heat production. They dismissed the notion that low T_b equates to "low" on the evolutionary scale, discussing instead the significance of T_b as a result of heat gain balancing heat loss and pointed out that in echidnas both heat gain and heat loss were well regulated.

On the other hand, faced with high T_a echidnas became hyperthermic with a T_b of about 37 ^{o}C at a T_a of 34 ^{o}C (Schmidt-Nielsen*, et al.*, 1966). Schmidt-Nielsen *et al.* declined to subject their experimental echidnas to higher ambient temperatures, fearing their T_b would become fatally high. However, their investigations at T_a of up to 34 ^{o}C revealed that heat loss by evaporation could only dissipate about one third (and a maximum of one half) of the metabolic heat produced. The rest, at this apparent equilibrium (of $T_b = T_a + 3$ ^{o}C), must be dissipated by physical heat exchange processes enhanced by a thermal conductance observed to increase five-fold at T_a above 20 ^{o}C (the lower critical temperature: Schmidt-Nielsen*, et al.*, 1966) from 0.63 $J.cm^{-2}hr^{-1}$ to 2.9 $J.cm^{-2}hr^{-1}$ at T_a of 34 ^{o}C. Schmidt-Nielsen *et al.* (1966) considered that such a large variation in conductance was unlikely to be the result of posture variation and piloerection alone and that there must be significant vasomotor adjustments in echidnas in spite of the conclusions of earlier workers (e.g. Martin, 1903; Robinson, 1954).

Augee (1976) and Augee and Grant (1976) conducted further experiments examining directly for sweat secretions of echidnas subject to high T_a. By applying Wada (1950 cited in Augee, 1976) reagents to the ventral surface, underside of the tail, soles of the feet, the ankles and the snout, Augee (1976) was unable to find any evidence of sweat. Neither was he able to induce sweating by injecting adrenalin subcutaneously, nor, as with other workers, could he find sweat glands from histological examination of these parts of echidna skins (Augee, 1976). Concurrent measurement of respiration rate failed to show any evidence of panting and while respiratory minute volume increased in (only) some echidnas, this was not sufficient to allow much cooling by evaporative water loss. Neither was evaporative cooling enhanced by echidnas licking themselves as occurs in, for example, macropods (Brown, 1984; McCarron & Dawson, 1989; Dawson*, et al.*, 2000a). Again, when subjected to high T_a, T_b rose consistently to lethal levels of 38 ^{o}C. These investigators claimed they had confirmed by direct measurement that echidnas do not sweat, pant or lick (Augee, 1976; Augee & Grant, 1976) and that they do not respond to heat by increased peripheral circulation (Augee & Grant, 1976) though Augee (1976) discussed the possibility of peripheral vasoconstriction restricting heat gain and delaying the "inevitable" rise in T_b.

Whether echidnas were still considered primitive or not, they had acquired a reputation as poor thermoregulators in hot conditions.

> Thus, when exposed to high ambient temperatures echidnas can do very little about it, speaking in a physiological sense – they can't pant or sweat, heat production increases at high T_b, and any increase in conductance is, apparently, not effective in preventing a further rise in T_b.
>
> (Griffiths, 1978)

Given that their range extends across one of the hottest continents (Linacre & Hobbs, 1977; Gentilli, 1986), it is apparent that echidnas are potentially exposed to high ambient temperatures on a regular basis. It is frequently stated, therefore, that echidnas must rely on behavioural mechanisms to avoid the heat (Griffiths, 1968; Augee, 1978; Griffiths, 1978). Martin (1903) noted that echidnas burrow deeply and are nocturnal on hot days and Robinson

(1954) stated that they need to burrow to "survive the hot Australian summers". Griffiths (1968) related how echidnas in Western Australia used caves that are up to 12 °C cooler than outside to avoid the heat while Grigg *et al.* (1992b) reported seeing an echidna utilise shade and possibly even water bodies apparently to cool down. Echidna activity definitely appears to be determined more by ambient temperature than by photoperiod or time of day (Augee*, et al.*, 1970), being restricted to temperatures below about 33 °C in many parts of their range (Augee*, et al.*, 1975; Abensperg-Traun & De Boer, 1992; Brice*, et al.*, 2002b). While echidnas are diurnal when active in cold weather (Grigg*, et al.*, 1989; Grigg*, et al.*, 1992b), and either diurnal or nocturnal in milder weather (Augee*, et al.*, 1975; Abensperg-Traun & De Boer, 1992), they are solely nocturnal in hot weather (Abensperg-Traun & De Boer, 1992; Brice*, et al.*, 2002a; Brice*, et al.*, 2002b).

In hot weather, echidnas seek refuge during the day in one of various types of shelters. These include burrows, caves, logs, tree stumps and piles of leaves, sticks and rubbish (Griffiths, 1968; Smith*, et al.*, 1989; Abensperg-Traun, 1991a; Wilkinson*, et al.*, 1998). Although predator protection is sometimes cited (Wilkinson*, et al.*, 1998), it is usually implicit that these sites provide protection from extremes of temperature (Griffiths, 1968; Abensperg-Traun, 1991a; Wilkinson*, et al.*, 1998). However few studies have attempted to relate the thermal environments of these sites to the heat tolerance of echidnas. Abensperg-Traun (1991a) compared the T_a within woodland logs to those within heathland burrows in Western Australia finding that the logs were 7 °C warmer in summer and 5 °C cooler in winter than the burrows. Wilkinson *et al.* (1998), working in the tablelands of south east Queensland found that, in contrast to the stable T_a within burrows, T_a within logs used by echidnas in summer and winter varied as much as shaded T_a outside. They noted that it was surprising that echidnas used these logs given their reputation in the heat, postulating that echidnas may be losing heat to the substrate or that echidnas were not "such poor thermoregulators in the heat as is currently believed"(Wilkinson*, et al.*, 1998). It was not until recently that microenvironmental temperatures were related to echidnas' thermoregulation directly and to their T_b. Brice *et al.* (2002a) found that T_a within logs used by echidnas may exceed 35 °C for 10 hours and reach 42 °C during hot weather at Idalia National Park in western Queensland. In fact, as with the hollow logs in south east Queensland (Wilkinson*, et al.*, 1998), the inside of these logs were just as hot as the shaded air temperature outside measured in a nearby Stevenson Screen (Brice*, et al.*, 2002a). Further, they found that although echidnas choose other cooler sites (caves and burrows) when mornings were warm (> 22 °C), they often used these logs on other mornings. They then tolerated the ambient temperatures up to 42 ^{o}C before emerging for an activity period at around 10 pm. when outside T_a finally dropped below about 33 ^{o}C.

Thus, contrary to the laboratory studies of Martin (1903), Robinson (1954), Schmidt-Neilsen *et al.* (1966) and Augee (1976), it seems that echidnas are able to tolerate at least day long exposure to heat in field situations. Brice *et al.* (2002a) speculated that this is related to their low metabolic rate. Indeed, perhaps Martin, Robinson and others were correct in that echidna thermoregulation is achieved primarily by varying metabolic rate. Grigg *et al.* (1989), having demonstrated that echidnas do in fact hibernate in the field in cold conditions, postulated that echidnas may also have the ability to aestivate in hot conditions. Their ability to hibernate has now been demonstrated from many parts of their distribution (Green*, et al.*, 1992; Nicol & Andersen, 1996; Rismiller & McKelvey, 1996; Beard & Grigg, 2000; Grigg & Beard, 2000; 2000), including the arid zone (Grigg & Beard, pers. com.) and the semi-arid zone (unpub. data), where conditions can become remarkably cold as well as extremely hot (Linacre & Hobbs, 1977). While long term torpor during summer months has not been found in echidnas, they have been observed to miss occasional activity periods by "taking the night off" (Brice*, et al.*, 2002b). Missed activity periods have been observed (sometimes consecutively) in mild weather from various parts of the echidna's geographical range and during these "days off" T_b has been observed to decrease to levels often associated with torpor (Grigg*, et al.*, 1989; Abensperg-Traun & De Boer, 1992; Rismiller & McKelvey, 1996; Beard & Grigg, 2000; Grigg & Beard, 2000; Kuchel, 2003). Brice *et al.* (2002b) speculated that the missed activity periods seen in hot weather are also torpor events but that the temperature effects of reduced metabolism are masked by the high microenvironmental temperatures heating the echidna passively through conduction, convection and radiation. They further hypothesised that, therefore, echidnas may also enter torpor or, given the lack of lethargy (pers. obs.), become hypometabolic during their daily inactive period, thus reducing one source of heat in a hot environment (Brice*, et al.*, 2002b).

This hypothesis can be tested only by measuring the metabolic rate of echidnas subject to the temperature fluctuations they experience in summer in the wild. While heart rate correlates to oxygen consumption and metabolic rate for echidnas (Argall, 1987; Wohlsen, 1997; Kuchel, 2003) and other mammals (Morhardt & Morhardt, 1971; McNairn & Fairall, 1979; Boyd*, et al.*, 1995), heart rate transmitters proved problematic when implanted into echidnas in trials for this project. This, as well as the need to determine heart rate vs. oxygen consumption relationships for each individual (Argall, 1987) over a wide range of ambient temperatures, discouraged the use of heart rate telemetry in favour of direct measurements of oxygen consumption in field conditions. Direct measurement also allowed concurrent determination of other physiological parameters such as evaporative heat loss and thermal conductance.

The role of thermal conductance in echidnas' heat tolerance is far from clear. Endotherms generally enhance the loss of metabolic heat by increasing their thermal conductance when T_a approaches T_b (Kleiber, 1961; Withers, 1992a). Mammals can vary their thermal conductance by altering their posture, degree of piloerection and/or peripheral circulation (Fleming, 1985; Withers, 1992a). Echidnas are capable of varying their conductance presumably through vasomotor control (Schmidt-Nielsen*, et al.*, 1966), and are capable of quite spectacular piloerection (pers. obs.). Augee and Grant (1976) claimed that echidnas do not respond to heat by increasing their peripheral circulation, which is not surprising since where T_a exceeds T_b, as in their experiments, such vasodilation would only enhance exogenous heat gain, a fact recognised by Augee (1976). In hot conditions where T_a exceeds T_b and conduction, convection and radiation act to heat the surface of the echidna, restricting peripheral blood flow reduces heat transport to the core and thus delays T_b rising to lethal levels. Echidnas inactive in hot logs display slowly increasing T_b throughout the day so that on emergence, these T_b actually reduce with the onset of activity (Brice*, et al.*, 2002a). If the rates of change of T_b were any higher (i.e. if their conductance was higher, or to put it another way, their thermal inertia was any lower) T_b might reach lethal levels before the day was out.

Review of Heat Budget Modelling

Clearly, understanding the heat tolerance of echidnas requires an understanding of the actions and interactions of several factors contributing to the T_b of an echidna in any given situation. One approach to understanding the dynamics of body temperature is to use the heat budget approach (Porter & Gates, 1969; Gates, 1980; Monteith & Unsworth, 1990). The heat budget approach recognises that T_b is a manifestation of the heat energy stored in the body and that changes in T_b represent heat added to or taken from that store. Heat added to or lost from a body through conduction, convection, radiation, latent heat exchange and metabolic heat production may be calculated and combined in a quantitative or mathematical model of heat exchange to predict T_b. Mathematical models in thermal biology, of which there are many, can usually be classified along a number of conceptual dimensions according to several criteria. Models are usually (i) predominantly either *a posteriori* or *a priori* though they may be some combination of both, (ii) either differentiated parameter models or integrated parameter models, or again some combination. Then, many of these models focus either on the thermal biology of ectotherms or on that of endotherms: relatively few consider both together. Finally, thermal biology models can be divided into those that incorporate environmental fluctuations and those that do not. Because pre-existing, suitable modelling was not readily found (see below), *a priori*, integrated parameter modelling was developed to predict the T_b of various animals in thermally diverse or fluctuating environments. While the primary focus was for an echidna, a heterothermic endotherm, resting in a log, this modelling has utility for a wide range of endotherms and ectotherms in different states of metabolic heat production (resting, active, thermoregulating, torpid etc.) in a wide range of thermal environments.

A posteriori vs. A priori

Models in thermal biology, or indeed in biology generally, derived from patterns in observed data may be considered *a posteriori*. For example Almirall (1997) mathematically described diurnal fluctuations in T_b based on data from some 60 studies in the human and “animal” literature. She showed that a two-term function may have advantages over the intuitive sine wave function when simulating diurnal T_b variations. Clearly this *a posteriori* model is highly valuable when the pattern of T_b needs to be mimicked without regard to the processes that produce that pattern. Perhaps T_b fluctuation or T_b as a function of time of day is an input into a study of some other parameter. However, although Almirall relates one term to heat gain and one to heat loss, this relationship is clearly an *a posteriori* fit of some mathematics to the observed patterns in T_b. There was no attempt to derive the relationships from biological or other theory.

The importance of *a posteriori* models should be recognised. For example, it is often assumed that convective heat loss varies with the square root of wind speed (Campbell & Norman, 1998) but this relationship is derived from engineering principles involving non-biological surfaces (Bakken*, et al.*, 2002). For a study of the thermoregulation of downy shorebird chicks on the windswept Arctic tundra, such an assumption was checked and found to be violated (Bakken*,* *et al.*, 2002). The variation of convective heat loss with wind speed was replaced with an empirically derived relationship (Bakken*,* *et al.*, 2002). This *a posteriori* relationship or model for thermal conductance can then be used in other models, including *a priori* ones, to predict aspects of the chicks' thermoregulation and energetics. However, it cannot be used for animals other than downy chicks as the thermal conductance of contour feathers varies according to yet other (empirically derived) relationships (Bakken*, et al.*, 2002). This limitation is a serious disadvantage for *a posteriori* models. In general, models derived by finding best fit equations for observations in one system cannot, with confidence, be applied to any other system: they may have no underlying theoretical foundation.

A priori models, on the other hand, seek to derive relationships based on theoretical knowledge or understanding of the systems involved: on the processes that produce the patterns. *A priori* thermal biology models may be used in attempts to quantify the heat exchange between an animal (or plant) and its environment. The processes considered usually include heat produced to a greater or lesser extent by metabolism, some of which may be lost through evaporative cooling and heat exchanged with the environment through conduction, convection and radiation. These processes are fundamentally common over a wide range of animals: what differs between them is the degree to which they occur. That is a mouse and an elephant both produce metabolic heat and exchange heat with the environment in fundamentally similar ways, just with different quantities. The mouse and the elephant might be modelled with the same set of equations but with different values for the parameters. Consequently *a priori* models are more likely to have wider utility than *a posteriori* models.

In reality, the processes of conduction, convection and radiation considered may be complex and not always easily modelled *a priori* (Gates, 1980; Campbell & Norman, 1998). Anatomy, including of the periphery of animals, may render the modelling of detailed heat exchange processes intractable. Consequently, many apparently *a proiri* models in thermal biology rely on *a posteriori* generalisations embodied in parameters such as Reynolds and Nusselt numbers (Kreith, 1965; Campbell & Norman, 1998). Depending on their purpose, these combined models may benefit from simplification. In some cases, all sensible heat exchanges may be integrated into a single (conceptual) process, often dispensing with the need for Reynolds, Nusselt and similar *a posteriori* parameters (see below).

Differentiated *vs.* Integrated Parameter

To some extent all models are simplifications: a 1:1 model is unwieldy and too time consuming to develop. The extent of this simplification varies between models but in thermal biology a definite dichotomy can be discerned between differentiated or distributed parameter models and integrated or lumped parameter models. This is particularly so with respect to how heat exchange between the environment and the animal is modelled and how temperature in the system is quantified. In differentiated parameter models, all the processes of heat exchange, metabolic heat production, evaporative heat loss, conduction, convection and radiation are modelled individually as separate elements. Because radiative heat exchange between an animal and its environment is dependent on surface temperature not on air temperature, differentiated parameter models often include a term for the animal’s surface temperature. Because convective heat exchange is complicated in endotherms by the presence of fur, feathers or down, many differentiated parameter models require separate terms for the skin temperature and for the radiative (or outer) surface temperature. The animal itself is usually simulated as a series of concentric cylinders representing body core, skin and fur or feathers, if present, and exchanges via the various processes between the various elements are calculated (Bakken, 1976; Gates, 1980). The resulting model equations are solved perhaps for skin surface temperature or for body temperature.

A key development in thermal biology was the modelling of Porter and Gates (1969). By quantifying evaporative heat loss with respect to humidity gradients between the animal and the environment, by quantifying convective heat loss with respect to wind speed and by frequently considering conduction as negligible they developed “climate spaces” for a variety of ectothermic and endothermic animals.

> The climate space is the four dimensional space which the animal can endure, formed of radiation, air temperature, wind and humidity, each acting simultaneously. It is the combination of these variables which will make the energy budget of the animal compatible with its body temperature requirements. ... If one knows the body temperature and the extreme values it can have for survival, the metabolic rate, the water loss, the absorptivity, the diameter, and the thickness of fat

> and of fur or feathers, one can determine the simultaneous set of values of climate factors for survival of the animal.
>
> (Porter & Gates, 1969)

Most predominantly *a priori* models in thermal biology are differentiated parameter models (Table 1.1) and many originate in the work of Porter and Gates (1969) or the subsequent text by Gates (1980). As conduction cannot always be ignored – not all animals spend all their time on long legs with little or negligible contact with the ground – Spotila *at al.* (1972) included conduction calculated from literature values of conductivity of fat and its thickness, the surface area of the animal (including that in contact with the ground) and the temperature difference between ground and body in their model focusing on the temperature relations of alligators, *Alligator mississippiensis*. Trites (1990) calculated the climate spaces for seal pups, *Arctocephalus galapagoensis*, and related these to different environments. Porter *et al.* (1994) developed a model for endotherms generally in which they assumed a stable T_b and solved the equations for metabolic rate, i.e. how much energy is required to maintain T_b and McCafferty *et al.* (2001) predicted metabolic heat production for barn owls, *Tyto alba*, in different roosts in different weathers and seasons.

Numerous differentiated parameter models exist, having been developed for a wide range of applications including in the thermal biology of ladybirds (De Jong*, et al.*, 1996), moths (Kingsolver, 1983; Kingsolver & Watt, 1983), clusters of bees (Watmough & Camazine, 1995), frogs (Tracy, 1976), lizards (e.g. Porter*, et al.*, 1973; Florides*, et al.*, 1999), cattle (McArthur, 1987; 1990) and other livestock (Turnpenny*, et al.*, 2000a; Turnpenny*, et al.*, 2000b), marine mammals (Hokkanen, 1990; Watts*, et al.*, 1993), dinosaurs (Spotila*, et al.*, 1973), bats (Webb*, et al.*, 1993) and humans (Buse & Werner, 1985). O'Connor (1999) incorporated blood flow in a model for lizards exhibiting heating/cooling hysteresis. Kettlewell and Moran (1992) investigated the heat production of poultry while being transported. In 1988 Tikuisis and colleagues modelled the heat lost through convection by humans immersed in cold water (Tikuisis*, et al.*, 1988) and in 1989 by humans (in cold water) with clothes on (Tikuisis, 1989). Werner *et al.* (1989) modelled the distribution of temperature throughout the human body using 54 elements while Werner and Webb (1993) modelled the distribution of temperature at different exercise levels in humans simulated as six juxtapositioned cylinders; head, trunk, arms, hands, legs and feet. Xu and Werner (1997) added to human based models by including clothing for their virtual subjects. Still other

Table 1.1 Classification of selected *a priori* biophysical heat exchange models into (i) differentiated or integrated parameter and (ii) ectotherm or endotherm models. Endotherm models are further divided according to whether T_b is assumed unchanging (Steady State) and those where endotherms might be heterothermic (Dynamic). Those marked **δ** allow for variable thermal environment

	Differentiated Parameter	Integrated Parameter
Ectotherm	Porter, *et al.* (1973) **δ**[1] Grigg, *et al.*(1979) Kingsolver (1983)[2] Kingsolver & Watt (1983) **δ** Grant & Porter (1992) **δ** De Jong, *et al.*(1996) Lactin & Johnson (1997) Florides, *et al.*(1999) **δ** O'Connor (1999) **δ** Dzialowski & O'Connor (2001) **δ** [3]	Bakken (1976) Dreisig (1984) **δ** Dreisig (1985) **δ**
Endotherm Steady State only	Porter & Gates (1969) Spotila, *et al.* (1972) Thorkelson & Maxwell (1974) Buse & Werner (1985) Brown & Gillespie (1986) McArthur (1987) [4] Werner, *et al.* (1989) McArthur (1990) Pal & Pal (1990) Trites (1990) Kettlewell & Moran (1992) Watts, *et al.* (1993) Webb, *et al.* (1993) Porter, *et al.* (1994) McCafferty, *et al.* (2001) **δ**	Bakken (1976) Bakken (1981) Buttemer, *et al.*(1986) Weathers & Sullivan (1989) Cartar & Morrison (1997)
Endotherm Dynamic solutions	Spotila, *et al.* (1973) **δ** Tikuisis, *et al.* (1988) Tikuisis (1989) Hokkanen (1990) Jardine (1992) Werner & Webb (1993) Parsons (1995)[5] Watmough & Camazine (1995) **δ** Bolton, *et al.* (1996) Xu & Werner (1997) O'Connor & Dodson (1999) **δ** Turnpenny, *et al.* (2000a) **δ**	Prothero & Jürgens (1986) Tikuisis, *et al.* (1988) Visser & Ricklefs (1993) Eppley (1994) [6]

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[1] with potential for endotherms

[2] steady state only

[3] but recognises that metabolism might be important in determining T_{eq}

[4] allows for heterothermy above thermoneutrality

[5] more tractable than many

[6] close in some respects to that in Chapter 3

models focused on details such as the heat loss through the feet of chickens (Hillman & Scott, 1989) or through the ears of elephants (Phillips & Heath, 1992) including cartoon elephants (Phillips & Heath, 2001) or, more seriously, the local temperature effects of burns on skin and subcutaneous tissue (Pal & Pal, 1990; and for even more detail see Pal & Pal, 1993) or the distribution of heat loss throughout the tissues during cold water immersion (Tikuisis, 2003). Yet others assess the risk of hyperthermia for infants in cots (Jardine, 1992; Bolton*, et al.*, 1996).

Clearly there can be no doubt as to the utility of these models in clinical and other arenae. Differentiated parameter models are also useful when it is important to differentiate between the different channels of heat exchange say for heliotherms and thigmotherms. Lactin and Johnson (1997) investigated the effects of radiation for nymphal migratory grasshoppers. However, for many such models the list of parameters required is long and quantifying them all may be challenging. Knowing:-

> the body temperature and the extreme values it can have for survival, the metabolic rate, the water loss, the absorptivity, the diameter, and the thickness of fat and of fur or feathers
>
> (Porter & Gates, 1969)

may not be as easy as it sounds when comparing animals which vary in absorptivity, size and amount of fat deposits, especially if these animals are studied in the field. McArthur (1990) listed 24 parameters that were required for his model including mean coat depth and wind penetration depth. A thermal environment often needs to be quantified, usually in terms of temperature, humidity, wind speed and so on. Because micro-meteorological data are rarely available, Porter *et al*. (1973) incorporated a micro-meteorological model into their model for the thermal relations of a desert lizard, *Dipsosaurus dorsalis*. Adding values for some parameters, such as those specifying blood flow within individuals, may rely on empirical data determined for different individuals or perhaps even for different species. Although this model for the desert lizard is generally applicable in principle, it is lucky that enough was known about the biology of this particular species for parameter values to be obtainable. For example, Porter *et al.* (1973) cite Templeton (1970) for values for blood flow. They cite Templeton (1970) again for values of evaporative heat loss including the ratio of respiratory evaporative heat loss to total evaporative heat loss at different temperatures. They cite

Dawson and Bartholomew (1958) for metabolic heat production. The number of parameters involved in this model runs to 68 and includes projected lizard surface area for reflected solar radiation, soil temperature as a function of depth, air temperature as a function of height, skin thickness and at least two radiation shape factors (Porter*, et al.*, 1973). Such detailed information may just not be available for a wide variety of species. Additionally, convective heat exchange can be particularly complex and depends upon whether convection is free or forced. To calculate expected convective heat exchange often requires the calculation of various dimensionless numbers such as the Nusselt number or the Reynolds number which in turn are determined by such parameters as wind speed and characteristic dimension of the animal (Gates, 1980; Campbell & Norman, 1998).

Such complexity is not always necessary. In fact, such complexity may be a disadvantage, especially if the modelling is overly sensitive to one or more poorly constrained parameters. In such cases, predictions based on that model may be wildly inaccurate. If all that is required is the overall sensible heat exchange with the environment, then there is no need to differentiate sensible heat flow into elements of conduction, convection and radiation. In some models, the number of required parameters is reduced by lumping several together. McArthur (1990) integrated the thermal environment into a single index which is defined with respect to an isothermal room. This index is the indoor temperature equivalent to that experienced by cattle outdoors and is therefore the same as operative environmental temperature, T_e, *sensu* Bakken (1976) and Bakken *et al.* (1985). That is, T_e integrates the total thermal environment, including the effect of insolation and so on, as experienced by the animal into a single parameter. T_e may also be considered as the temperature an animal would achieve in its environment in the absence of any physiology or physiological thermoregulation. Because of this, T_e can be measured, for small animals (< 100 g) at least, simply by using physical models designed to mimic the animal's posture and surface thermal characteristics (reviewed by Bakken, 1992). Otherwise T_e may be calculated (Robinson*, et al.*, 1976; Campbell & Norman, 1998) including, from first principles (Bakken, 1976). O'Connor and Dodson (1999) calculated T_e in an otherwise differentiated parameter model.

Recognising problems in applying differentiated parameter models to field studies, Bakken (1976) proposed an approach where conductive, convective and radiative heat exchange are combined conceptually into a single channel. This channel is probably best described as dry environmental heat exchange to distinguish it from the latent heat of evaporative heat loss on the one hand and from the sensible heat produced, and possibly stored or released within the body tissues, on the other. Because conduction and convection are usually modelled as linear functions directly proportional to temperature gradients and because radiation can usually be modelled as such (Bakken, 1976), dry environmental heat exchange may also be modelled as a linear function directly proportional to the gradient between T_b and T_e. The proportionality coefficient describing the rate of dry environmental heat exchange down this gradient is defined as overall thermal conductance, K_o (Bakken, 1976). It integrates conductive, convective and radiative coefficients. As with T_e, K_o can be calculated (Bakken, 1976) or, in some cases, measured by physical models of animals (Bakken*, et al.*, 1983). In other cases and for a variety of technical reasons, determining K_o and T_e in complex environments can be challenging, especially for medium to large animals > 2000 g (Bakken, 1976; 1992; Wiersma & Piersma, 1994; Walsberg & Wolf, 1996b; a; Larochelle, 1998; Bakken*, et al.*, 1999; Bakken*, et al.*, 2000).

The real advantages of an integrated parameter model are two-fold. Firstly, unless the details of each avenue of heat loss via convection, conduction etc. are important, it is easier to conceptualise the animal environment system when it is simplified. This is achieved when the dry heat exchange is lumped into a single process, when the rate of exchange is quantified by a single conductance value and when the temperature is lumped into a single measure. The response of an animal to low temperature may be a focus irrespective of whether the animal is losing heat to the substrate (conduction) or to the air stream (convection), in which case there is no need to tangle with details such as tissue conductivity, fur length, characteristic dimension and shape factor. Similarly, a heterotherm's response to high temperature might involve thermal inertia, a time lag induced by the rate at which heat is absorbed by the tissues from the environment. It may be sufficient to characterise the environmental temperature as T_e and the rate at which at the body at T_b absorbs heat as a function of the gradient ($T_e – T_b$) and the overall thermal conductance, K_o. The *caveat* to this, of course, is the difficulty with which T_e and K_o may be quantified. Although T_e and K_o may be measured directly in smaller animals with physical models (Bakken, 1992) it is sometimes necessary to calculate T_e and K_o from first principles in medium and large animals. This is because an accurate measurement of T_e requires physical models with negligible thermal inertia and no internal thermal gradients – technically difficult when physical models are large (Bakken, 1992; Walsberg & Wolf, 1996a; Bakken*, et al.*, 1999). So in spite of the advantages of using an integrated parameter model, details of convection, conduction and radiation may still be required to calculate T_e. Nevertheless, with an integrated parameter approach, the parameters can be calculated separately from first principle knowledge of conduction etc. (Bakken, 1976; Campbell & Norman, 1998). In such situations, integrated parameter models still have advantages because the detail, the sensitivity to detail and the sensitivity to error or to precision of the detail or indeed to lack of knowledge or understanding of the detail (e.g. Kettlewell & Moran, 1992) can be resolved separately.

The second real (and practical) advantage of integrated parameter models lies in their relative tractability. By minimising the number of parameters involved in a model and, therefore, the number of functions and calculations, a computer program can be written more easily by a student with limited programming experience and without a sophisticated language compiler. The simpler relationships inherent in models using integrated parameters may be incorporated into simple programs with confidence and form the foundations for refinements until a surprisingly sophisticated program is developed (Chapter 3). Programming differentiated parameter models is far more daunting.

While explicit integrated parameter models are few by comparison to differentiated parameter models (Table 1.1 and below), there is a whole body of work in thermal biology based on (valid) assumptions where integration of parameters is implied. In any closed isothermal environment T_e equals T_a because there is zero radiation exchange between all the internal surfaces. By definition, they all have the same temperature. For any animal within and in equilibrium with that environment, its perception of that environment, T_e, equals T_a. These special cases are not particularly uncommon. Caves, burrows and similar retreats usually approximate isothermal environments (Vispo & Bakken, 1993; Cooper, 1999), as would many air conditioned offices. In laboratories, controlled temperature rooms and cabinets are designed to be isothermal and metabolic chambers are almost always assumed to be isothermal, again by design. Therefore, all the respirometry studies of animals, especially of endotherms, where metabolic rate is related to T_a within a metabolic chamber and where the results are discussed with respect to “Newton’s law of cooling” are in fact making use of an integrated parameter model (despite Strunk, 1971). Whenever a thermal conductance is determined or discussed in these numerous studies, whether it is a wet thermal conductance or a dry thermal conductance, this thermal conductance is an integrated parameter because it quantifies the rate of heat exchanged with the environment through a combination of conduction to the substrate, convection to the air stream, radiation to the chamber walls and, in the case of wet thermal conductance, evaporative heat loss.

Ectotherm *vs.* Endotherm

Although models may be generalised (see below), many investigators use them to consider either ectotherms or endotherms (Table 1.1); few consider them together. This dichotomy reflects the traditional views that (i) ectotherms are dependent on external temperature for variation in their T_b and that, when at rest at least, the contributions of metabolic heat production and evaporative heat loss are negligible (Bakken, 1976; Tracy, 1982; Dreisig, 1984) and (ii) endotherms have stable T_b, that in non-torpidators at least, vary only to a negligible degree in different T_e, maintained by high levels of metabolism and/or evaporative heat loss (Bakken, 1976; Wiersma*, et al.*, 1993; McCafferty*, et al.*, 2001). The presence of T_b fluctuation and the absence of metabolism in one group and the absence of T_b fluctuation and the presence of metabolism in the other tend to reinforce the treatment of the two groups as if they were mutually exclusive. However, the minimum of careful consideration is enough to realise that they are not and that the separate treatment is merely one of convenience.

For many small ectotherms, assuming that metabolic heat production and evaporative water loss is negligible may be valid. This allows the application of Newton's law of cooling to determine T_b variations over time without concern for the effect varying metabolic rate has on heating and cooling curves (Bakken, 1976), and is the basis for the determination of thermal time constants and heating/cooling hysteresis in a whole range of ectotherms (Grigg*, et al.*, 1979). Using Newton's Law, Dreisig (1984) modelled the thermoregulatory and foraging behaviour of tiger beetles, *Cicindela hybrida*, on the basis that equilibrium T_b (whether maximum or minimum) was equivalent to T_e. This is only true when T_b is determined solely by T_e and is unaffected by metabolic heat production or evaporative heat loss. Adequate for insects, this was also assumed to be adequate for small (~ 20 cm) lizards, *Lacerta agilis* (Dreisig, 1985). However, an integrated parameter model applied to data from a restrained, resting 480 g agamid lizard, *Pogona barbata* (Bartholomew & Tucker, 1963), showed that metabolic rate is likely to contribute 0.1 ^{o}C to equilibrium T_b in a T_a of 20 ^{o}C and 0.2 ^{o}C in a T_a of 40 ^{o}C (Chapter 3). Small as this increment is, there was enough of a discrepancy between predictions based on Newton's Law and the observed changes in T_b for

Bartholomew and Tucker (1963) to include a correction factor for metabolic rate. Metabolic rate was found to be a significant factor in the heating and cooling of varanid lizards of up to 4.4 kg (Bartholomew & Tucker, 1964). Although Seebacher and Grigg (1997) successfully considered the thermoregulation of crocodiles, *Crocodylus johnstoni*, without including metabolic rate, it is clear that there are circumstances in which metabolic rate in ectotherms should not be ignored.

Of course, it is well recognised that all endotherms, even small ones, have significant metabolic heat production. Buttemer *et al.* (1986) used doubly labelled water derived estimates of metabolic rate in a model that demonstrated the utility of using T_e rather than T_a in energy budget studies of budgerigars, *Melopsittacus undulatus*, in outside aviaries. In an in-depth study of the energetics of adult and juvenile yellow-eyed juncos, *Junco phaeonotus*, Weathers and Sullivan (1989) used an integrated parameter model to demonstrate how newly independent juveniles were more likely to be constrained by energy shortages than established adults. Cartar and Morrison (1997) calculated T_e in a similar way to that provided by Campbell and Norman (1998) in their model to estimate the daily metabolic rates of calidridine sandpipers across time and space in the Arctic. In these examples, T_b is more or less maintained and it is valid to treat T_b as constant. However, not all endotherms maintain or are able to maintain a stable T_b. Some, including echidnas, enter daily or seasonal torpor (Geiser & Ruf, 1995; Geiser, 2004). Prothero and Jürgens (1986) used a simplified model to demonstrate the energetics of daily torpor. They concluded that daily torpor is likely to be confined to endotherms of less than about 1 kg. A number of endotherms lack the ability to maintain a stable T_b early in life. Visser and Ricklefs' (1993) model explores the rate at which neonate shorebirds cool when exposed to low T_a. Although to some extent this is an *a posteriori* model, it does combine metabolic rate and evaporative heat loss with cooling curves in a study of birds that might be considered part way between being ectothermic and endothermic. Similarly, Eppley (1994) developed an *a priori*, integrated parameter model for endotherms that were as yet not fully developed. To do this she combined characteristics typical of ectotherm models, such as a storage term describing the rate at which heat is exchanged with the body, with characteristics typical of endotherms, such as the variable metabolic heat production. Because of the likely temperature effect, Eppley incorporated an equation where metabolic rate varied with T_b according to a Q_{10} of 2.5 and solved iteratively in a manner similar to the modelling in this project (Chapter 3). While Eppley's model has potential for generalisation it differs in several respects to that used in Chapter 3. It is only applied to neonates subject to cold environments and therefore only considers cooling. Because evaporative cooling is minor or negligible for most endotherms in cold environments Eppley's model does not incorporate evaporative heat loss. Of greater significance though is that her model assumes a constant environment: it is tested in a metabolic chamber where T_e is controlled.

Static T_e *vs.* Dynamic T_e

Commonly, plants and animals live in fluctuating environments characterised perhaps by diurnal (and seasonal) variations in T_e. Incorporating the dynamics of T_e into the modelling allows its application outside the simple environments of laboratories. Not all available models in thermal biology allow this. Of all the *a priori* models considered, none combine (i) the use of integrated parameters, (ii) the possibility of heterothermic endotherms, and (iii) the possibility of dynamic environments. In Table 1.1, only differentiated parameter models allow for both heterothermic endotherms and variable T_e. Spotila *et al.* (1973) used modelling to investigate the effect of size in dinosaurs on T_b. Given the effect of radiation and T_a (determined separately in a differentiated parameter model), they concluded that while large size, even with a small metabolic rate, would confer stable T_b over a few days, it would not confer the same T_b throughout all the seasons of the year. In their dinosaur model, O'Connor and Dodson (1999) calculated T_e separately on the basis of radiation and T_a regimes. However, this is considered a differentiated parameter model because it divides the animal into two elements, a homogenous core surrounded by a substantial shell, and considers the effect of blood flow on heat transfer between the two (O'Connor & Dodson, 1999). While internal temperature gradients are likely to be important components over large tissue thickness in these animals, they are assumed to be less significant in smaller ones. In, for example, echidna sized and smaller animals, by assuming an isothermal core T_b and a thin shell, the integrated parameter, K_o, incorporates all heat flow from the core to the environment including that from the core to the shell and from the shell to the environment. This retains tractability in the conceptualisation and programming (see above) forming a foundation for incorporating other factors considered more relevant to this study. On the other hand, O'Connor and Dodson (1999) did incorporate metabolic heat production which allowed them to consider different levels of both endothermy and activity. At the other size extreme Watmough and Camazine (1995) developed a sophisticated model to simulate bee behaviour in swarms. The model includes their small but significant (when combined over 30,000 individuals) metabolic rate affected by a temperature effect, and resulted in temperature profiles varying in response to a T_a fluctuating daily between −15 °C and +15 °C.

Suitable Model Availability

Modelling allows both the development of quantitative hypotheses and the analyses of quantitative data in a wide range of biological fields, including thermal biology. A tractable, flexible model would aid in the consideration of heat tolerance in echidnas as identified above. However none of the models considered combine all the features desirable for such a task without substantial modification. From well over 2000 publications in the biological literature that include models of any sort and thermal biology terms (such as “body temperature”, “metabolic rate”) more than 180 were selected as being potentially relevant. Of these, models that mathematically solved for T_b or some other thermal parameters on the basis of physiological (e.g. metabolic rate, evaporative heat loss etc.) and environmental (temperature, humidity etc.) inputs in whole animals were selected on the basis of availability (language and access). Of these, about three quarters were differentiated parameter models in that, where considered, conduction, convection and radiation were quantified separately. Only about one quarter of the selected models specified the integration of environmental temperatures as the single parameter T_e and were therefore categorised as integrated parameter models. None of these models combined all the features listed as desirable although some combined some of them. A model (Tikuisis*, et al.*, 1988) to predict hypothermia in humans allows for variation of T_b in an endotherm with significant metabolic heat and, in the sense that the temperature of the cold water is equivalent to T_e, this is partly an integrated parameter model. While it has features in common with that developed in Chapter 3, this is a specific model and differentiates the body into spheres and cylinders, so in this sense it is a differentiated parameter model. Eppley’s (1994) is a dynamic model for use in a steady, metabolic chamber environment (though it could conceivably be developed to incorporate environmental dynamics) to predict the time course of T_b for young endotherms that are still developing their capacities. It has been noted that while it incorporates many features in common with that developed (in Chapter 3), it does not incorporate situations other than those where animals are cooling. Neither does it, nor do the other integrated parameter models considered, simulate how the dynamics of T_b are affected by the dynamics of fluctuating T_e. And fluctuating T_e is such a dominant factor in the thermal environment of so many organisms!

Consequently, in this project (Chapter 3) an integrated parameter modelling approach to predict the T_b, and the variation of T_b, in a heterothermic endotherm such as an echidna, given different metabolic rates, variations in thermal conductance and variations in environmental temperature, is described. Where necessary, it incorporates evaporative cooling either as total evaporative cooling or as respiratory evaporative cooling, in which case it may incorporate variations in ventilation rate. Where appropriate, it incorporates a range of temperature effects on metabolic rate. It can be applied to such animals as they rest, forage (different metabolic rates), enter torpor or arouse from torpor and to animals in the heat, the cold or, importantly, in fluctuating thermal conditions. Fortuitously, this *a priori* modelling is also applicable to a range of ectotherms and endotherms. It was able to aid in the analysis of data collected from echidnas subject to temperature variations similar to those encountered in the field and provided insight into the physiological mechanisms allowing echidnas to tolerate heat.

Summary and Implications

In summary, then, this project is a combination of experimental and modelling studies, each separate but complimentary, focussing on the thermoregulation of echidnas. Together, they demonstrate (i) how echidnas are physiologically competent in the heat even without the common evaporative cooling mechanisms of panting, sweating or saliva spreading, (ii) how constitutional eurythermy and heterothermy allow echidnas the physiological flexibility to be indifferent to the cold, as well as (iii) the utility of simple, integrated parameter, *a priori* modelling in understanding the thermoregulation of this and other animals in complex, variable situations. These studies add to previous studies to increase the understanding of thermoregulation, not just in echidnas, but in mammals generally. They show how echidnas represent alternative thermoregulatory strategies to those classically associated with mammals, strategies that may be echoes of prototherian thermoregulation and echoes of stages in the evolution of “classical” (homeothermic) endothermy. Whether echoes or not, such strategies are energetically economical and likely give echidnas resilience in the face of large and broad scale changes in the Australian landscape and possibly give to them advantages unavailable to many other contemporary mammals. Thus, this physiologically based project may add insight into the ecology of Australian mammals as well.

Chapter 2: Thermoregulation in a eurythermal mammal, the short-beaked echidna, *Tachyglossus aculeatus*

I wish to acknowledge Danielle Levesque who collected some of the data reported in this chapter under my supervision as part of an assignment while visiting from McGill University, Canada.

Abstract

Although mammals are generally considered to thermoregulate as homeothermic endotherms with body temperature varying by less than two to three degrees each day, short-beaked echidnas are now known to be highly heterothermic in the field with body temperatures varying by as much as ten degrees each day. While echidnas do enter torpor, daily reductions in body temperature may have more to do with their ability to forage even with body temperatures as low as 20 ^{o}C. In the light of this “constitutional eurythermy”, this study investigated thermoregulatory responses of echidnas to ambient temperatures from 13 ^{o}C to 31 ^{o}C.

Individual echidna thermoregulatory responses were highly variable, especially at ambient temperatures below thermoneutrality. Responses to ambient temperatures below 25 ^{o}C varied from hypometabolism and torpor to vigorous shivering thermogenesis. Unusually for mammals, echidnas also showed intermediate metabolic heat production at these ambient temperatures, resulting in highly variable metabolic rates and body temperatures. Thermoneutrality was difficult to define in echidnas. This was probably due to (i) further individuality amongst echidnas, (ii) complications arising from different metabolic rates being associated with different body temperatures being defended and (iii) the possibility that echidnas were varying thermal conductance within, as well as between, ambient temperatures. While thermoneutrality may have been as high as 27.9 ^{o}C, correlating with a metabolic rate of 0.86 W.kg^{-1}, between ambient temperatures of 25 ^{o}C and 27.5 ^{o}C, metabolic rates varied from 0.53 W.kg^{-1} to 1.37 W.kg^{-1}. The mean metabolic rate in this range (1.04 W.kg^{-1}) was the most consistent for all ambient temperatures investigated. At higher temperatures there was also the possibility that echidnas varied thermal conductance within as well as between ambient temperatures. Nonetheless, echidnas share with other mammals

an increase in metabolic rate and body temperature above thermoneutrality, although they lack effective evaporative cooling.

The diversity of responses, particularly to low ambient temperatures, and the physiological flexibility implied, may have implications for the evolution of endothermy and probably has implications for the yearly energy budget of a mammal that has survived where other similarly sized mammals have not.

Introduction

The most widespread current model of thermoregulation in endotherms can be attributed largely to work published in 1950 by Scholander and his colleagues. In a pioneering comparison between the heat production in arctic and tropical mammals and birds they developed what has come to be known as the Scholander or Scholander-Irving model of endothermic thermoregulation (Scholander*, et al.*, 1950a). In this model, Scholander *et al.* emphasised the role of insulation in the thermoregulation of endotherms as well as the role of thermogenesis. Insulation, derived largely (but not wholly) from hair in mammals and feathers in birds, restricts heat flow between the animal and the environment; that is this insulation lowers thermal conductance, K. Thermogenesis is an increase in metabolic rate, M, which adds heat to a resting animal and may be shivering or non-shivering (Withers, 1992a). In essence, the Scholander model (Scholander*, et al.*, 1950a) states that in order to maintain its body temperature, T_b, in increasingly cold environments a resting endotherm initially (and primarily) decreases its K. Thermal conductance obtains a minimum value at some critical value of air temperature, T_a: a commonly used surrogate for operative (environmental) temperature, T_e, in experimental situations (Chapter 1). Should T_a (or T_e) reduce further below this critical temperature, T_{lc}, then an endotherm needs to increase its M in order to maintain its T_b; it cannot, in theory, reduce its K further. The range of temperatures above T_{lc} was referred to as the zone of thermoneutrality; Scholander *et al.* (1950a) did not consider responses to higher T_a in their study. The zone of thermoneutrality was wider, that is T_{lc} was lower, in arctic mammals than in tropical ones essentially due to their higher insulation (lower K) provided by longer coats etc. (Scholander*, et al.*, 1950a; Scholander*, et al.*, 1950b). The concept can be expanded to include the upper critical temperature, T_{uc}, where K is at its maximum (insulation is at its minimum). If no cooling mechanisms are present, T_b rises at $T_a > T_{uc}$ (Kleiber, 1961; Withers, 1992a). In this case, *M* increases due to the effect increased T_b has on *M* generally. In the presence of cooling mechanisms such as panting, sweating and saliva spreading, *M* increases before T_b as these cooling mechanisms require energy expenditure (Withers, 1992a). The zone of thermoneutrality becomes the zone of T_a between T_{lc} and T_{uc}, now more often called the thermal neutral zone, TNZ. Within the TNZ, *M* for resting, post-absorptive, stress-free endotherms is basal, *BMR*, there being no thermogenesis, cooling or additional temperature effect (because T_b is constant).

An alternative strategy exists where $T_a < T_{lc}$ when thermogenesis may not be energetically economical. Instead of increasing *M*, many mammals and some birds become hypometabolic, allowing both *M* and T_b to drop below "normothermic" or euthermic levels. If when hypometabolic, mammals or birds are also lethargic, then they are considered torpid (IUPS Thermal Commision, 2003). Torpor may be short-term "daily" torpor or long term hibernation (Geiser & Ruf, 1995; Geiser, 2004). In any case, hypometabolism may be utilised by some or all members of a population, presumably according to local weather conditions, resource availabilities and/or breeding imperatives (Davis, 1976; Hudson, 1978; Heldmaier & Steinlechner, 1981; Geiser & Baudinette, 1987; Pulawa & Florant, 2000). Rewarming from a hypometabolic state is energetically expensive, especially for large animals, so heterothermic endotherms are generally small, especially if they are heterothermic daily (Prothero & Jürgens, 1986; Geiser, 2004).

Body temperature results from variations in *K* and *M* (amongst others) so that where T_e (T_a in isothermal environments) is less than T_{uc} (that is within the TNZ and below), T_b remains constant according to the Scholander model. In many mammals, for example, T_b varies by less than two or three degrees on a daily basis and these mammals are considered homeothermic (Refinetti & Menaker, 1992; Refinetti, 1999). In homeothermic mammals, distributions of T_b for all $T_a < T_{uc}$ are expected to be unimodal about their constant normothermic values. For species where some individuals are heterothermic, bimodal distributions of T_b are apparent at $T_a < T_{lc}$, one mode corresponding to the T_b for normothermic individuals and one for the hypometabolic ones (e.g. Geiser & Baudinette, 1987; Song, *et al.*, 1997; Hosken & Withers, 1999). Unimodal T_b result within the TNZ ($T_{lc} < T_a < T_{uc}$).

These patterns of T_b, K, and M variation with T_a occur in many mammals to various degrees. Examination of just some of the data reported from studies of mammalian thermoregulation gives an idea of how much T_b varies within thermoneutrality, and how both T_b and K vary below thermoneutrality for normothermic individuals across a wide range of species (Table 2.1). Both the platypus, *Ornithorhynchus anatinus*, and the least weasel, *Mustela rixosa*, are notable for their homeothermy with T_b remarkably constant over various T_a in experimental situations. Most other mammals sampled in this cursory review (Table 2.1) vary T_b by less than 2 ^{o}C both within and below their TNZ, but fossorial mammals such as the naked mole-rat, *Heterocephalus glaber*, are notable exceptions. However in many, including the most homeothermic, there is significant reduction in K below the identified T_{lc} (Table 2.1). Presumably in many mammals, further decreases in K can be achieved even when thermogenesis is initiated. Nevertheless, the primary prediction of the Scholander model that most normothermic mammals maintain a fairly constant T_b over a range of T_a is supported by a large body of experimental data. Presumably, the predictions apply in the field as well, so that over a wide range of T_e, mammals maintain a fairly constant T_b except when torpid or hypometabolic.

This is not the case for short-beaked echidnas, *Tachyglossus aculeatus*. Short-beaked echidnas are prototherian mammals extant throughout Australia and parts of New Guinea with adult body weights of two and half to more than five kilograms (Strahan, 1983). They have very labile T_b. Except when incubating (Beard*, et al.*, 1992), T_b of echidnas in the field vary by as much as 5 ^{o}C to 10 ^{o}C on a daily basis (Augee*, et al.*, 1970; Grigg*, et al.*, 1989; Grigg*, et al.*, 1992b; Nicol & Andersen, 2000; Kuchel, 2003). Although echidnas do utilise torpor over various time frames (Grigg*, et al.*, 1989; Grigg*, et al.*, 1992b; Nicol & Andersen, 1996; Kuchel, 2003), the daily variation of their T_b is not necessarily analogous to the daily torpor observed in many smaller mammals and some birds. As echidnas have been observed to be active and fully competent foragers with T_b as low as 20 ^{o}C (Brice*, et al.*, 2002b; Kuchel, 2003; Grigg*, et al.*, 2004), they may be categorised as constitutional eurytherms *sensu* Eisentraut (1960). The rises in their T_b correlate with, and may be initiated by, periods of activity and the falls with periods of rest (Augee*, et al.*, 1970; Grigg*, et al.*, 1992b; Brice*, et al.*, 2002b; Nicol*, et al.*, 2004). It is almost as if the maintenance of some stable T_b is of little importance to echidnas.

This study was initiated to investigate the thermoregulation of echidnas in the context of their constitutional eurythermy. If echidna T_b are so labile in the field then perhaps they do not conform to the Scholander model of endotherm thermoregulation in laboratory experiments. Specifically the study investigated how echidnas responded to various air temperatures, T_a, from 13 ^{o}C to 31 ^{o}C while at rest, attempted to define the extent of the TNZ for these mammals, and quantified values and variations in both their K and resting M. Although similar studies were completed nearly four decades ago, they pre-date the realisation that echidnas may not maintain stable T_b in their normal daily routines and were therefore not interpreted in the light of these now extensive field data. For example, Schmidt-Nielsen *et al.* (1966) (i) undertook a study re-examining the view then current that the echidna was primitive with respect to its thermoregulation, and (ii) concluded that echidnas have a wide TNZ which does imply the maintenance of stable T_b. On the other hand, Augee (1969) found that echidnas do not maintain stable T_b and that at lower T_a both M and T_b vary widely with level of activity.

Methods

Six echidnas previously caught from various mesic and semi-arid areas of Queensland and implanted with temperature sensitive radio transmitters (Sirtrack) were selected from a captive population maintained in a semi-natural enclosure at the University of Queensland Veterinary Farm in South East Queensland. The echidnas had been in captivity for several months and had become habituated to human handling. They were routinely fed (a mixture of lean beef mince, eggs, dextrose, wheat bran, and olive oil with supplements of calcium carbonate and multivitamins (after Bellamy, 1994)) in the afternoon or evening which helped retain their natural rhythms of crepuscular to nocturnal activity alternating with rest from early morning often till afternoon.

Echidnas were selected while feeding, one at a time, and transported to a controlled temperature, CT, room pre-set to either 12 ^{o}C, 15 ^{o}C, 18 ^{o}C, 21 ^{o}C, 24 ^{o}C, 27 ^{o}C or 30 ^{o}C, T_{CT}. Because numerous previous studies showed echidnas tolerate heat poorly in laboratory experiments (Martin, 1903; Robinson, 1954; Augee, 1976), higher T_a were not used in this study. In the CT room, echidnas were allowed to equilibrate overnight in a 400 l pvc tub filled to a depth of 15 cm with clean, dry leaf litter. Generally, echidnas buried themselves in

Table 2.1 Various parameters from selected mammals' responses to a range of T_a manipulated in laboratory studies. TNZ, as determined by range of T_a over which M approximates BMR, extends from T_{lc} to T_{uc} with mean T_b at T_{lc} and T_{uc} as given: all temperature data being in ^{o}C. The difference in these T_b divided by the difference between T_{uc} and T_{lc} gives $\delta T_b/\delta T_a$ where a value of 0 implies constant mean T_b throughout the TNZ ("perfect thermoregulator") and a value of 1 implies mean T_b varying in proportion to T_a ("perfect thermoconformer"). T_a and mean T_b at lower T_a (5 ^{o}C below the TNZ) and equivalent $\delta T_b/\delta T_a$ calculated to indicate relative variation of mean T_b under mild cold stress for non-torpid individuals (only) : data presented in order of increasing mean $\delta T_b/\delta T_a$ within the TNZ and below. Alternative metabolic responses (M res) to cold T_a observed given as '+' for animals that increase M above BMR, 't' for animals that enter torpor, '-' for animals that exhibit $M < BMR$ but remain coordinated and responsive ("constitutional eurytherms") and/or '~' for animals that exhibit $M \approx BMR$ at $T_a < T_{lc}$ (and see text for clarification). Increase in variation of M and T_b in resting non-torpid animals at low T_a as calculated from the ratio of the maximum standard deviation or standard error reported (or calculated from reported data where errors are not explicit) of M and T_b respectively at any $T_a < T_{lc}$ to the maximum standard deviation or standard error (as appropriate) of M and T_b respectively at any $T_{lc} < T_a < T_{uc}$: SD M ratio and SD T_b ratio respectively. Continuing decrease in K at T_a below TNZ quantified as the ratio of minimum K (K_{min}) to K at $T_a = T_{lc}$ (K_{lc}) where K is either K_{dry} or K_{wet} as reported or K_{wet} as calculated from reported values of M and T_b at different T_a when not explicitly reported by original authors.

Animal	Reference	M res	5 below TNZ			TNZ					SD M ratio	SD T_b ratio	K_{min}/ K_{lc}
						T_a range		T_b range					
			T_a	T_b at $T_{lc} - 5$	δT_b/ δT_a	T_{lc}	T_{uc}	T_b at T_{lc}	T_b at T_{uc}	δT_b/ δT_a			
Platypus *Ornithorhynchus anatinus*	(Grant & Dawson, 1978)	+	20	32.0	0.00	25	25	32.0	32.0	0.00	3.00	n/a	0.63
Least weasel *Mustela rixosa*	(Casey & Casey, 1979)	+	23	39.5	0.00	28	>33	39.5	39.5	0.00	1.21	0.92	0.77
Platypus *Ornithorhynchus anatinus*	(Smyth, 1973)	+	10	30.8	0.02	15	25	30.9	30.8	-0.01	1.00	n/a	n/a
Grey kangaroo *Macropus giganteus*	(Dawson, *et al.*, 2000a)	+	10	36.5	0.00	15	>33	36.5	36.9	0.02	1.86	1.00	0.81
White-tailed jackrabbit *Lepus townsendii*	(Rogowitz, 1990)	+	7	38.2	0.00	12	>23	38.2	38.5	0.03	1.50	1.50	0.95
Australian water rat *Hydromys chrysogaster*	(Dawson & Fanning, 1981)	+	20	36.4	0.00	25	30	36.4	36.6	0.04	1.27	1.00	0.77
Black-tailed tree rat *Thallomys paedulcus*	(Lovegrove, *et al.*, 1991)	+	22	37.8	-0.02	27	>35	37.7	38.2	0.06	1.67	1.88	0.86
Cape porcupine *Hystrix africaeaustralis*	(Haim, *et al.*, 1990)	+	19	37.0	0.16	24	27	37.8	37.6	-0.07	1.38	1.20	0.89

Animal	Reference	*M* res	5 below TNZ			TNZ					SD *M* ratio	SD T_b ratio	K_{min}/K_{lc}
						T_a range		T_b range					
			T_a	T_b at $T_{lc} – 5$	$\delta T_b/\delta T_a$	T_{lc}	T_{uc}	T_b at T_{lc}	T_b at T_{uc}	$\delta T_b/\delta T_a$			
Hyrax *Procavia capensis*	(McNairn & Fairall, 1984)	+	20	39.7	0.12	25	>30	40.3	40.2	-0.02	2.60	n/a	0.97
European hedgehog *Erinaceus europaeus*	(Shkolnik & Schmidt-Nielsen, 1976)	+,t	22	34.3	0.02	27	31	34.4	34.8	0.10	1.39	0.80	0.94
Brown lemur *Lemur fulvus*	(Daniels, 1984)	+,~	25	38.1	0.02	30	40	38.2	39.2	0.10	2.87	1.44	0.17
Dog *Canis familiaris*	(Hammel, 1966)	+	21	37.8	0.04	26	33	38.0	38.6	0.09	1.64	1.43	0.80
Red panda *Ailurus fulgens*	(McNab, 1988)	+,-,~	20	37.4	0.04	25	36	37.6	38.6	0.09	4.80	0.91	0.16
Pygmy rabbit *Brachylagus idahoensis*	(Katzner, *et al.*, 1997)	+	12	38.2	0.02	17	25	38.3	39.2	0.11	0.89	1.54	0.70
Ghost bat *Macroderma gigas*	(Leitner & Nelson, 1967)	+	25	37.1	0.06	30	35	37.4	37.8	0.08	1.33	0.98	0.71
Golden bandicoot *Isoodon auratus*	(Withers, 1992b)	+	25	33.1	0.14	30	>30	33.8	33.8	0.00	1.33	2.00	0.82
Long-nosed potoroo *Potorous tridactylous*	(Hudson & Dawson, 1975)	+	19	35.8	0.08	24	32	36.2	36.8	0.07	1.90	1.33	0.79
Southern brown bandicoot *Isoodon obesulus*	(Larcombe, 2002)	+	20	33.9	-0.10	25	35	33.4	36.0	0.26	2.40	1.00	0.66
Sugar glider *Petaurus breviceps*	(Fleming, 1980)	+,-	22	36.3	0.00	27	31	36.3	37.0	0.18	3.03	6.43	0.90
Lesser long-eared bat *Nyctophilus geoffroyi*	(Geiser & Brigham, 2000)	+,t	24	34.8	0.18	29	33	35.7	35.7	0.00	6.81	2.08	1.70
Coyote *Canis latrans*	(Golightly & Ohmart, 1983)	+	17	36.9	0.06	22	26	37.2	37.7	0.13	2.01	0.95	0.99
Kowari *Dasyuroides byrnei*	(Geiser & Baudinette, 1987)	+,t	23	34.0	0.20	28	>33	35.0	35.0	0.00	3.25	2.00	0.66
Brown lemur *Lemur fulvus*	(McNab, 1988)	+,~	27	38.0	0.00	32	>38	38.0	39.2	0.20	5.25	5.72	0.16

Animal	Reference	M res	5 below TNZ			TNZ					SD M ratio	SD T_b ratio	K_{min}/K_{lc}
						T_a range		T_b range					
			T_a	T_b at $T_{lc} - 5$	$\delta T_b/\delta T_a$	T_{lc}	T_{uc}	T_b at T_{lc}	T_b at T_{uc}	$\delta T_b/\delta T_a$			
Spectacled hare-wallaby *Lagorchestes conspicillatus*	(Dawson & Bennett, 1978)	+	20	36.0	0.00	25	35	36.0	38.2	0.22	2.90	0.70	0.61
Marsupial weasel *Lutreolina crassicaudata*	(McNab, 1980)	+	22	36.0	-0.02	27	35	35.9	37.9	0.25	2.06	1.38	0.98
Matschie's tree-kangaroo *Dendrolagus matschiei*	(McNab, 1988)	+,-,~	22	36.2	0.08	27	37	36.6	38.2	0.16	3.60	0.97	0.14
Namaqua rock mouse *Aethomys namaquensis*	(Lovegrove, *et al.*, 1991)	+	22	38.0	-0.04	27	>35	37.8	40.2	0.30	1.63	1.00	0.90
Eastern pygmy-possum *Cercartetus nanus*	(Song, *et al.*, 1997)	+,t	24	34.0	0.14	29	33	34.7	35.2	0.13	3.37	1.17	0.71
Common mole rat *Cryptomys* sp.	(Marhold & Nagel, 1995)	+	28	34.6	0.31	33	33	36.1	36.1	0.00	7.63	1.67	0.58
Wagner's gerbil *Gerbillus dasyurus*	(Haim, 1987)	+	27	38.2	0.10	32	35	38.7	39.4	0.23	4.67	3.00	1.20
Lesser bulldog bat *Noctilio albiventris*	(Chappell & Roverud, 1990)	+	25	35.3	0.18	30	>35	36.2	37.0	0.16	1.50	3.01	1.48
Tasmanian devil *Sarcophilus harrisi*	(Nicol & Maskrey, 1980)	+	23	34.7	0.02	28	32	34.8	36.1	0.33	7.51	1.72	0.82
Giles' planigale *Planigale gilesi*	(Geiser & Baudinette, 1988)	+,-	24	32.5	0.10	29	>33	33.0	34.0	0.25	6.80	6.91	1.02
Eastern barred bandicoot *Perameles gunnii*	(Ikonomopoulou & Rose, 2003)	+	20	33.9	0.38	25	30	35.8	35.8	0.00	4.00	1.00	1.00
Kultarr *Antechinomys laniger*	(Geiser, 1986)	+,t	25	34.0	0.00	30	>35	34.0	36.0	0.40	1.33	4.00	0.92
Stripe-faced dunnart *Sminthopsis macroura*	(Song, *et al.*, 1995)	+,t	26	34.8	-0.10	31	34	34.3	35.8	0.50	2.96	2.19	0.67
Swamp rat *Rattus lutreolus*	(Collins, 1973)	+	25	36.6	0.02	30	32	36.7	37.7	0.50	3.33	1.75	0.70

Animal	Reference	M res	5 below TNZ			TNZ					SD M ratio	SD T_b ratio	K_{min}/ K_{lc}
						T_a range		T_b range					
			T_a	T_b at $T_{lc} – 5$	$\delta T_b/\delta T_a$	T_{lc}	T_{uc}	T_b at T_{lc}	T_b at T_{uc}	$\delta T_b/\delta T_a$			
Round-tailed ground squirrel *Spermophilus tereticaudus*	(Wooden & Walsberg, 2002)	+,(t,-)	25	33.2	0.20	30	35	34.2	36.2	0.40	2.25	1.00	0.92
Numbat *Myrmecobius fasciatus*	(Cooper & Withers, 2002)	+	25	33.5	0.10	30	>33	34.0	35.4	0.56	8.00	3.00	0.66
Naked mole-rat *Heterocephalus glaber*	(McNab, 1966)	+,~	27	30.8	0.50	32	36	33.3	35.4	0.53	2.94	9.21	0.28
Blossom-bat *Syconycteris australis*	(Geiser, *et al.*, 1996)	+,t	25	33.0	0.20	30	>33	34.0	36.5	0.83	2.60	0.87	0.47
Short-beaked echidna *Tachyglossus aculeatus*	(this study)	+,-,~	20	27.8	0.44	25	28	30.0	31.7	0.68	5.66	3.82	0.53
Lesser long-eared bat *Nyctophilus geoffroyi*	(Hosken & Withers, 1999)	+,t	29	32.0	0.60	34	35	35.0	35.9	0.90	2.88	2.30	0.60
Namib Desert golden mole *Eremitalpa granti*	(Fielden, *et al.*, 1990)	+,-,~	26	29.4	0.73	31	36	33.0	37.9	0.98	2.33	2.00	0.79
Naked mole-rat *Heterocephalus glaber*	(Buffenstein & Yahav, 1991)	+,-,~	26	27.0	1.02	31	34	32.1	35.1	1.02	2.07	1.00	n/a
Pygmy shrew *Sorex minutus*	(McDevitt & Andrews, 1995)	+	20	n/a	--	25	30	n/a	n/a	--	1.63	n/a	n/a
Western quoll *Dasyurus geoffroii*	(Arnold & Shield, 1970)	+	23	n/a	--	28	33	n/a	n/a	--	1.47	n/a	n/a
Northern marsupial mole *Notoryctes caurinus*	(Withers, *et al.*, 2000)	+	T_{lc} not known – T_b varies from 22.7 ($T_a = 15$) to 30.8 ($T_a = 30$)										
Long-beaked echidna *Zaglossus bartoni*	(Dawson, *et al.*, 1978)	~	T_{lc} not known – $32 < T_b < 32.2$ for all $15 < T_a < 30$ – K varies										
Common brushtail possum *Trichosurus vulpecula*	(Dawson, 1969)		M not measured – $T_b = 35.9$ at $22 < T_a < 26$. $T_b = 36.9$ at $T_a = 10$ and $T_a = 41.9$										

the leaf litter, and were found at rest the next morning.

Between 7 am. and 8 am. the next morning, the echidna was gently placed into a 13 l cylindrical metabolic chamber 29 cm long, containing 800 g of kitty litter (pellets of crushed, shredded recycled paper: Fibrecycle Pty Ltd), weighed and connected to the experimental plumbing. On some occasions, a movement sensor (Sigma Delta Technologies) was attached to the spines of the echidna using tape or, more easily, Blu-Tack (Bostik Australia Pty. Ltd.). This sensor, together with visual observations of the echidna, allowed detection of both shivering and breathing movements of the body wall. This whole procedure usually took less than five minutes and care was taken to disturb the animal as little as possible. Previous trials had shown that the presence of the kitty litter allowed the echidna to partially bury itself as it often does in the field and that, therefore, echidnas were more likely to be at rest during experiments. Nevertheless, one echidna was dropped from the study as it was consistently active in the chamber both with and without the kitty litter. Data were collected from the remaining five echidnas. For each echidna at each T_{CT}, the experiment was terminated when the echidna consistently tried to escape the metabolism chamber (often at about the normal feeding time), showed signs of heat stress, or at the end of the day (~ 5 pm.), whichever occurred first. The echidna was then fed and returned to the farm. Occasionally, echidnas were used on consecutive days, in which case they would be fed and retained in the CT room (at a different T_{CT}). However, this was unusual and would not occur unless the echidna appeared relaxed and stress-free.

Dry, compressed air was drawn through the experimental apparatus at a rate of 1 $l.min^{-1}$ by a mass flow controller (Sable Systems MFS-1 Mass Flow System) after being scrubbed of CO_2 by soda lime. Incurrent air entered the metabolism chamber at the base of one end and below a perspex panel perforated with 49 holes of 12 mm diameter. This panel was included to act as a floor for the echidna and to disturb the air flow through the chamber. This enhanced complete mixing of air before it exited the chamber through a vent at the top of the opposite end. Excurrent air was drawn through a dew point meter (Sable Systems RH-100/RH Dewpoint Meter) where water vapour pressure was measured. Water vapour and CO_2 were then removed from the air stream, by drierite and soda lime respectively, before it entered the flow controller. Two hundred millilitres per minute of this dried, CO_2 free air was sub-sampled after passing the flow controller and its O_2 concentration measured using an O_2 analyser (Sable Systems PA-1B O_2 Analyser). Ambient air temperature, T_a, in the chamber was measured by a calibrated T-type thermocouple (interpreted by a Sable Systems TC-1000 Thermocouple Meter) situated in the space above, but out of reach of, the animal. This indicated that the actual T_a of relevance to the animal was often a degree or two above T_{CT} and the difference was attributed to metabolic heat lost by the animal. T_b was measured by converting the implanted transmitters' signals as received by a receiver (Telonics Model TR-2) into temperature according to previously obtained calibration curves for each individual. All raw data, including excurrent oxygen concentration, $F_{E.O2}$, water vapour pressure, *WVP*, T_b radio peaks, T_a, flow rate, and 2 data channels from the movement sensor (when used) were recorded on an AD Instruments PowerLab 8 SP using Chart 4.2 (AD Instruments) for later analysis.

Because technical problems sometimes resulted in variations of flow rate, an additional channel was added where oxygen consumption, V_{O2}, was calculated according to Withers' (1977) equation 4a from $F_{E.O2}$ and flow rate. This was purely to provide a visual aid to the selection of data according to the following criteria. The lowest value of V_{O2} that pertained for five continuous minutes was selected as representative of minimal, potentially basal metabolic rates. However, animals at rest shivered to various degrees with V_{O2} clearly greater than these minima. So additional data to include these instances of higher V_{O2} were selected. These data were selected from when V_{O2} (at any value) was (nearly) constant for five minutes or more. However, instances of only 3.5 minutes of continuous vigorous shivering which occurred occasionally were also included so that overall metabolic costs for resting animals should not be under-estimated. As V_{O2} stabilised quickly in this experimental set-up, no O_2 washout corrections were considered necessary to relate directly T_b, shivering and V_{O2}.

For analysis, raw data were used to calculate V_{O2} and $V_{E(3)}$ as per Withers (2001). However, because CO_2 and H_2O were removed from the excurrent air stream before the mass flow controller $V_{E(1)}$ *sensu* Withers (2001) was required and calculated as:

$$V_{E(1)} = V_{CO2} + \frac{V_{E(3)}}{\left(1 - F_{E.H2O}\right)}$$

V_{CO2} was calculated assuming an *RQ* of 0.82 (below) and $F_{E.H2O}$, the fractional concentration of water vapour in the excurrent air, was determined by multiplying the absolute humidity, h_a

(in $g.l^{-1}$), of the excurrent air by the volume of an ideal gas and dividing by the molecular weight of water (Barrow, 1973). Absolute humidity, h_a, was calculated from the total water vapour pressure of the excurrent air as:

$$h_a = \frac{WVP}{R_w(273.15 + T_a)}$$

where $R_w = 461.5$ $J.kg^{-1}K^{-1}$ (Barrow, 1973).

V_{O2} as calculated using Withers' (2001) equation 4a was used to calculate M in W ($W.animal^{-1}$) using an energy equivalence of 20.6 $J.ml\ O_2^{-1}$. This is consistent with an RQ of 0.82 found for these and similar echidnas resting in other studies (unpub. data).

Because of the presence of ambient humidity in the CT room at the start of the experiment, i.e. before the chamber was sealed, only part of the h_a measured during the experiment can be attributed to evaporation from the animal. When investigated in the absence of an echidna, it was found that this humidity took as long as four hours to washout of the system. This was attributed to water absorbed by the kitty litter during storage prior to being used in these experiments. To account for the washout of this humidity throughout the experiments, a dry run (!) was conducted at each T_a where the animal was replaced by a coffee tin estimated to be of approximately equal volume. Kitty litter was included as per the experiments. WVP was measured at the same flow rate, and using the same source air and general setup as during the experiments. The resulting trace (on Chart) of WVP versus time when adjusted for the correct starting ambient humidity was used to estimate residual ambient absolute humidity for all data selected. The difference between this and the total absolute humidity was used to calculate the animals' net evaporative water loss, EWL (in $g.min^{-1}$): the product of the net absolute humidity (in $g.l^{-1}$) and the flow rate, $V_{E(l)}$ (in $l.min^{-1}$). To convert EWL into evaporative heat loss, E (in $W.animal^{-1}$), EWL was multiplied by the latent heat of evaporation for the midpoint T_b of the animal for each sample (see below). This assumes that all the evaporative heat loss is respiratory (i.e. occurs at core body temperature) which is valid since echidnas neither sweat nor pant (Augee, 1976). In any case, the difference that would occur by using skin or surface temperature is very minor.

Because echidnas are heterothermic, it was assumed that some heat would be exchanged with that stored in the body tissues. The amount so exchanged, S (in W), was calculated as the product of the rate of change of T_b, $\delta T_b/\delta t$, the specific heat capacity of tissues, 3.43 J.g^{-1o}C^{-1}(Bartholomew & Tucker, 1963), and the animal's mass at the start of the experiment. $\delta T_b/\delta t$ was calculated as the difference in T_b (if any) between that at the start of the 5 (or more) minute sample and that at the end divided by the duration of the sample (in sec). For other purposes, such as recording actual T_b for sample data or calculating latent heats of evaporation, the midpoint of these two temperatures was used in each case.

Thermal conductance was calculated in three different ways. Wet thermal conductance:

$$K_{wet} = \frac{M}{(T_b - T_a)}$$

Dry thermal conductance:

$$K_{dry} = \frac{(M - E)}{(T_b - T_a)}$$

Overall thermal conductance:

$$K_o = \frac{(M - E - S)}{(T_b - T_a)}$$

Finally the parameters relevant for analysis (M, E, S, K_{wet}, K_{dry}, K_o) were converted into mass specific units (W.kg^{-1} or W.kg^{-1}.^{o}C^{-1}).

Results

Considering data with respect to minimal values of V_{O2} first, M varied from a mean across all five echidnas of 0.652 W.kg^{-1} when the CT room was set to 12 ^{o}C (T_{CT}) to 1.389 W.kg^{-1} across four echidnas when T_{CT} was 30 ^{o}C (Figure 2.1). T_b measured concurrently with these values of M varied between a mean of 24.8 ^{o}C and 32.3 ^{o}C. Concurrent E amounted to a mean of 7.7 % of M across all T_{CT} and never to more than 11.4 % for any T_{CT}. Because of this there was little difference between K_{wet} and K_{dry} across all T_{CT}: both increased as T_{CT}

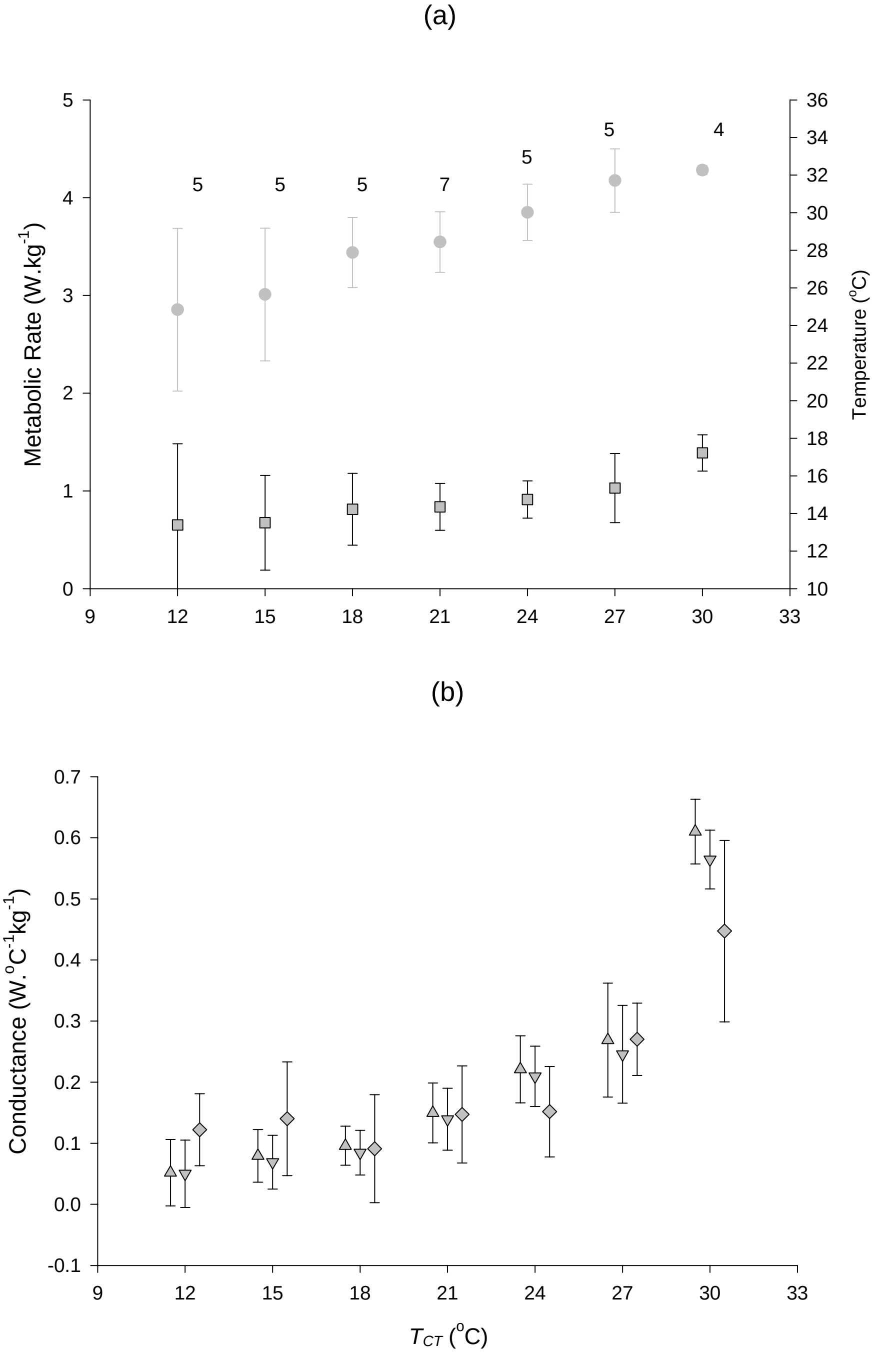


Figure 2.1 (a) Mean T_b (grey points) ± 1SD and mean M (closed squares) ± 1SD for echidnas over the range of T_{CT} as sampled when VO_2 was minimal and steady for ≥ 5 minutes. Numbers indicate numbers of echidnas in each treatment although at T_{CT} = 21 ^{o}C, experiments were repeated for two echidnas while at T_{CT} = 30 ^{o}C one echidna was excluded. (b). K_{wet} (upward pointing triangles) ± 1SD, K_{dry} (downward pointing triangles) ±1 SD and K_o (diamonds) ±1 SD calculated concurrently with the data in (a).

increased. K_o was approximately constant at 12 ^{o}C ≤ T_{CT} ≤ 24 ^{o}C at about 0.13 W.^{o}C^{-1}kg^{-1} but increased at higher T_{CT} to 0.447 W.^{o}C^{-1}kg^{-1} at T_{CT} = 30 ^{o}C. While the standard deviations were fairly consistent for all measures of K, those for both T_b and M were obviously much greater at lower temperatures indicating likely variation in responses to low temperatures between echidnas (Figure 2.1).

Considering all data sampled for individual echidnas at rest, differences in physiological responses to differing T_a both between and within echidnas were apparent. For example, with echidna IB13, when T_{CT} was 12 ^{o}C, M was higher than for all other T_{CT} resulting in T_a of about 15 ^{o}C and T_b > 29 ^{o}C; similar to that at higher T_a (Figure 2.2). Further, on that day at that T_a, M varied between 2.11 W.kg^{-1}and 3.57 W.kg^{-1}; a factor of 1.7. With this and other echidnas at rest, changes in M appeared to correlate well with changes in the degree of shivering discernable both to the naked eye and to the movement sensors when attached. However, as orientation of echidnas affected the movement sensor signal, shivering intensity was not possible to quantify consistently.

In contrast to IB13, at T_a < 15 ^{o}C RL28 showed relatively low and variable M (1.48 W.kg^{-1} to 0.15 W.kg^{-1}). Correlating with this low M at a low T_a, T_b < 20 ^{o}C throughout the day (Figure 2.2). However, when T_a was approximately 16 ^{o}C RL28 initially had a high metabolic rate (> 3.5 W.kg^{-1}) maintaining a T_b > 26 ^{o}C, but as the experiment progressed, M decreased to low levels (0.173 W.kg^{-1}) whence T_b dropped to 18.7 ^{o}C. On this day, as on some others, the echidna showed every sign of entering into torpor although the experiments were stopped prior to this occurring.

A yet different pattern was exhibited by echidna GB20 over various temperatures featuring fewer extreme rates of M, although the lowest was 0.19 W.kg^{-1} when T_a was 17.2 ^{o}C (Figure 2.2). Nevertheless, metabolic rates were closer to the median value of M for all T_a. At the lowest T_a at least, these metabolic rates were associated with mild and intermittent bouts of shivering, occurring predominantly during actual breathes. However, a T_b of about 28 ^{o}C seems to have been maintained, presumably by an occasional bout of high M (> 2 W.kg^{-1}) which was associated (both observed and from movement sensor data) with a bout of vigorous and continuous shivering.

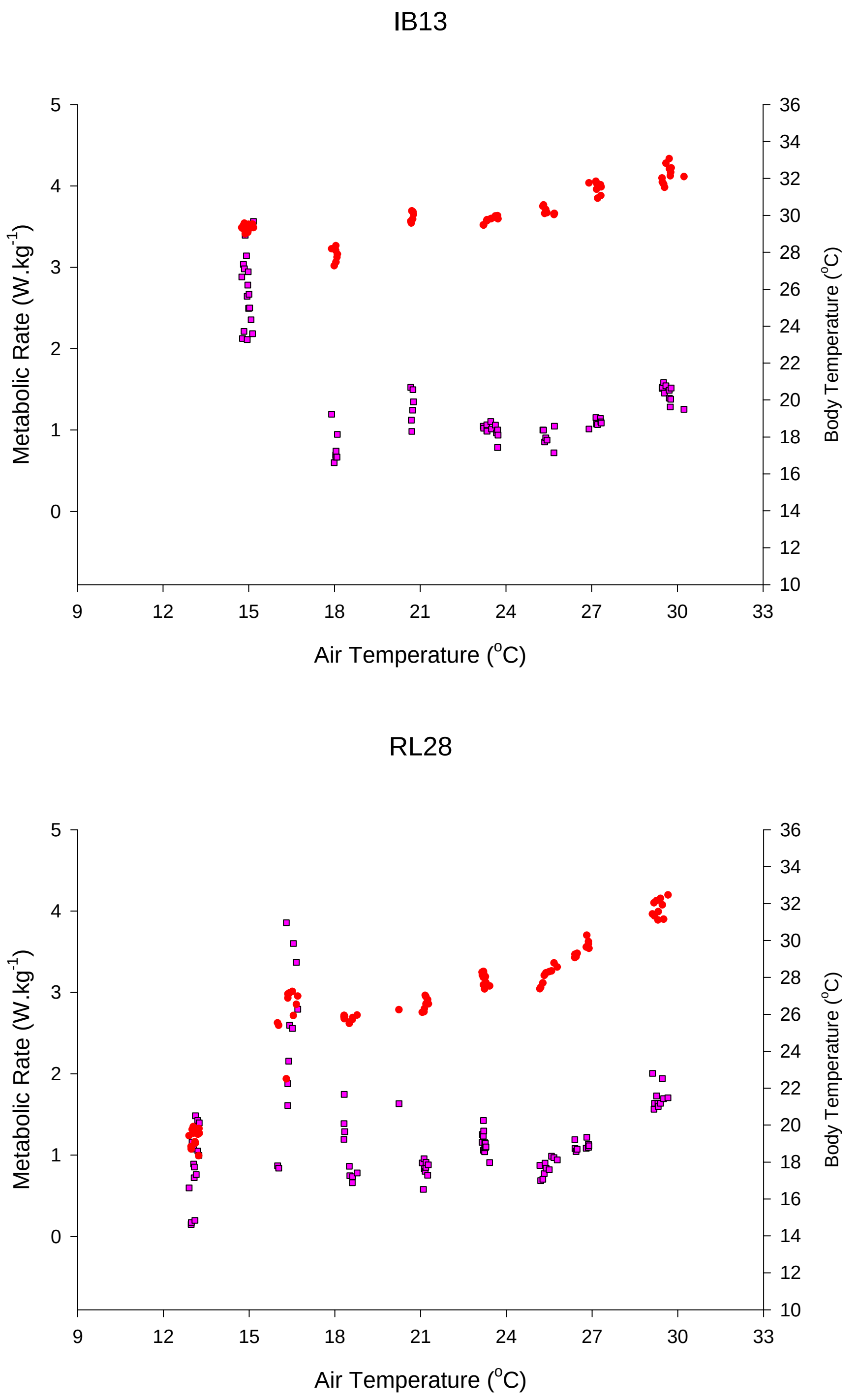
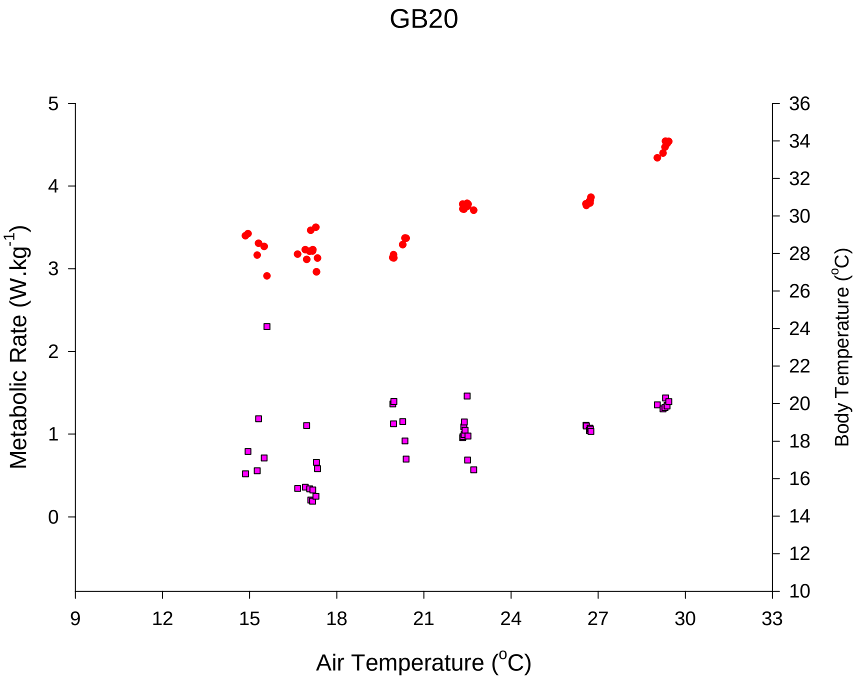


Figure 2.2 T_b (red points) and M (purple closed squares) for selected echidnas (IB13, RL28, GB20) and for all echidnas pooled over the range of T_a. Includes all data sampled from echidnas at rest.



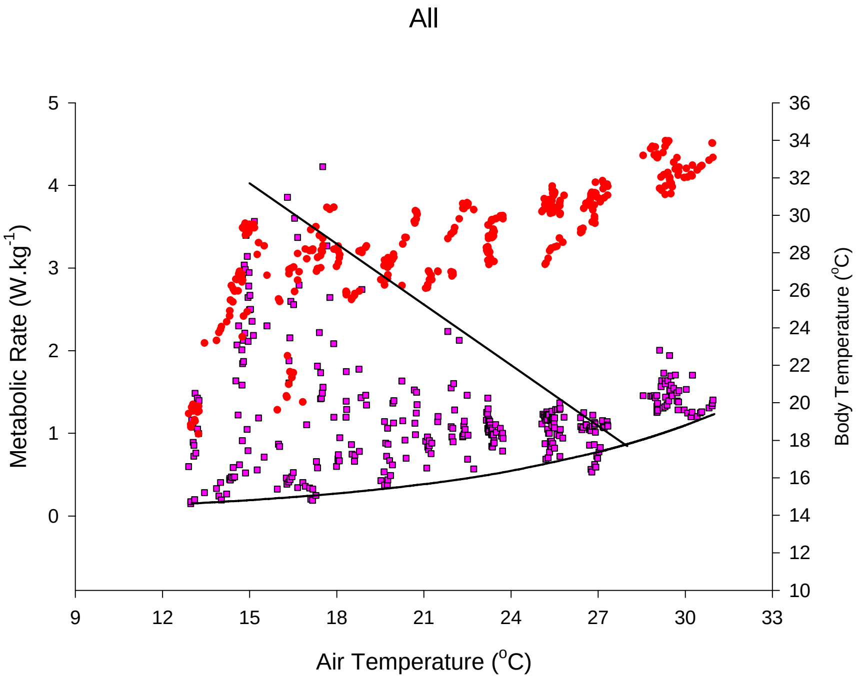


Figure 2.2 continued

Combining all the data sampled from all five echidnas at rest reveals an overall pattern (Figure 2.2). Between a T_a of about 25 ^{o}C to 28 ^{o}C no resting echidna exhibited a metabolic rate of greater than 1.37 W.kg^{-1} or less than 0.53 W.kg^{-1}. The mean M for 24 ^{o}C < T_a < 27 ^{o}C is 1.04 W.kg^{-1}. The standard deviation for M is lower for this range of T_a than for any other examined and about one fifth the standard deviation at lower T_a (Table 2.2). A similar cluster of points occurred at higher T_a with a similarly low standard deviation, but M was higher ranging from 1.20 W.kg^{-1} to 2.01 W.kg^{-1}. Below a T_a of about 28 ^{o}C, resting metabolic data are distributed across an area of (T_a, M) space. By adapting the edge detection techniques of Blackburn *et al.* (1992), this (T_a, M) space could be delineated by two lines. A linear regression of the maximum M, $M_{edge.u}$, in each of six equal T_a classes for T_a < 28 ^{o}C gave a straight line:

Table 2.2 Parameter values based on all data sampled from echidnas when seen to be resting for periods of ≥ 5 minutes. Data for all echidnas were pooled and distributed into 3 ^{o}C increments of T_a (as measured in the metabolic chamber) except the last which includes 12 observations at 30 ^{o}C < T_a < 31 ^{o}C. “No” refers to sampled observations of resting echidnas across all echidnas for given T_a increment. Note five-fold increase in SD of M and T_b at low T_a.

T_a (^{o}C)	No	T_b (^{o}C)		M (W.kg^{-1})		E (W.kg^{-1})		S (W.kg^{-1})		K_{wet} (W.kg^{-1o}C)		K_{dry} (W.kg^{-1o}C)		K_o (W.kg^{-1o}C)	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
12 to 14.9	54	24.58	3.86	1.306	0.946	0.022	0.023	-0.052	1.211	0.1227	0.0732	0.1209	0.0737	0.1112	0.1031
15 to 17.9	51	26.78	2.97	1.522	1.131	0.074	0.033	-0.168	1.180	0.1496	0.1115	0.1410	0.1115	0.1546	0.1014
18 to 20.9	45	27.59	1.31	1.033	0.464	0.088	0.029	-0.228	0.514	0.1261	0.0552	0.1152	0.0559	0.1358	0.0597
21 to 23.9	65	28.62	1.39	1.063	0.278	0.071	0.029	0.076	0.338	0.1836	0.0503	0.1713	0.0498	0.1605	0.0543
24 to 26.9	72	30.21	1.01	1.042	0.200	0.058	0.031	0.086	0.270	0.2531	0.0503	0.2386	0.0690	0.2103	0.0823
> 27	62	32.50	0.89	1.384	0.224	0.112	0.057	0.177	0.268	0.4771	0.1971	0.4409	0.1956	0.3596	0.1537

$$M_{edge.u} = 7.6952 - 0.2445T_a \quad (r^2 = 0.8160)$$

to demarcate the maximum metabolic rate for T_a < 28 ^{o}C. An exponential regression of the minimum M, $M_{edge.l}$, in each of eight equal T_a classes for all T_a resulted in a curved line:

$$M_{edge.l} = 0.0333e^{\,0.1165Ta} \qquad (r^2 = 0.9151)$$

to demarcate the minimum metabolic rate for all T_a. These lines intercepted at a T_a of approximately 27.9 ^{o}C, equivalent to M of approximately 0.86 W.kg^{-1}(Figure 2.2). Maximum thermogenesis exhibited by this group of echidnas increased linearly with decreasing T_a while the minima metabolic rates exhibited decreased with decreasing T_a. However, on many occasions, echidnas at rest showed metabolic rates and levels of thermogenesis between these two extremes.

Discussion

While a cursory review of the data implies that echidnas are not obligate conformers to the classical model of endotherm thermoregulation, a deeper analysis reveals that they have the physiological flexibility to conform or not, depending upon circumstances that still may not be clear. This in turn supports conjecture as to the role of echidnas, or animals with echidna-like physiology, as models for proto-endotherms (e.g. see Grigg & Beard, 2000; Grigg*, et al.*, 2004).

Throughout this study, no consistent T_b was maintained across various T_a either between or within individuals (Figures 2.1 & 2.2 & Table 2.2). Compared with other mammals subject to standard respirometry-based studies into thermoregulation (Table 2.1), T_b was labile both within (see below) and below thermoneutrality. Variation of T_b with T_a was comparable to that in poorly thermoregulating fossorial mammals, including naked mole-rats, as well as to that in some bats. For a constitutional eurytherm this should be of little consequence. Records from other studies of echidnas active with T_b as low as 20 ^{o}C are accumulating (Brice*, et al.*, 2002b; Kuchel, 2003; Grigg*, et al.*, 2004) and such constitutional eurythermy was exhibited here. On one occasion, echidna RL28 was seen moving and looking around the metabolic chamber in a coordinated, albeit slow, manner prior to measurements being started when its T_b was 17.2 ^{o}C. On another, IE24 was seen engaging in somewhat more vigorous movement at a T_b of 22.3 ^{o}C.

Body temperature should be considered the result of a number of physiological processes and mechanisms, whether or not changes in T_b trigger those mechanisms. For example, thermogenesis results in a higher T_b than would occur otherwise. In this study, differing intensities of shivering thermogenesis were observed both between and within individuals at differing $T_a < 25$ °C and, allowing for a time lag, the levels of thermogenesis correlated with values for T_b. Maximum thermogenesis increased with decreasing T_a for all echidnas (Figure 2.2) but M often took any value between this and some minimum associated with no thermogenesis at all. This capacity to set M between a minimum and a maximum, and to do so at any $T_a < 25$ °C, is rare amongst mammals. Of the studies reviewed, only a few reported intermediate values of M (endorsed "~" in Table 2.1), and in all cases except this study, those intermediate values were exhibited under limited conditions. For arboreal mammals, intermediate M were exhibited only at particular T_a and in association with spectacular and probably relatively short-lived low values of K, resulting in stable, normothermic T_b (Daniels, 1984; McNab, 1988). For fossorial mammals, intermediate M were associated with high K and declining T_b, and with failures of thermogenesis in animals subject to experimental T_a outside the narrow range of burrow temperatures normally experienced in the wild (Fielden, *et al.*, 1990; Buffenstein & Yahav, 1991). In their thermally neutral and stable burrows the need for thermogenesis would be minimal. Interestingly, the lack of thermogenic capability in three-toed sloths, *Bradypus griseus*, was also attributed to it being superfluous in their thermally stable rainforest habitats (Britton & Atkinson, 1938). Clearly, over time such animals have either lost their thermogenic capabilities or have never evolved them. In contrast, at any of various $T_a < 25$ °C echidnas exhibit differing degrees of thermogenesis. Whether this has evolutionary significance is unclear, but in a sense an echidna's physiology may be (and has been: Grigg, *et al.*, 2004) used as a model for the evolution of endothermy. It may mimic one or more stages in the transition of a heterothermic ectotherm into a homeothermic endotherm via a eurythermal prototype. If mammalian body temperatures have advantages, it seems logical that this evolution may be accomplished by incremental stages in thermogenic capability. The exhibition of variable thermogenesis in echidnas seems more supportive of this sequence than an alternative whereby hypometabolism evolved in thermogenically competent mammals. This in turn would imply that hypometabolism, if not torpor, is plesiomorphic as also suggested by Grigg and Beard (2000) and Grigg *et al.* (2004).

This variability in M, and therefore T_b, at low T_a adds patterns to data from echidnas not apparent in data from other mammals. As well as the strongly bimodal distribution of M and T_b exhibited by heterotherms, some of whom defend normothermia while others enter torpor (Geiser & Baudinette, 1987; Song, *et al.*, 1997; Hosken & Withers, 1999), in echidna data there are intermediate M and T_b. As well as the intermittent shivering which is possibly related to sleep patterns in some mammals (Nicol & Maskrey, 1980; Cooper & Withers,

2002) occurring all day, there are instances of shivering being reduced and abandoned during the day and of no shivering at all. The patterns in these data also add to those found by Augee (1969). He found great variability in T_b and M in echidnas, but in his case for echidnas kept at constant T_a over several days. Augee found that echidnas' T_b declined with rest and increased with activity both for those in his experiments and for those kept in outside pens. He further interpreted the variation in T_b from echidnas in the pens with respect to minimum values of T_a during the T_b cycle: T_b reached lower values on colder mornings (Augee, 1969; Augee*, et al.*, 1970). In this current study, the variability was obtained from resting, not active, echidnas. It was as if some were defending one T_b one day, a different T_b (not necessarily dependent on T_a) a different day, or simply not defending a T_b at all. Why particular T_b should be defended on different occasions is not clear even for a constitutional eurytherm. Perhaps there are differing optimal T_b for various physiological states and processes not considered in this study: reminiscent of variable preferred T_b in some ectotherms (Heatwole, 1976)?

Shivering thermogenesis was clearly demonstrated by echidnas in this study. Shivering thermogenesis is typical in mammals exposed to the cold, so whether they choose to exercise it or not, the echidnas were clearly capable of this classic response. Although not always exercised (e.g. IB13 at $T_a \sim 18$ °C, RL28 at $T_a \sim 13$ °C and GB20 generally: Figure 2.2), echidnas utilised metabolic heat production which maintained T_b at various values in this (IB13 with $T_b \sim 30$ °C at $T_a \sim 15$ °C: RL28 with $T_b \sim 28$ °C at $T_a \sim 16$ °C and even GB20 with $T_b \sim 29$ °C at $T_a \sim 15$ °C for a short bout: Fig 2.2) and in other studies (Schmidt-Nielsen*, et al.*, 1966; Augee, 1969), even with T_a as low as 5 °C. When the echidnas did utilise shivering, maximum metabolic rate declined with increasing T_a, which conforms to the Scholander model. Echidnas conform to the Scholander model of endotherm thermoregulation in other ways as well. In addition to the low values of K correlating with insulation, echidnas together with other mammals (and birds) exhibit a wide range of K when compared to, say, comparably sized lizards. It is by varying K across this range that allows endotherms to maintain a T_b and a (near) basal M over a range of T_a. According to the Scholander model, K should be low at $T_a = T_{lc}$ and increase throughout the TNZ. In this, echidnas conform (Figure 2.1) to the same extent as a range of other mammals (Table 2.1). However, and contrary to the classical model, many mammals continue to reduce K at $T_a < T_{lc}$ in concert with increasing thermogenesis, and in this, echidnas are no different (Table 2.1). It is easy to imagine ever tighter huddling into a K-minimising posture together with shivering more intensely as T_a drops and drops. Over the range of T_a examined in this study, K_o reduced to

53 % of that at $T_a = T_{lc}$, a greater reduction than most but not much greater than for mammals as diverse as spectacled hare-wallabies, *Lagorchestes conspicillatus*, and common mole-rats, *Cryptomys* sp. (Table 2.1).

Other features of the Scholander model are not so apparent in echidnas. Identifying any thermal neutral zone from these data proved to be a matter of interpretation. On the one hand, there was a range of T_a from about 25.0 °C to 27.5 °C where M was relatively low and consistent for all echidnas (Figure 2.2). Ignoring for the moment that K_o continued to reduce below thermoneutrality, the Scholander model predicts that for an endotherm defending a T_b of 30.2 °C with $0.2103\ \text{W.}^o\text{C}^{-1}\text{kg}^{-1} < K_o < 0.3596\ \text{W.}^o\text{C}^{-1}\text{kg}^{-1}$ and a *BMR* of 1.04 W.kg^{-1} the TNZ should extend from 25.6 °C to 27.5 °C. This appears to conform to the empirical data (Figure 2.2 and Table 2.2). On the other hand, there was still significant variation in M at these T_a that needed to be explained. Although the standard deviation of M over this range of T_a was lower than for any other range of T_a examined (Table 2.2), M still varied from 0.53 W.kg^{-1} to 1.37 W.kg^{-1}. Four factors may combine to explain this variation: (i) experimental error and noise in the data, (ii) theoretical considerations pertaining to constitutional eurythermy, (iii) variation in K_o over and above that expected from the Scholander model alone, and/or (iv) increased metabolism in some individuals casting doubt as to the true value for *BMR* in echidnas.

Errors and noise are likely to occur in all experimental data. The extent of such is usually quantifiable as variation about a mean. Assuming that a mean is meaningful normally implies that all individuals respond in the same way to various stimuli, such as variations in T_a. Clearly, echidnas respond individually to at least some T_a (Figure 2.2), so while the presence of error must be assumed, other factors warrant investigation.

A major assumption behind the presence of a TNZ emerging in the Scholander model is the maintenance of some high and stable, single T_b. For a constitutional eurytherm, any given value of T_b may be of little importance, so different individuals may exhibit different T_b on different occasions. The temperature effect of T_b on M predicts that different standard metabolic rates, *SMR*, pertain for different T_b and that, therefore, thermoneutrality in constitutional eurytherms is characterised by various *SMR*/*BMR* correlating with various T_b. Evidence that this occurred in this study comes from the relationship between M and $T_b <$ 30°C in echidnas resting at 25.0 °C < T_a < 27.5 °C (Figure 2.3). In a subsequent study (Brice,

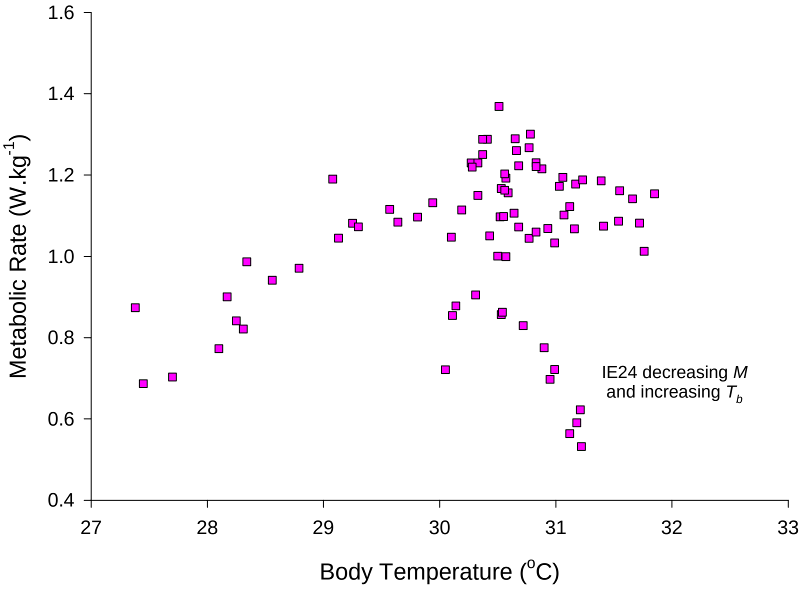


Figure 2.3 Metabolic rates of echidnas at rest during open circuit respirometry as function of body temperature at 25 °C < T_a < 27.5 °C.

in prep.) multiple values for *SMR*/*BMR* emerged at thermoneutrality for a hypothetical endothermic constitutional eurytherm defending preferred T_b that ranged from 30 °C to 35 °C.

The relationship between *BMR* and T_b appears to break down at T_b > 30 °C (Figure 2.3). The data from the bottom right of the figure (T_b > 30 °C & M < 0.9 W.kg^{-1}) derived from a single individual on a single occasion. Although most echidnas were in thermal equilibrium at these T_a, this individual increased its T_b (from 30.4 °C to 31.2 °C) while it decreased its M (from 1.25 W.kg^{-1} to 0.53 W.kg^{-1}). This could only be achieved by varying K_o, which changed from about 0.317 W.°C^{-1}kg^{-1} to about 0.141 W.°C^{-1}kg^{-1} during the experiment. This represents a change in K_o from above mean values (mean K_o = 0.210 W.°C^{-1}kg^{-1}: Table 2.2) to below mean values occurring at a single T_a (26.5 °C), and as such, is a significant departure from expectations derived from the Scholander model. Typically, K from similar studies are reported as means with standard deviations or standard errors, giving the impression that one value (more or less, depending on experimental error) of K pertains at any particular T_a. Such an interpretation is intuitive, given that for any single T_b (and therefore single *BMR*), an endotherm's heat budget is balanced only by a single value for K (assuming insignificant variations in E). However, the assumption of a single T_b is clearly invalid, at least for echidnas. In consequence, the variation of K_o with T_a, in echidnas at least, may be more complex than predicted by the Scholander model, and may be more than due to experimental error alone. Echidnas may be manipulating K_o (as well as M) within various T_a. The maintenance of nearly constant M at $T_b > 30$ °C at 25 °C < T_a < 27.5 °C (the other data in Figure 2.3) is explicable by variations in K_o. In these cases, however, K_o apparently is varied so that a range of T_b (> 30 °C) results from a constant M of more or less 1.1 W.kg^{-1}.

Finally, variations in M at 25.0 °C < T_a < 27.5 °C may simply derive from shivering or other thermogenesis. If this occurs, then technically, these T_a are not thermoneutral and these M are not basal. Some, but not all, echidnas at these T_a exhibited some twitching or slight, barely discernable, shaking which may have been low intensity shivering. Of the five echidnas, this was noted in two, one at T_a of 25.3 °C and 27.3 °C, the other at T_a of 25.6 °C. For another echidna at T_a of 25.3 °C, close observation failed to reveal even slight shaking, while for the others, observation may not have been close enough to discern such movements. For some echidnas, therefore, T_a as high as ~ 27 °C may be lower than T_{lc}. The linear relationship of maximum M (shivering thermogenesis) over all T_a below thermoneutrality extrapolates to intercept the curvilinear relationship of minimum M over all T_a at a T_a ~ 27.9 °C (Figure 2.2). Thus, the TNZ may be higher and narrower, while *BMR* may be lower than superficial examination of the data implies.

In reality, a combination of all four factors may pertain, especially given the individuality of echidna responses to lower T_a (noted above) and their constitutional eurythermy. While some echidnas may increase M, others may accept a lower M. Additionally, echidnas may be manipulating K_o in unexpected or unusual ways. These mechanisms, together with experimental error, seem to have contributed to the observed variability in both M and T_b at 25.0 °C < T_a < 27.5 °C.

At $T_a > 28.0$ °C, there was an increase in mean M, as expected for a classical endotherm lacking significant evaporative cooling (Kleiber, 1961; Withers, 1992a). However, as with lower T_a, M showed significant variation even though T_b was more constant at these T_a than at any other (Table 2.2). Unlike at 25 °C < T_a < 27.5 °C, M decreased with T_b at higher T_a > 28 °C, although the relationship was weak ($r^2 = 0.2208$: Figure 2.4). This is surprising since theory suggests that at $T_a > T_{uc}$ and in the absence of significant evaporative cooling, increased M should occur due to the effect T_b has on M. Given that increased M does not correlate with increased T_b at these highest T_a, only experimental error or variation in K_o

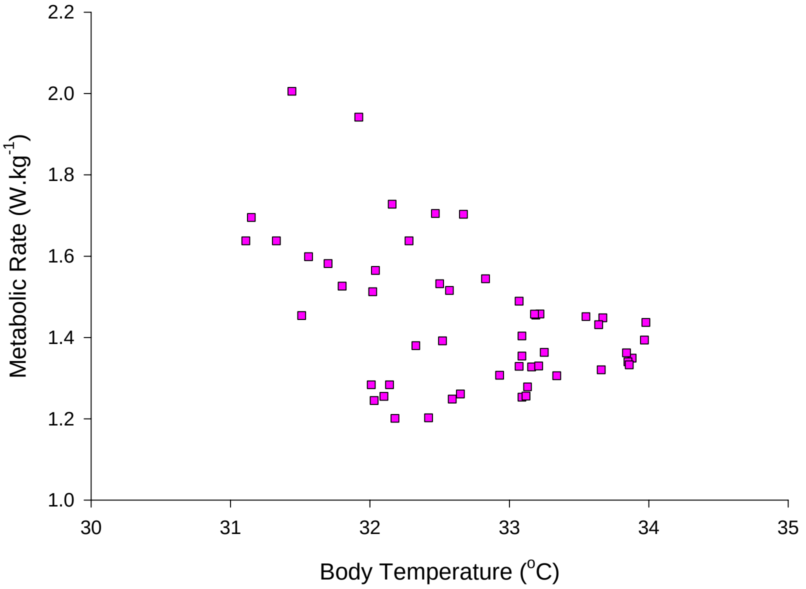


Figure 2.4 Metabolic rates of echidnas at rest during open circuit respirometry as a function of body temperature at $T_a > 28$ °C.

likely explain the range of M. Thermal conductance (by any measure; K_{wet}, K_{dry} or K_o) was more variable at the highest T_a than at any others (Table 2.2). Because they were determined from all the other parameters measured, estimates of K_o were especially sensitive to experimental error, particularly at high T_a where the $(T_b - T_a)$ gradients were small. Thus, experimental error may explain this variation in K_o (Table 2.2). However, experimental error is insufficient to explain the ranges of M and T_b, both of which were determined with some precision. Although evaporative cooling was greatest at highest T_a, it was still insufficient to balance metabolic heat production (mean $E/M = 0.09$; range 0 to 0.18). Therefore, only changes in K_o can explain the unexpected reduction of M with increased T_b. Variations in K_o may be used to compensate, or even over-compensate, for what would otherwise be increased M and T_b at $T_a > 28$ °C. This adds credibility to the idea that some of the variation in K_o observed is real and biologically significant, rather than just experimental error. Even for a mammal lacking evaporative cooling, these patterns are not predicted from classical theories about thermoregulating endotherms (e.g. Kleiber, 1961).

Clearly, these data show the great flexibility in echidna thermoregulatory physiology. At one level, the echidna may conform to the classical models of endotherm physiology, but need not. Thermal conductance is adjusted over different T_a to manage heat loss and maximum shivering thermogenesis increases with decreasing T_a below “thermoneutrality”. This results in the ability to maintain high and stable T_b over a wide range of T_a. It is probably this facultative conforming to the Scholander model that allows T_b to be maintained at about 30 °C to 32 °C by incubating echidnas during winter in the wild (Beard & Grigg, 2000). However, as constitutional eurytherms, the maintenance of T_b seems to be of little importance at other times. Instead, a range of T_b (possibly preferred T_b) may be maintained by less than maximum thermogenesis, and instances of this were apparent in the data. Alternatively, echidnas allow M, and therefore T_b, to drop, sometimes to levels associated with torpor. *SMR* varies with T_b at all T_a. Therefore, as predicted by classical models of endotherm thermoregulation, M and T_b increase at T_a above any TNZ in the absence of cooling mechanisms. At a more detailed level, K_o seems to be varied within T_a, as well as between T_a, especially at and above a range of T_a that might be thermoneutral. Within this range, various *SMR* and T_b are related to give what could to be a multi-tiered TNZ, although other interpretations are possible. The TNZ may be narrower than implied by some data (this study; Schmidt-Nielsen*, et al.*, 1966) at least for some individuals that may shiver slightly at these T_a. However, by no means is energetically expensive thermogenesis exercised as a matter of course. Numerous combinations of M, K_o and T_b are available to echidnas so that what emerges is a picture of a constitutional eurytherm, without energetically expensive cooling mechanisms and with optional shivering thermogenesis. Such a picture implies advantages for echidnas in the wild if resources are scarce or erratic. Apparently not in decline throughout their range, including the arid zone of Australia where so many similarly sized therian mammals have declined, it is interesting to speculate about the significance of echidnas’ flexibility and energy economy in their continued survival in such environments. Declines in formally sympatric therian mammals in the arid zone of Australia are usually attributed to ecological rather than physiological factors (Burbidge & McKenzie, 1989; Morton, 1990; Johnson, 2006) but a mammal apparently physiologically indifferent to the cold is likely to have better energy economy in the field than one which must devote (possibly limited) resources to staying warm. Add to this the low metabolic rate echidnas have anyway (Dawson*, et al.*, 1979) and the hibernation they utilise from late summer until their mid-winter breeding season (Augee*, et al.*, 1970; Grigg*, et al.*, 1992b; Brice*, et al.*, 2002b; Kuchel, 2003; Nicol*, et al.*, 2004) and it is clear how efficient these mammals might be in a multitude of environments.

Chapter 3: Simply modelling complexity: thermal exchange of animals in dynamic environments

Abstract

Equations commonly used in thermal biology and physiology were combined in a computer program to include endothermy, heterothermy, temperature effects and thermal inertia in the prediction of body temperature for ectotherms and endotherms in stable, multiple or variable thermal environments. These equations were solved iteratively for body temperature when inputs, metabolic rate, thermal conductance, respiratory minute volume (or total evaporative water loss), humidity and operative environmental temperature, were variously either constant, functions of time or functions of each other as appropriate. Results of simulations involving animal environment systems that were in equilibrium, that were dynamic in a simple way and that were dynamic in complex manners were compared to equivalent situations reported in the literature and to novel experimental data from a medium sized heterothermic endotherm to confirm the accuracy of the programming and of various simulation solutions. Simulations comparing modelled data to further empirical data are included to demonstrate further analytical applications of this modelling approach. Prototypes of the program will be available from the author on request.

Introduction

Temperature is undoubtedly the most important abiotic factor influencing living organisms through its effects on biochemical processes. In terrestrial environments especially, temperature often varies widely on a daily and seasonal basis so that plants and animals are routinely challenged both by cold and by heat. The importance of the thermal environment for organisms is recognised by an extensive literature and a proliferation of modelling in thermal biology. However for analysing or predicting thermal relations of organisms in the field, much of this modelling is of limited use. Three quarters of readily available thermal modelling sampled from the literature failed to incorporate fluctuations of ambient temperature (Chapter 1). Other model short-comings include limited applicability of *a posteriori* modelling to organisms other than those for which they were developed, requirements for huge arrays of difficult to obtain parameter values for detailed, comprehensive models and/or failures to recognise the possibility of heterothermy in endothermic mammals and birds or the possibility of significant metabolic heat production in some (active) ectotherms. Such problems ensure that understanding the thermal relations of at least some animals in their natural environments is either inadequate or perhaps lost in unnecessary detail (Chapter 1).

These issues are addressed by modelling developed and presented as part of a wider study into the thermoregulation of echidnas, *Tachyglossus aculeatus*. While motivated by the need to understand the heat fluxes of an echidna in various environments (Chapter 1), the modelling actually has remarkably wide applicability to a variety of other animals both ectothermic and endothermic. This is due to the fact that as heterothermic endotherms, echidnas demonstrate labile body temperatures, a salient feature of ectotherm thermal biology, and significant metabolic rate, a salient feature of endotherm thermal biology (Chapter 2). By adding evaporative water loss, necessary because respiratory evaporative water loss in echidnas is significant (even if insufficient for cooling: Chapter 4), and by integrating sensible heat fluxes, this modelling allows a complete suite of thermoregulatory mechanisms and strategies to be examined for a wide variety of animals. By incorporating fluctuations in temperature (both ambient and body), thermoregulation in dynamic situations can be modelled. As it is *a priori* in nature, different organisms can be represented by different values (or functions) of parameters such as thermal conductance, metabolic heat production etc.

The development and testing of this *a priori*, integrated, lumped parameter thermal modelling developed *ab initio* in the computer program QBASIC is reported here. The modelling combines equations commonly used in thermal biology and physiology and solves these iteratively for body temperature and other parameters as appropriate. The modelling is simple, intuitive, requires relatively few inputs and is applicable to a wide variety of animals in a wide variety of thermal environments, static and dynamic. It might be used to predict the body temperature of a medium to large ectotherm (or endotherm) moving between two fluctuating environments, or to quantify the contribution of metabolic heat production to the thermal relations of a heterothermic mammal active, resting, torpid or arousing in a variable thermal environment. It incorporates a Q_{10} effect of body temperature on metabolism when necessary. It incorporates thermal inertia according to the size of the animal and its thermal conductance. It therefore recognises that, for many animals, thermal equilibrium with their environments may rarely, if ever, be reached, though situations where equilibrium is achieved are also included. To cope, the modelling was developed in three stages each building upon the previous. The stages are (i) steady state, where an animal is in equilibrium with an unchanging environment, (ii) simple dynamics where changes in parameter values are limited to changes from one constant value to another constant value and (iii) complex dynamics where parameters change throughout, either as functions of time or as functions of each other. Few, if any, published modelling exercises combine these attributes.

The Model

The model itself is very simple:

$$M = K_o(T_b - T_e) + E + C\frac{dT_b}{dt}$$

Eq 3.1

where M is the metabolic heat production of the whole animal, T_b is its overall core body temperature assuming no internal temperature gradients, E is the total evaporative heat lost by the animal and C is its total heat capacity; the product of its mass and weighted average tissue heat capacity usually assumed to be 3.35 $J.g^{-1}.^{o}C^{-1}$ (Schmidt-Nielsen, 1990; Schleucher*, et al.*, 1991) while t is time. T_e is the operative environmental temperature and K_o is the animal's overall thermal conductance in that environment; both parameters being *sensu* Bakken (1976). The model simply states that in an animal, metabolic heat production is balanced by dry environmental (sensible) heat exchange, latent heat exchange and heat exchanged with the body store quantified by changes in T_b. In essence this is an integrated, lumped parameter model commonly used for both homeothermic endotherms ($dT_b/dt = 0$) and heterothermic ectotherms ($M \rightarrow 0$, $E \rightarrow 0$) (Bakken, 1976). The distinction is somewhat artificial and the combined model (eq 3.1) may be used for heterothermic endotherms and for ectotherms with significant metabolic heat production and evaporative cooling as well.

Here, latent heat exchange or evaporative cooling, E, may be calculated according to one of two methods. The simplest is as a product of total evaporative water loss, *TEWL*, and latent heat of evaporation, λ_{Tb}. For animals that do not sweat or spread saliva, cutaneous evaporative water loss, *CEWL*, may be negligible, in which case E is the result of respiratory water loss, *REWL*, alone. In these cases:

$$E = \lambda_{Tb} V_i \left(\chi_{(Tb-Tue)} - h_{Ta} \chi_{Ta} \right)$$

Eq 3.2

where λ_{Tb} is the latent heat of evaporation at temperature T_b, V_i is the respiratory minute volume, h_{Ta} is the relative humidity at temperature T_a, and χ_T (in g $H_2O.m^{-3}$) is the absolute humidity of air saturated at temperature T. The temperatures specified in the subscripts include T_a and T_{ue}. Here T_a is distinct from T_e which is an integrated, lumped measure of the thermal environment of an animal incorporating the effects of radiation, conduction and so on. T_a is air temperature which is the parameter of relevance for determining the saturation of inspired air during respiration. T_{ue} is defined as the effect on the temperature of expired air of any nasal turbinates the animal may possess and is measured in degrees Celsius. Thus E is calculated according to the difference in water vapour density between inspired and expired air (assumed to be saturated) and the volume of such air that passes through the respiratory tract per unit time.

Although there are equations describing the water vapour pressure (e.g. Buck, 1981; Blackadar, 1983; Abbott & Tabony, 1985) it is convenient for programming purposes to define χ_T according to a fifth order polynomial equation fitted to values derived from tables (Beer, 1990) for temperatures from 275 K to 370 K in five degree increments (Appendix 1). Similarly, λ_T is a (weak) function of temperature and here is taken to be defined according to a third order polynomial fitted to values from temperatures over the same range. Evaporation is taken to occur at T_b assuming most of the respiratory tract is at this temperature. For animals where $CEWL > 0$, evaporation is also assumed to occur at T_b for all *TEWL* (= *CEWL* + *REWL*). This retains the simplicity of the modelling and programming without sacrificing too much precision in the thermal balance. λ_{Tb} is only a weak function of T_b and the difference between deep body temperature and skin temperature is likely to have only a minor effect on the calculations.

The programming is designed to solve equation 3.1 for T_b. Because E is a high order polynomial function of T_b, equation 3.1 cannot be solved by simple algebra. Therefore the program was written to solve the model by iteration. The iterative approach is extended (below) when simulations in which all the terms of equation 3.1 (including M) are considered as functions of T_b.

Stage (i) – A Steady State Equilibrium/Starting Point

The easiest situation to simulate is, perhaps, that of a classical homeothermic endotherm in a constant environment. By definition, a homeotherm does not change its T_b so $dT_b/dt = 0$ and equation 3.1 becomes

$$M = K_o(T_b - T_e) + E$$

Eq 3.3

Equation 3.3 is often used for endotherms studied in well designed metabolic chambers. These chambers are isothermal so in these special circumstances $T_e = T_a$. While the model equations (Equations 3.1 & 3.3) are well established and need no further verification, the algorithms used in the programming to solve them require testing. In order to verify the programming to this stage, including the accuracy of both algorithms to determine E and the iteration to solve for T_b, simulations were compared with data from the literature for various endotherms in steady state, including marmots, spinifex pigeons and kangaroos.

Marmots

Armitage *et al.* (1990) compared the thermoregulation of montane-mesic and lowland-xeric populations of yellow-bellied marmots, *Marmota flaviventris*, on restricted and *ad libitum* water supplies. Only the results for animals with *ad libitum* water supply are considered here. Armitage *et al.* measured oxygen consumption, V_{O2}, *TEWL* and T_b for the animals in respirometry chambers, reporting most of these parameters for a wide range of T_e (= T_a in metabolic chambers). Their values of V_{O2} were converted into M using the mass of the animals (4200 g for montane-mesic animals and 2790 g for lowland-xeric animals) and oxygen equivalence of 20.9 J.ml O_2^{-1} (assuming an *RQ* of 0.88: Withers, 1992a). Armitage *et* *al*. measured T_b telemetrically. Although wet thermal conductance, K_{wet}, and dry thermal conductance, K_{dry}, were reported approximately by Armitage *et al.* (1990) there is enough information to calculate K_{dry} (= K_o in homeotherms in an isothermal metabolic chamber) precisely using their (constant) value for λ (2411.2 J.g H_2O^{-1}). Because K_{dry} (= K_o) were calculated from M, E and T_b in any given T_e, a certain circularity is implied when using K_o, M, and E to predict T_b in that T_e. However, it should be noted this is of little relevance here as it is the programming code, not the well established modelling, that is being tested. It is the algorithms written to solve the model iteratively that need to be checked (before proceeding) to ensure that for different combinations of parameter inputs, the correct (expected) T_b is calculated. Therefore, calculated values of M, K_o and E (from λ and $TEWL$) were used as inputs into the program to determine expected values of T_b (Table 3.1) which were compared to actual T_b observed by Armitage *et al.* (1990). In both populations of marmots considered over a wide range of T_e the programming solved for T_b within 0.1 °C for the parameter values used.

Table 3.1 Parameters input to determine expected T_b from modelling compared to observed T_b for various marmots in steady state at different T_e.

	M (W)	K_{dry} (W.°C^{-1}) (calc)	$TEWL$ (g.hr^{-1})	**Exp T_b**	**Obs T_b**
Montane-mesic					
5	18.63	0.585	0.378	**36.4**	**36.4**
10	13.85	0.515	0.462	**36.3**	**36.3**
15	10.22	0.475	0.588	**35.6**	**35.7**
20	10.24	0.611	0.882	**35.8**	**35.8**
25	8.00	0.695	1.050	**35.5**	**35.5**
30	9.58	1.432	1.260	**36.1**	**36.1**
Lowland-xeric					
5	16.15	0.499	0.335	**36.9**	**36.9**
10	12.80	0.465	0.558	**36.7**	**36.7**
15	8.44	0.369	0.753	**36.5**	**36.5**
20	8.63	0.484	1.172	**36.2**	**36.2**
25	5.15	0.378	1.479	**36.0**	**36.0**
30	5.10	0.640	1.786	**36.1**	**36.1**

Spinifex Pigeons

Withers and Williams (1990) conducted a study of nine spinifex pigeons, *Geophaps plumifera*. From their data they presented a regression equation for V_{O2} of pigeons at T_a (= T_e) below their thermoneutral zone. This equation, together with the oxygen equivalence value used by Withers and Williams (20.1 J.ml O_2^{-1}) was used to calculate M for individual birds of mean mass, 89 g. Withers and Williams (1990) also provided regression equations for both K_{dry} (= K_o) and *TEWL* which together with M and T_e could be used to predict T_b. These expected T_b were compared with the observed T_b of the pigeons over a range of T_e.

Withers and Williams (1990) also determined ventilatory parameters from whole body plethysmography and from this were able to provide regression equations for V_i at T_e < 30 °C. In addition they measured the temperature of the air expired by the pigeons, T_{ex}, so it was also possible to determine values for T_{ue} (= Observed $T_b - T_{ex}$). Together with an assumed h_{Ta} of 15 % at each T_a, these parameters were used to predict T_b of pigeons using the V_i as an input.

There was a complication, however, in that spinifex pigeons have significant *CEWL* (Withers & Williams, 1990). Therefore several steps were necessary to verify the algorithms that use V_i as an input (that is when using equation 3.2). The first was to use the program to see if the observed T_b were predicted using values of *TEWL*. If using *TEWL* accurately predicted the observed T_b then the second step was to predict the T_b the pigeons would obtain if there were no *CEWL*, that is by using *REWL* as if it were *TEWL*. The final step was to use the ventilatory parameters to predict T_b using V_i, again ignoring *CEWL*. Two criteria should be met to verify the respiratory algorithms; (i) the T_b predicted by using *TEWL* should match the actual T_b observed and (ii) the T_b predicted from using V_i (h_{Ta} and T_{ue}) should match those predicted from the *REWL* alone using the *TEWL* algorithms.

For the spinifex pigeons, the T_b predicted (Exp T_b) by the program were within 0.3 °C of the observed T_b (Obs T_b) over all the T_e selected when using *TEWL* to determine E (Table 3.2) and within 0.1 °C for T_e between 10 °C and 30 °C. On the basis of *REWL* alone, these same algorithms predict T_b between 2.1 °C and 4.5 °C higher (Table 3.3) (incidentally giving some indication of the effect of *CEWL*). When using the ventilation parameters of V_i, h_{Ta} and T_{ue} as inputs, the respiratory algorithms of the program predicted T_b 0.5 ^{o}C or less lower than those predicted by the *TEWL* algorithm based on *REWL* alone for the same animals (Table 3.3).

Table 3.2 Parameters input to compare expected T_b from modelling to observed T_b for pigeons in steady state at different T_e.

T_e	M (W)	K_{dry} (W.^{o}C^{-1})	$TEWL$ (g.hr^{-1})	**Exp T_b**	**Obs T_b**
0	1.645	0.0383	0.153	**40.2**	**40.5**
10	1.302	0.0381	0.214	**40.4**	**40.5**
20	0.959	0.0378	0.274	**40.5**	**40.5**
30	0.616	0.0375	0.335	**40.4**	**40.5**

Table 3.3 Parameters input to compare expected T_b from modelling with *REWL* to expected T_b from modelling with V_i for pigeons in steady state at different T_e.

T_e	M (W)	K_{dry} (W.^{o}C^{-1})	$REWL$ (g.hr^{-1})	**Exp T_b ($REWL$)**	V_i (ml.min^{-1})	h_{Ta} (%)	T_{ue} (^{o}C)	**Exp T_b (V_i)**
0	1.645	0.0383	0.032	**42.3**	77.9	15	28.9	**42.0**
10	1.302	0.0381	0.041	**43.4**	68.3	15	22.0	**42.9**
20	0.959	0.0378	0.062	**44.2**	58.7	15	15.1	**43.7**
30	0.616	0.0375	0.080	**44.9**	49.1	15	8.2	**44.4**

Kangaroos

Dawson *et al.* (2000a) and Dawson *et al.* (2000b) collected comparable data from red, *Macropus rufus*, and grey kangaroos, *M. giganteus*, over a variety of T_e. For both red and grey kangaroos, expected T_b as predicted by the program using *TEWL*, were within 0.1 ^{o}C of those observed for all T_e selected (Table 3.4). Using *REWL* alone in the same algorithms, the program predicted T_b up to 7.7 ^{o}C higher in red kangaroos and between 1.7 ^{o}C and 4.0 ^{o}C higher in grey kangaroos for different T_e, again quantifying the predicted cooling effect of *CEWL* (Table 3.5). Using respiratory parameters as input, the program predicted T_b within 0.2 ^{o}C of these elevated T_b for four out of five T_e with red kangaroos and for three out of five T_e with grey kangaroos. At the other two T_e with the grey kangaroos, this difference between the predicted T_b according to the two different algorithms in the model was 0.6 ^{o}C.

These examples show that the program solves the steady state model for T_b well enough. Especially when using *TEWL*, the program predicts T_b within a few tenths of a degree of that observed in a variety of taxa across a large size range (89 g pigeons to 26000 g kangaroos). Although when using V_i (in equation 3.2) the program performs less well, it still predicts T_b within 0.5 ^{o}C on 11 out of 14 occasions examined and within 0.6 ^{o}C on 13 out of 14 occasions. It frequently predicts T_b to within 0.2 ^{o}C (Tables 3.1 to 3.5).

Table 3.4 Parameters input to determine expected T_b from modelling compared to observed T_b for kangaroos in steady state at different T_e.

T_e	Mass (kg)	*M* (W)	K_{dry} (W.^{o}C^{-1})	*TEWL* (g.hr^{-1})	**Exp T_b**	**Obs T_b**
Red kangaroo						
-4.2	23.3	57.784	1.322	5.89	**36.5**	**36.5**
16.3	23.6	35.164	1.460	8.43	**36.5**	**36.5**
25.2	23.6	25.960	1.616	11.93	**36.3**	**36.3**
34.5	24.1	29.884	6.507	25.10	**36.5**	**36.5**
44.8	23.8	32.368	2.975	83.56	**36.8**	**36.8**
Grey kangaroo						
-5.2	26.5	52.735	1.135	7.88	**36.6**	**36.6**
15.1	26.0	34.32	1.385	6.96	**36.5**	**36.5**
26.1	26.4	31.68	2.071	15.71	**36.3**	**36.3**
33.4	25.8	32.25	4.718	23.41	**36.9**	**36.9**
43.5	26.2	36.156	3.398	86.13	**37.1**	**37.1**

Table 3.5 Parameters input to determine expected T_b from modelling compared to observed T_b for kangaroos in steady state at different T_a using respiratory parameters and ignoring *CEWL*.

T_e	*M* (W)	K_{dry} (W.^{o}C^{-1})	*REWL* (g.hr^{-1})	**Exp T_b (*REWL*)**	V_i (ml.min^{-1})	h_{Ta} (%)	T_{ue} (^{o}C)	**Exp T_b (V_i)**
Red kangaroo								
-4.2	57.78	1.322	5.199	**36.9**	3471	20	8.5	**36.7**
16.3	35.16	1.460	5.617	**37.8**	2856	20	4.0	**37.7**
25.2	25.96	1.616	4.915	**39.2**	2407	20	3.3	**39.1**
34.5	29.88	6.507	10.755	**38.0**	4965	20	2.2	**38.1**
44.8	32.37	2.975	49.921	**44.5**	26299	20	1.8	**42.0**
Grey kangaroo								
-5.2	52.74	1.135	2.760	**39.7**	3949	20	20.4	**39.1**
15.1	34.32	1.385	3.481	**38.2**	2392	20	10.5	**38.2**
26.1	31.68	2.071	3.535	**40.3**	2429	20	4.3	**39.7**
33.4	32.25	4.718	10.747	**38.7**	5366	20	2.9	**38.7**
43.5	36.16	3.398	64.309	**41.5**	27405	20	1.9	**41.7**

That the program performs better when using the *TEWL* algorithm is due to the relative simplicity of that algorithm. *E* is simply the product of the λ_{Tb} and *TEWL* where *TEWL* is easily measured in most experimental set-ups. λ_{Tb} is a relatively weak function of the temperature at which the vaporisation occurred; most authors assume a constant value such as 2450 $J.g^{-1}$ H_2O (Withers & Williams, 1990) or 2420 $J.g^{-1}$ H_2O (Dawson*, et al.*, 2000a). That the program calculates λ_{Tb} as a function of temperature in this simpler algorithm is only a consequence of the program calculating both λ_{Tb} and χ_T as functions of temperature in the more complex V_i algorithm. When using V_i, h_{Ta} and T_{ue} as inputs, the calculations are more complex, involving high order polynomials of various temperatures, one of which (T_b) is unknown (equation 3.2). Also of consequence in the present context, is the uncertainty of some parameters in the literature. As well as h_{Ta} being only approximately known (Withers & Williams, 1990), V_i is estimated and, if determined from the product of tidal volume and respiration rate, associated with errors of perhaps > 20 % (Maloney & Dawson, 1994b). Added to this may be complications of gaining accurate measurements of T_{ex} (Withers & Williams, 1990; Maloney & Dawson, 1994b). Incorporating all of these factors into the iteration to solve for T_b would lead to greater probability of inaccuracy. Additionally, in these examples, the predictions of the program using ventilatory parameters are compared to the predictions using *TEWL* which, while accurate, still have (rounding and precision) errors. Despite all this, the program predicts T_b to within 0.5 ^oC or 0.6 ^oC almost always, and to within 0.2 ^oC frequently.

Almost all laboratory studies of thermoregulation in endotherms below thermoneutrality meet the criteria for a stage (i) simulation, providing a wealth of data for similar exercises. Stage (i) simulations predicted T_b to within a mean of 0.3 ^oC for neonate mallee fowl, *Leipoa ocellata* (Booth, 1984), to within a mean of 0.6 ^oC for numbats, *Myrmecobius fasciatus* (Cooper & Withers, 2002), to within a mean of 0.7 ^oC for Australian diamond doves, *Geopelia cuneata* (Schleucher*, et al.*, 1991), and less accurately for tawny frogmouths, *Podargus strigoides* (0.9 ^oC) (Bech & Nicol, 1999), emus, *Dromaius novaehollandiae* (1.4 ^oC) (Maloney & Dawson, 1994b; a) and a species of neotropical bat, *Noctilio albiventris* (1.7 ^oC) (Chappell & Roverud, 1990). In every case, these predictions can be improved by adjusting one or more input parameter values slightly and by increments less than those attributable to experimental error. That is, adjusting either *M*, or K_o, or *TEWL*, or V_i by a value less than the reported standard error of that parameter is sufficient for the model to predict T_b exactly. Examining the magnitude of these adjustments highlighted the sensitivity of the (commonly used physiological equations in the) modelling to various parameters: small adjustments in K_o had a particularly large effect while large adjustments in parameters associated with evaporative cooling (V_i, $TEWL$) had very little effect on T_b. In some of these cases, input parameters were estimated from data depicted graphically and this adversely affected the accuracy of model predictions.

Stage (ii) – Adding Simple Dynamics

Stage (ii) simulates the response of T_b to simple, single step function changes in one or more of the other parameters. It does this by calculating the time course of T_b towards an equilibrium. This equilibrium is defined by a stage (i) type simulation (above) to solve:

$$M_{eq} = K_{o.eq}\left(T_{b.eq} - T_{e.eq}\right) + E_{eq}$$

Eq 3.4

for equilibrium body temperature, $T_{b.eq}$, where M_{eq}, $K_{o.eq}$, $T_{e.eq}$ and E_{eq} are the endpoint equilibrium values of M, K_o, T_e and E given that one or more of these parameters has changed once. For any system to approach this equilibrium when (importantly) M_{eq}, $K_{o.eq}$, $T_{e.eq}$ and E_{eq} change no further, T_b at time t, $T_{b.t}$, is given by:

$$T_{b.t} = T_{b.eq} - \left(T_{b.eq} - T_{b.t-1}\right)e^{-\delta t/\tau}$$

Eq 3.5

according to Newton's law of cooling where τ is the thermal time constant and $\tau = C/K_{o.eq}$ (Bakken, 1976). Thus, if the initial T_b, $T_{b.0}$, is known, a time course for $T_{b.t}$ can be calculated as the animal approaches a new equilibrium with its environment. This is the basis of the heating and cooling curves derived when, for example, a lizard is placed into an environment of a particular temperature (below). Note, however, that the equilibrium temperature is a body temperature, not an environmental one.

To verify simulations produce accurate values for $T_{b.t}$ when lizards are placed in different environments, predicted $T_{b.t}$ for simulated lizards were compared to actual $T_{b.t}$ reported in the literature. Once again, these are tests of the programming code, not of the well established model equations.

Bearded Dragons

The thermoregulation of the bearded dragon, *Pogona barbata* (formerly *Amphibolurus barbatus*), was studied by Bartholomew and Tucker (1963) who demonstrated that it heated faster than it cooled. In their study, Bartholomew and Tucker allowed lizards to reach thermal equilibrium at a T_e of 20 ^oC. They then placed the animals in a T_e of 40 ^oC and measured T_b, respiration rate and heart rate until the lizards once again reached equilibrium. Then they reversed the procedure, moving the animals from a T_e of 40 ^oC back to one of 20 ^oC. V_{O2} was measured separately at various T_e between 15 ^oC and 40 ^oC. In order to include evaporative cooling in simulations of such situations, it was necessary to assume that all evaporative heat loss was respiratory, and calculate V_i for the animals based on the information given in the study. Assuming h = 70 % at T_a = 40 ^oC and T_{ex} = 20 ^oC (conditions that pertain to the start of the simulation when an animal is first placed into T_e = 40 ^oC), expired air is saturated and 20 % of O_2 inspired is used as 150 cc.hr^{-1} of metabolism (Bartholomew & Tucker, 1963), V_i can be solved from:

$$\begin{aligned} 0.2\times[O_2]\times V_i &= 150\text{cc.hr}^{-1} \\ 0.2\times 0.20946\times V_i &= 150\text{ml.hr}^{-1} \\ V_i = \frac{150}{0.2\times 0.20946} &= 3581\text{ml.hr}^{-1} = 59.68\text{ml.min}^{-1} \end{aligned}$$

Eq 3.6

for a 500 g bearded dragon. Mean values of K_{dry}, as calculated by Bartholomew and Tucker (1963), and values of M, as regressed and converted (to W.animal^{-1}), were used along with V_i, h_{Ta} and T_e to simulate the heating and cooling of a 480 g bearded dragon between equilibria when T_e = 20 ^oC, when T_e = 40 ^oC and when T_e = 20 ^oC again. However, given that air that is 70 % saturated at 40 ^oC would be super-saturated at 20 ^oC (Beer, 1990) and that Bartholomew and Tucker (1963) allowed room air to enter the experimental chamber, it would be more appropriate to assume that air was 70 % saturated at both temperatures.

To determine $T_{b.t}$, $T_{b.0}$ and $T_{b.eq}$ are required for the cooling curve (equation 3.5). To determine $T_{b.0}$ in each case, it was assumed that the animal was in equilibrium at the start and a stage (i) simulation was used to solve for $T_{b.0}$. Thus, for a measured M of 0.12 W when resting and a V_i of 57.3 ml/min (59.68×480/500), a dragon at 20 °C would have a $T_{b.0}$ of 20.1 °C. At a T_e of 40 °C, the simulated dragon with an increased M of 0.45 W as measured and a proportional increase in V_i has a $T_{b.eq}$ of 40.2 °C. This change in M and T_b represents a Q_{10} of 1.93 which compares to a Q_{10} of 1.95 as determined for real bearded dragons by Bartholomew and Tucker (1963). Thus three parameters, M, V_i and T_e, were all changed together as step functions. Bartholomew and Tucker (1963) calculated a K_{dry} of 2.06 W.°C^{-1} when the animals warmed and 1.59 W.°C^{-1} when they cooled. In the simulation of a warming animal, these values gave a thermal time constant, τ, of 13.3 minutes and near equilibrium (where dT_b/dt < 0.1 °C per hour) was reached in 92 minutes. When the dragons cooled from $T_e = 40$ to $T_e = 20$, τ became 17.3 minutes and the virtual lizard took 114 minutes to approach equilibrium.

When presented in the same manner as Bartholomew and Tucker (1963, their Fig 3), the heating and cooling curves for a 480 g simulated lizard are highly reminiscent of those for a real one (Figure 3.1). The warming lizard reached within 1 °C of 40 °C after about 38 minutes

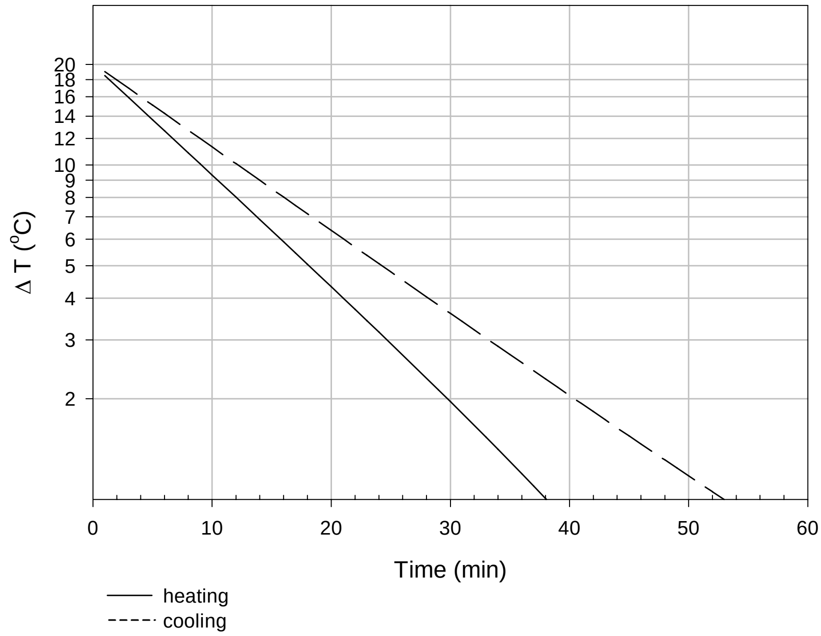


Figure 3.1 Relation of difference between T_b and T_a (ΔT) to time in a modelled 480 g *Pogona barbata* during heating and cooling. During heating, T_a = 40 °C; during cooling, T_a = 20 °C.

both in the simulation and in reality. The cooling lizard reached within 1 ^{o}C of 20 ^{o}C after about 52 minutes in reality and 53 minutes in the simulation. As the heating animal passed 30 ^{o}C, the (instantaneous) rate of change of T_b was 0.71 ^{o}C.min^{-1} in the virtual lizard and 0.72 ^{o}C.min^{-1} in the real one. When cooling, the rates of change were 0.61 ^{o}C.min^{-1} in the virtual lizard and 0.62 ^{o}C in the real one as they passed 30 ^{o}C (Bartholomew & Tucker, 1963).

Tuatara

To check that similar results obtained when only one or two parameters were changed as step functions, the warming and cooling of a tuatara, *Sphenodon punctatum*, was modelled and compared to data collected by Wilson and Lee (1970). They measured heart rate, V_{O2} and K_{wet} (but not *TEWL*) for tuataras in warming and cooling environments. In one such experiment a 520 g tuatara initially at equilibrium in a T_e of 26 ^{o}C was transferred to a T_e of 4 ^{o}C and allowed to cool towards a new equilibrium. This was simulated. *TEWL* was input as 0 throughout which is valid when K_{wet} (incorporating evaporative heat loss) is used in lieu of K_o. The initial $T_{b.0}$ was predicted using a stage (i) type simulation, as the system started in equilibrium, while $T_{b.eq}$ was predicted by a second stage (i) simulation. Because heart rate was observed to remain at levels associated with activity in the real animal while it cooled, *M* was not changed between beginning and end so that, in this case, only one parameter, T_e, was varied throughout the simulation. T_e was set to 4 ^{o}C for all $t > 0$. The model predicted T_b would reach 6 ^{o}C (within 2 ^{o}C of ambient at 4 ^{o}C) after about 82 minutes which compares favourably with the 82 minutes observed in the real tuatara (Wilson & Lee, 1970).

The same animal was later placed back into the warm environment but in this case, heart rate increased as the animal warmed (Wilson & Lee, 1970). Therefore in the simulation to mimic this experiment, two variables, *M* as well as T_e, were changed in a step function fashion. The simulation predicted 71 minutes required for the tuatara to reach 24 ^{o}C: in reality it took 73 minutes (Wilson & Lee, 1970).

A 340 g tuatara was put through the same routine (Wilson & Lee, 1970) but with this individual, heart rate decreased during cooling and increased during heating. When simulated, both T_e and *M* were varied as single step function changes as appropriate. When cooling, both the simulated and the real tuatara reached 7 ^{o}C in about 73 minutes. The simulated 340 g tuatara warmed to 23 ^{o}C (within 3 ^{o}C of ambient) in 60 minutes whereas the real one took 61 minutes (Wilson & Lee, 1970).

Stage (ii) simulations are of limited practical use as parameters are constrained (unrealistically) to single changes at the start of the simulation. Stage (ii) is only a development stage to set the foundations for stage (iii) type simulations. Thus data available for verification of stage (ii) modelling are more limited. The best studies to use are probably heating/cooling hysteresis studies conducted on ectotherms less than 1 kg as used above. Studies of larger animals, even in simple heating and cooling experiments, become complicated by the increased magnitude of their physiology. For example, once metabolic rate, likely to change during and throughout the experiment, becomes significant, the study violates the assumptions of a stage (ii) simulation. It was only after incorporating more complex (non-step function) changes to K_o that a simulation predicting the heating curve of a 1060 g varanid, *Varanus gouldii*, became accurate (Bartholomew & Tucker, 1964). That is a stage (iii) simulation was more appropriate, even for this simple experiment.

Stage (iii) – Adding the Complexity

A stage (ii) simulation, where values of parameters may change only once, is clearly generally inadequate. Parameters such as M and E may change in a complex way with T_b such that they actually change (several times/continuously) during the simulation rather than at the beginning. In animals where M is a significant contributor to heat balance, such changes become particularly important. Additionally, in natural situations at least, environmental parameters, such as T_e, are likely to change before equilibrium is achieved. Should any or all of the parameters (M_{eq}, $K_{o.eq}$, E_{eq} and $T_{e.eq}$) that define the equilibrium T_b, $T_{b.eq}$, change, then at that point, a new equilibrium needs to be determined by solving equation 3.4 for the new values of M_{eq}, $K_{o.eq}$, E_{eq} and $T_{e.eq}$. Once a new equilibrium has been determined, equation 3.5 can be used to determine $T_{b.t}$ as it approaches the new equilibrium. As parameters may change frequently, continuously, or at any time t, this process is repeated for all values of $t = 1,2,...t_{max}$, where t_{max} is the length of the simulation (as desired by the user), to give a new equilibrium $T_{b.eq}$ for each (and every) t, $T_{b.eq.t}$, from changing values of M_{eq}, $K_{o.eq}$, E_{eq} and T_e, $M_{eq.t}$, $K_{o.eq.t}$, $E_{eq.t}$ and $T_{e.t}$ (= $T_{e.eq.t}$) respectively. Note that it is not necessary for the simulation to actually reach equilibrium at any time.

Introducing the complexities of a dynamic thermal environment and of the dynamics of metabolic heat production, evaporative heat loss and so on is simply a matter of defining these parameters as functions of time or as functions of other parameters. For example, $T_{e.t}$ can be defined as a constant through time, as a step function of time, as a linear function of time, as a sinusoidal function of time, etc. (Appendix 1). V_i can be defined as a function of M such that ventilation increases directly (Stahl, 1967; Withers, 1992a; Chappell & Dawson, 1994) or indirectly (Mortola & Frappell, 2000; Hicks & Bennett, 2004) with metabolic rate (see below). Otherwise, V_i can be defined as a function of $T_{b.t\text{-}1}$ increasing at high T_b to simulate panting in appropriately equipped animals. Alternatively, V_i can be defined as a combination of these two functions. K_o can be defined to respond to given set point temperatures and/or to body temperature history ($T_{b.t\text{-}1}$ and $T_{b.t\text{-}2}$) to enhance heat gain or heat loss in particular situations. M can be adjusted to maintain homeothermy in classical endotherms (resting metabolic rate, *RMR*), or to simulate activity, torpor or arousal in a wide variety of animals. In many animals, M is subject to an effect of temperature; the so called Q_{10} effect. Thus, while $T_{b.eq.t}$ is a function of $M_{eq.t}$ according to equation 3.4, $M_{eq.t}$ is also a function of $T_{b.eq.t}$ in these situations. To incorporate this feedback loop when required

$$M_{eq.t} = M_{eq.0} Q_{10}^{\frac{\left(Tb_{eq.t} - Tb_{eq.0}\right)}{10}}$$

Eq 3.7

is solved simultaneously, and iteratively, with equation 3.4 for $M_{eq.t}$ and $T_{b.eq.t}$. Sometimes this is not possible (Chaui-Berlinck*, et al.*, 2002) unless V_i is also defined as a function of M (equation 3.11 below).

In all cases, once $T_{b.eq.t}$ is known for all t, equation 3.5 can be used to determine $T_{b.t}$, which is the primary output. Once $T_{b.t}$ is known, secondary output can also be calculated by substituting $T_{b.t}$ into:

$$M_t = K_{o.t}(T_{b.t} - T_{e.t}) + E_t + S_t$$

Eq 3.8

(where $M_t = M$ at time t etc.) via the defined dynamics, or otherwise, of these separate parameters. The program currently outputs metabolic rate, M_t, dry environmental heat exchange, $K_{o.t}(T_{b.t} - T_{e.t})$, evaporative heat loss, E_t, heat absorbed or released by the animal, $S_t = CdT_{b.t}/dt = M_t - K_{o.t}(T_{b.t} - T_{e.t}) - E_t$, $T_{e.t}$ and $K_{o.t}$ as well as $T_{b.t}$.

Equation 3.7 provides a range of $M_{eq.t}$ and $T_{b.eq.t}$ that satisfy a given Q_{10} when compared to $M_{eq.0}$ and $T_{b.eq.0}$. $M_{eq.0}$ and $T_{b.eq.0}$ are equilibrium values of M and T_b that pertain at the start of the simulation, and are equivalent to starting values of M and T_b respectively only if the simulation starts in equilibrium. For simulations that do not, known equilibrium values of M_{eq} and $T_{b.eq}$ may be substituted (for $M_{eq.0}$ and $T_{b.eq.0}$ respectively), and all $M_{eq.t}$ and $T_{b.eq.t}$ satisfying the Q_{10} with respect to these values may be determined. Thus, if equilibrium for some later time, $t = n$ say, is known or can be determined from a stage (i) simulation, then $M_{eq.n}$ and $T_{b.eq.n}$ may be substituted for $M_{eq.0}$ and $T_{b.eq.0}$ respectively. Alternatively, if the standard metabolic rate, *SMR*, at a given T_b is known for the animal being simulated, then these values may be used in lieu of $M_{eq.0}$ and $T_{b.eq.0}$. For endotherms, basal metabolic rates at normothermic T_b or torpid rates at torpid T_b may be used. In any case where Q_{10} is important, equations 3.7 and 3.4 may be (and should be) used to determine transient equilibria against any known (or hypothetical) reference.

Driving functions (independent variables) may be selected according to the particulars of the simulation. For example, where ectotherms respond directly to variations in T_e, T_e becomes the independent variable driving the simulation. Other parameters might vary interactively as a result, such as K_o to optimise heat exchange with the environment, M due to the Q_{10} effect and V_i with M. Alternatively, especially for endotherms, M may be the independent variable. Changes of M independent of any temperature effect may drive the simulation, resulting in changes in predicted T_b, again after taking into account any interacting changes in K_o, V_i, T_e etc.: arousal from torpor in a hibernator provides an example. Any variable or combination of variables may be chosen to drive the simulation. Thus, the effects of vasodilation (changing K_o), of panting (changing V_i), of activity or shivering (changing M) may be determined in isolation or in combination either in static or in dynamic thermal environments (changing T_e).

Stage (iii) simulations perform correctly if they accurately predict the dynamics of body temperature in animals when subject to disruptions of equilibria. For example, when a torpid mammal in a cold room arouses using endogenous heat production, then T_b responds transiently to complex changes in metabolic rate. Equilibrium may be approached, in this example, if M reaches RMR after a period of time. A more complex dynamic situation may occur if several of the parameters that determine T_b change concurrently. For example, some mammals may enter torpor in response to a change in T_e. Here, T_e, M, and V_i all change concurrently to produce complex, transient changes in T_b, which may never reach equilibrium if T_e continues to change. Both these examples are used to compare $T_{b.t}$ predicted by the modelling with actual $T_{b.t}$ determined empirically, firstly for a small mammal arousing from torpor in a constant T_e, and secondly for a larger mammal entering torpor in a variable T_e.

Syrian hamster

The arousal of a Syrian hamster, *Mesocricetus auratus*, in a constant environment was investigated by Lyman (1948). He documented changes in V_{O2} and T_b after an individual had been stimulated by manipulation. Figure 4 in Lyman's paper depicts the V_{O2}, the cheek pouch temperature, T_{cp}, and the rectal temperature, T_{rect}, in an individual arousing from torpor in an isothermal ($T_e = T_a$) environment where T_e is about 4 °C. V_{O2} increases approximately exponentially to a peak of 8920 cc $O_2.kg^{-1}hr^{-1}$ about 163 minutes after the torpid hamster was stimulated. T_{cp} increases to reach the normothermic value of about 37 °C after approximately 178 minutes. T_{rect} increases more slowly to reach a normothermic value slightly less than T_{cp} after about 200 minutes. The lag between T_{rect} and T_{cp} was interpreted as due to differential blood flow favouring oxygenation of the anterior brown adipose tissue and anterior organs in the hamster during arousal setting up an internal gradient in T_b (Lyman, 1948). This gradient was absent during entry into torpor (Lyman, 1948).

For the simulation assume the following initial conditions:

(i) the torpid animal starts in equilibrium with $T_e = 4$ °C,
(ii) $TEWL$ (not measured by Lyman) is negligible for a torpid mammal in this cold environment, $\therefore TEWL = 0$,
(iii) K_o is minimal; for a mammal this size $K_o = 0.0744$ W.°C^{-1} (Withers, 1992a), and
(iv) torpid $M = 0.0356$ W converting Lyman's (1948) original data assuming an oxygen equivalence of 20.8 J.ml O_2^{-1}.

Equation 3.3 solves for an equilibrium T_b of 4.5 °C, within 1 °C of T_e as noted by Lyman (1948). For the arousal itself assume this is the starting point and T_e and K_o remain unchanged throughout while *TEWL* stays negligible:

(i) $T_{b.0} = 4.5$ °C,

(ii) $M_{eq.0} = M_0 = 0.0356$ W,

(iii) $T_{e.t} = 4$ °C and $K_{o.t} = 0.0744$ W.°C^{-1} for all *t*, and

(iv) $TEWL_t = 0$ for all *t*.

It remains to specify the dynamics of $M_{eq.t}$. The arousal was due to a rapid increase in endogenous heat production preceding (and causing) a change in T_b. Such an increase is, therefore, over and above the Q_{10} effect and cannot be simulated by the Q_{10} effect alone. The relative contributions to *M* from non-shivering and shivering thermogenesis are not known. Neither are they important in testing the dynamics of stage (iii) simulations. For this integrated parameter modelling, and in this example, the change in T_b is driven by the incremental change in *M*, no matter how this comes about. Therefore, it is sufficient to mimic the dynamics of *M* as determined empirically by Lyman (1948). As *M* changes (incrementally) $T_{b.t}$ responds to each M_t as if each M_t defines a new equilibrium. Thus, $M_{eq.t} = M_t$. Let M_t mimic that observed by Lyman (1948, Fig 4), that is increase exponentially from M_0 to a peak of 4.535 W at about $t = 163$ which is maintained as $T_b \rightarrow 37$ °C and then revert to *RMR*. Still required are the rate of increase of M_t during the exponential rise and *RMR*. A rate of increase in M_t of 3 %.min^{-1} (from trial and error) has $M_{164} = 4.535$ W, one minute later than in Lyman (1948). In this case the *RMR* required to maintain (constant, stable) $T_b = 37$ °C may be determined from equation 3.1 for a mammal with known K_o in a known T_e, $E = 0$, $dT_b/dt = 0$. Thus $RMR = 2.455$ W.

$\Rightarrow$

$$
\begin{aligned}
M_t &= 1.03M_{t-1} \ \text{ for } 1.03M_{t-1} < 4.535 \text{ and } T_{b.t-1} < 36.8 \\
M_t &= 4.535 \ \text{ for } 1.03M_{t-1} > 4.535 \text{ and } T_{b.t-1} < 36.8 \\
M_t &= 2.455 \ \text{ for } T_{b.t-1} \geq 36.8
\end{aligned}
$$

Eq 3.9

noting that for tractability and in anticipation of thermal inertia leading to a slight overshoot, the T_b threshold is set slightly < normothermic T_b.

The output for this simulation for $t = 1,2 \ldots 300$ minutes is depicted in Figure 3.2. The modelling predicts a normothermic T_b of 37.2 °C after 188 minutes; this being part way between the 178th minute observed for T_{cp} and the 200th minute observed for T_{rect} in the real animal and is as expected for the integrated parameter T_b. The shape of the curve $T_{b.t}$ as a function of t closely reflects a T_b curve between T_{cp} and T_{rect} in Lyman's Figure 4. Other output in the upper panel confirms that T_e and K_o remained constant throughout the modelled arousal. In the lower panel, M_t closely reflects that given by Lyman (1948, Fig 4) and does so only partly by design. While the maximum M obtained, the initial rate of increase in M and the torpid metabolic rate were determined from Lyman's data, K_o and, therefore, *RMR* for the hamster were determined using allometric equations for mammals (Withers, 1992a). This has led to a satisfactory simulation of the arousal in this individual. The other (secondary) outputs depicted include evaporative heat loss (zero throughout), heat absorbed by the body (as T_b increases) which is transient, and dry environmental heat exchange which balances metabolic heat production once equilibrium has been attained. In this panel positive values are heat

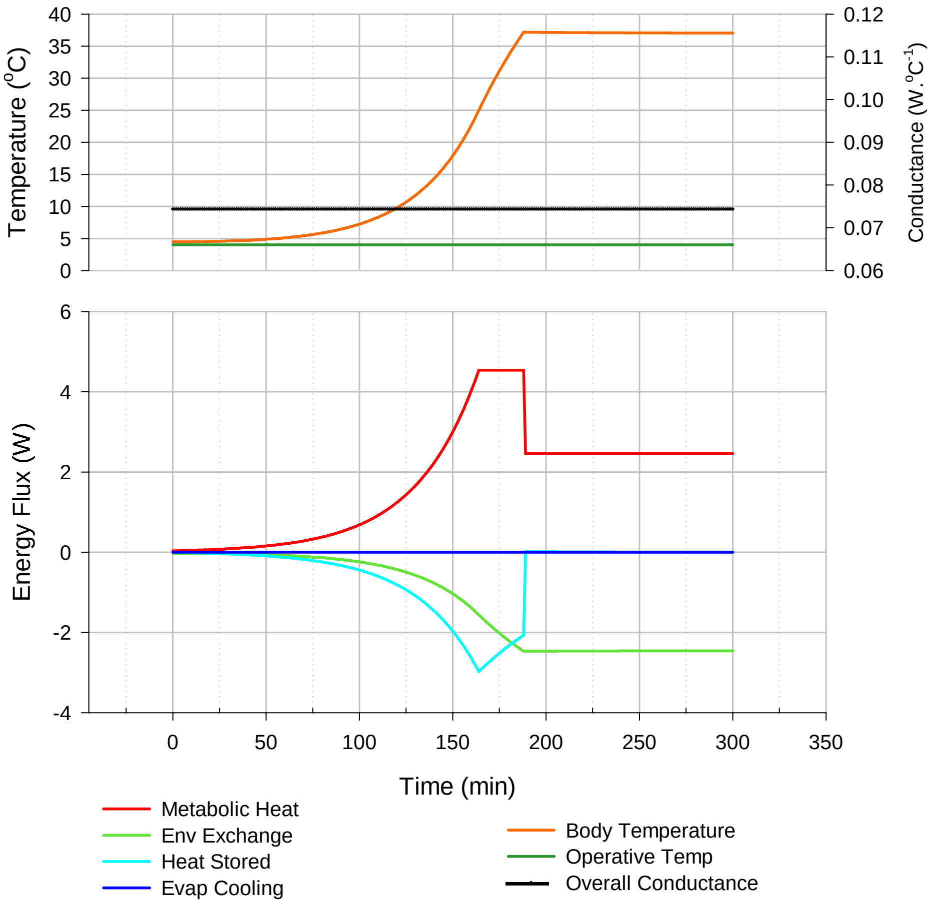


Figure 3.2 T_b, M and other parameters from a modelled arousal from torpor in a hamster

sources (metabolic heat production) while negative values are heat sinks (the body store transiently and the environment).

Echidna Entering Torpor

During a pilot study (which was not pursued: Chapter 1), some echidna heart rates were correlated with V_{O2}. Echidnas were placed in a 13 litre metabolic chamber submerged in a water bath where water temperature could be changed rapidly with 0.5 ^{o}C precision. While subject to flow rates between 0.5 l.min^{-1} and 5.0 l.min^{-1}, water temperature, and therefore T_e within the chamber, was manipulated to induce various V_{O2}. On one occasion, a 2.86 kg echidna responded to decreasing T_e with decreasing V_{O2}. Recognising a potential opportunity to record V_{O2} for a torpid echidna, T_e was manipulated (not always downwards) over some 13 hours to promote the lowest possible values of V_{O2}. Although it is doubtful that true torpor was actually reached (Figure 3.3 and below), this situation provided an opportunity to test a complex stage (iii) simulation. Here was a large heterothermic endotherm with significant thermal inertia in a dynamic thermal environment. T_e was continually changing, altering the heat balance of the animal resulting in complex interactions between M, V_i (presumably) and T_b. Thus, changes in T_e, M and V_i, together with thermal inertia, all contributed to transient, complex changes in T_b of an animal that did not reach equilibrium during 13 hours of measurement. Predicted $T_{b.t}$ from a stage (iii) simulation were compared to observed $T_{b.t}$ for this situation.

For the modelling to mimic changes in T_e, the driving variable, (Figure 3.3) let:

$$\begin{aligned}
&T_{e.0} = 26.9, \text{the initial } T_e \text{ of the experiment}\\
&T_{e.t} = 26.9 - 0.02005t \ \text{ for } t \le 400 \therefore T_{e.400} = 18.88\\
&T_{e.t} = 18.88 + 0.03739(t-400) \ \text{ for } 401 \le t \le 512 \therefore T_{e.512} = 23.12\\
&T_{e.t} = 23.12 - 0.03130(t-512) \ \text{ for } 513 \le t \le 790 \therefore T_{e.790} = 14.43
\end{aligned}$$

Eq 3.10

and let h = 30 % when T_a = 20 ^{o}C. Assume the animal starts with a T_b of 32 ^{o}C in equilibrium with its environment which may be thermoneutral (Chapter 2). Therefore, let M_0 = BMR = 3.0 W (Chapter 2).

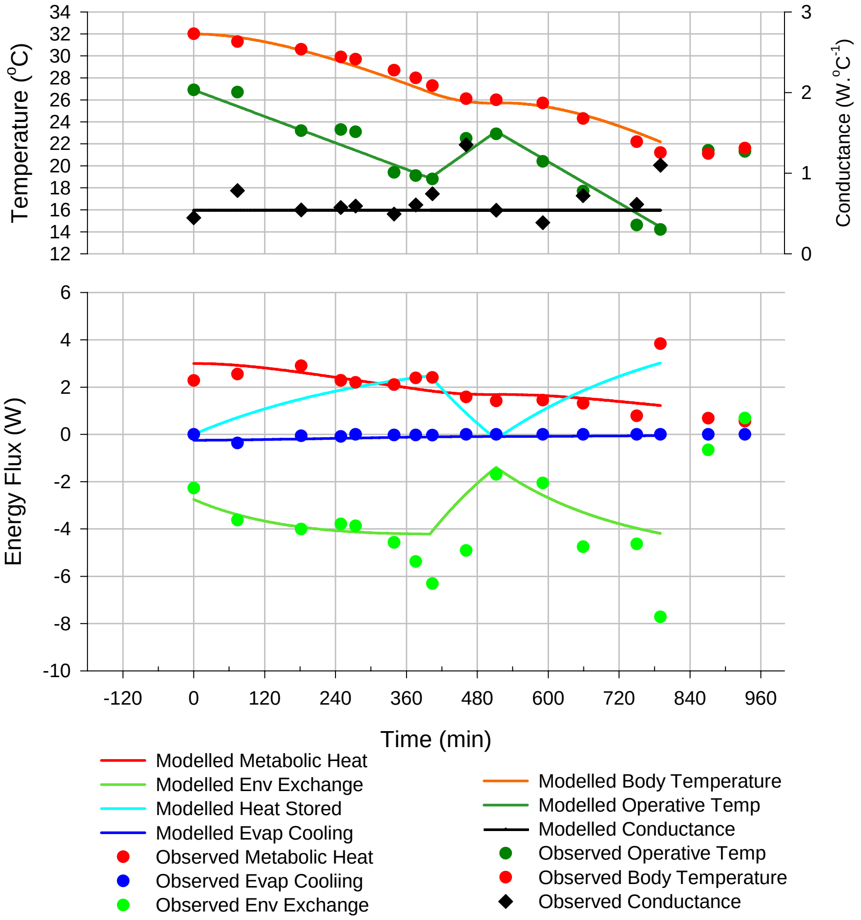


Figure 3.3 T_b, *M* and other parameters from an echidna entering torpor compared to the output of a modelled echidna entering torpor with a Q_{10} of 2.5 in the same thermal environment.

Assume that as the echidna enters torpor there is no metabolic inhibition and *M* and T_b reduce with a Q_{10} of 2.5. Let $V_{i.0} = 212$ ml.min^{-1} when $M_t = BMR$ (Parer & Hodson, 1974) and reduce in direct proportion to M_t. Thus $V_{i.t}$ is a function of M_t:

$$V_{i.t} = fpM_t + V_{i.0} - fp \times BMR$$

Eq 3.11

where *p* is the initial proportion of $V_{i.0}$ to *BMR* and *f* is a proportionality factor. If $f = 1$ then V_i varies in direct proportion to *M* (*p* retained throughout), if $f < 1$ then relative hypoventilation occurs when *M* increases and if $f > 1$ then relative hyperventilation occurs when *M* increases.

Let $K_{o.0} = \dfrac{M_0 - E_0}{T_{b.0} - T_{e.0}} = 0.54$ W.^{o}C^{-1}. This is slightly lower than expected for an echidna in thermoneutrality though not as low as occurs with thermogenesis in colder environments (Chapter 2). Although this slows their rate of entry (Snapp & Heller, 1981), several other mammals retain relatively low values of K_o when entering torpor (Wilz & Heldmaier, 2000; Geiser, 2004). Despite speculation that torpor is an energy saving strategy (Grigg & Beard, 2000; Nicol & Andersen, 2000), maximising K_o to enter torpor more quickly is likely beyond the physiological capabilities of echidnas and other mammals since high values of K_o are associated with vasodilation and normothermic heart rates (Kamau & Maloiy, 1982; Milsom, *et al.*, 1999). Therefore, let $K_{o.t} = 0.54$ W.^{o}C^{-1} for all *t*.

The modelling solves for $T_{b.eq.t}$ by solving:

$$\begin{aligned} M_{eq.t} &= 3.0 \times 2.5^{\frac{T_{b.eq.t}-32}{10}} \\ M_{eq.t} &= 0.54\left(T_{b.eq.t} - T_{e.t}\right) + E_{eq.t} \end{aligned}$$

Eq 3.12

where

$$E_{eq.t} = \lambda_{Tb.eq.t} V_{i.t}\left(\chi_{\left(T_{b.eq.t}-T_{ue}\right)} - h_{Ta}\chi_{Ta}\right)$$

Eq 3.13

(and $V_{i.t}$ is solved by equation 3.11) simultaneously for $M_{eq.t}$ and $T_{b.eq.t}$, and then solves equation 3.5 for each $T_{b.t}$. When incorporating the temperature effect M_t is generally assumed to be determined by $T_{b.t}$ (not $T_{b.eq.t}$) according to the Q_{10}. Therefore M_t is determined as per equation 3.7 but using $T_{b.t}$ instead of $T_{b.eq.t}$. Other secondary output can then be determined using equation 3.8.

Considering the complexity of $T_{e.t}$ and the incorporation of the Q_{10} effect with its need to solve equations simultaneously, the modelling predicts $T_{b.t}$, M_t, $K_{o.t}$ and E_t well (Figure 3.3). Although the actual echidna started slightly hypometabolic, its M_t and its $T_{b.t}$ declined according to the predicted Q_{10} effect with relatively minor fluctuations over the 790 minutes of the experiment. $K_{o.t}$ was observed to fluctuate about the predicted 0.54 W.^{o}C^{-1} throughout most of the experiment. Observed E_t, though insignificant in the heat balance, was observed to decrease according to predictions.

Stage (iii) simulations are designed to predict T_b for animals in complex situations, including animals for which metabolic heat is a significant contributor to thermal balance. Most heterothermic endotherms are small, so most of the data available to test this modelling are for animals smaller than needed in this project. While this modelling predicted T_b well for hibernators such as the Syrian hamster (above) and the eastern pygmy possum, *Cercartetus nanus* (Song*, et al.*, 1997), experimental and rounding errors are likely to be particularly important for small animals. For example, in a simulation of heterothermy in 8 g hummingbirds, T_b was found to change too quickly (Bech unpub. data) until a higher value of 3.9 $J.g^{-1}.^{o}C^{-1}$ (Withers, 1992a) was used for *C*. Such problems are exacerbated by the program's default value for time intervals, *t* (one minute). While this might easily be changed, there was still a need to test the modelling for larger heterothermic endotherms in order to be of use addressing the questions of the next chapter; questions that were the primary aim of this whole project. Thus, the modelling was tested primarily against novel data from echidnas collected independently of the experiments reported in the next chapter.

For all that, the utility of modelling is greater than merely predicting parameter values correctly. Modelling can be used to increase understanding of a system by highlighting discrepancies apparent in that system, and/or by comparing the results of various inputs into that system. Reported below are some examples of how this modelling might help test hypotheses, resolve discrepancies and highlight various aspects of thermal biology.

Applied Examples

Q_{10} and K_o for Endotherms Entering Torpor in Dynamic Environments

This example shows how different hypotheses might be evaluated on the basis of comparing modelled data to empirical data.

Sometimes, hibernation is distinguished from daily torpor by the occurrence of metabolic inhibition which, in turn, is correlated to the Q_{10} between normothermic and torpid animals being greater than 2 to 3 (Geiser, 2004). Values of Q_{10} should normally only be determined from animals that are in equilibrium with their environments and for mammals as large as echidnas this may occur rarely in nature and only after long, protracted experiments at constant temperature in laboratories. While echidnas are often recognised as hibernators, Q_{10} values ranging from < 2 (Kuchel, 2003) to > 6 (Nicol, *et al.*, 1992) are cited. For the echidna above that became hypometabolic in a fluctuating but cooling thermal environment, incorporating a Q_{10} of 2.5 in a modelling exercise provided an adequate fit to the empirical data. Had a Q_{10} of 5 been incorporated, the fit would have been poorer (Figure 3.4). Neither M_t nor $T_{b.t}$ fit so well. In this way variations in Q_{10} can be determined from animals not necessarily in equilibrium, with implications for the interpretation of empirical data not only from echidnas.

Similarly, a K_o of 0.54 W.^{o}C^{-1} was applied to the modelled echidna throughout which resulted in a good prediction of both the real K_o (given the errors inherent in determining K_o) and the real T_b. This value of K_o is greater than the minimum that can be achieved by echidnas

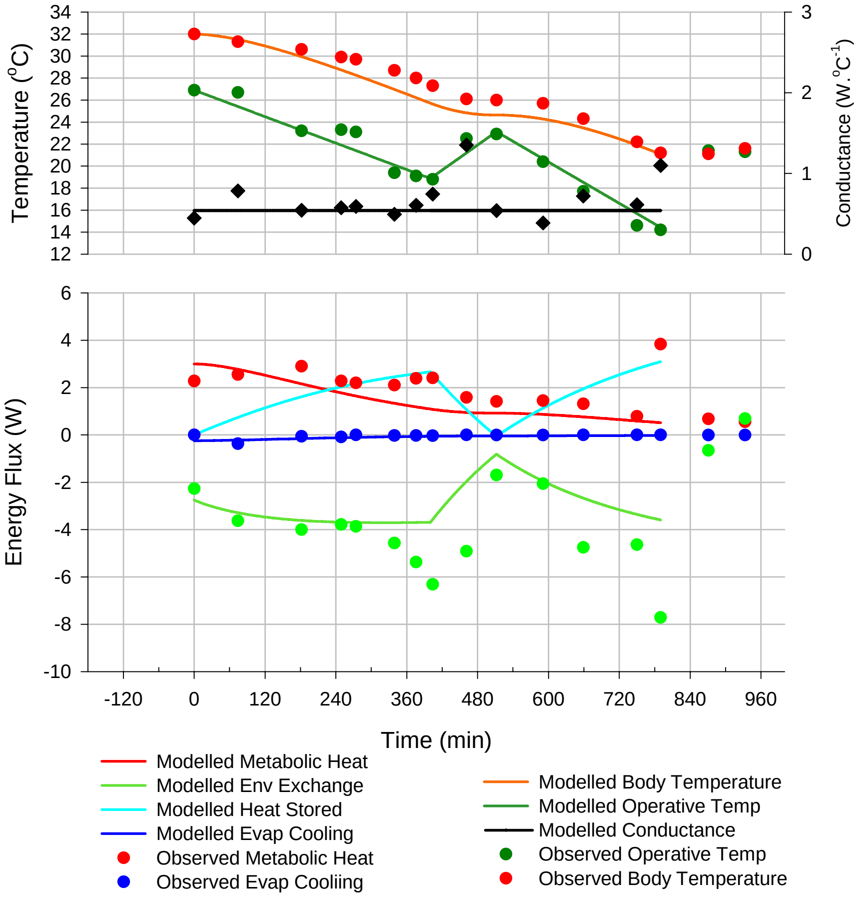


Figure 3.4 T_b, *M* and other parameters from an echidna entering torpor compared to the output of a modelled echidna entering torpor with a Q_{10} of 5.0 in the same thermal environment.

(Chapter 2) but using lower values in the modelling also results in a poorer fit to the empirical data (not shown). Hypotheses about how endotherms optimise their sensible heat exchange when entering torpor may be difficult to test in anything other than fluctuating environments. Thus, this modelling approach can be used to interpret data collected from animals not in steady state with their possibly fluctuating environments, and to distinguish between different hypotheses.

Classical Thermoregulation

This example shows how modelling can highlight inconsistencies within and between previous studies into the physiology, here specifically, of echidnas. It demonstrates how the thermoneutral zone of an echidna might not be as wide as predicted by Schmidt-Nielsen *et al.* (1966) though they did note more data were required. Additionally, it illuminates differences in respiration for resting echidnas found in several studies and aids in the interpretation of those data.

Sometimes echidnas thermoregulate in a classical way, or at least nearly so (Chapter 2). Within thermoneutrality, echidnas vary their K_o which regulates heat exchange with the environment resulting in the maintenance of a nearly stable and high T_b (Schmidt-Nielsen*, et al.*, 1966), as do many mammals (Scholander*, et al.*, 1950a). When subject to $T_e < T_{lc}$ (where T_{lc} is the lower limit of thermoneutrality), such echidnas should increase their metabolic rate and should T_e reduce even further, then in theory echidnas should increase their M still further.

Data for a 2.50 kg echidna that was apparently thermoregulating in this way (through observable shivering) were obtained when its V_{O2}, $TEWL$ and T_b was measured in a metabolic chamber submerged in a water bath as described above (Echidna Entering Torpor). Initially $T_e = 22$ °C which (when set) was assumed to be within thermoneutrality (Schmidt-Nielsen*, et al.*, 1966): these data pre-date subsequent investigation of thermoneutrality in echidnas (Chapter 2). After 370 minutes, T_e was reduced (Figure 3.5) according to equations previously determined for this particular experimental setup.

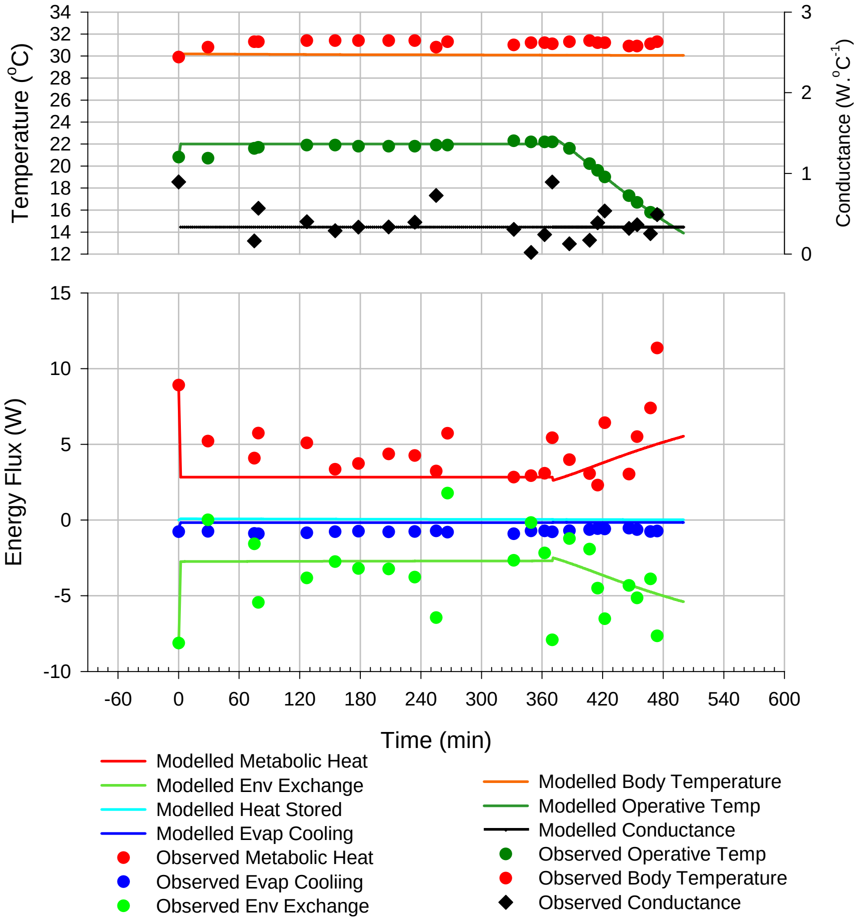


Figure 3.5 T_b, M, E and K_o for a classically thermoregulating echidna compared to initial predictions from modelling.

To model classical thermoregulation in general:

(i) $T_{b.eq.t}$ = preferred or set T_b, T_{set},

(ii) K_o may vary between limits, $K_{o.\min}$ and $K_{o.\max}$,

(iii) below thermoneutrality, $K_o = K_{o.\min}$ and M increases, and

(iv) above thermoneutrality, $K_o = K_{o.\max}$ at least while $T_e < T_b$ and M and T_b increase according to a Q_{10} effect, unless affected by dramatic increases in *EWL* (sweating or panting: panting may incorporate dramatic increases in V_i instead) with an extra cost in M.

In this situation, thermoregulation above thermoneutrality does not arise so only the following is required:

$$K_o = \frac{BMR - E_{BMR}}{T_{b.eq.t} - T_{e.t}} \text{ for } K_{o.\min} \le K_o \le K_{o.\max}$$
$$M_{eq.t} = K_o\left(T_{b.eq.t} - T_{e.t}\right) + E_{eq.t} \text{ for } K_o = K_{o.\min}$$

Eq 3.14

where $E_{eq.t}$ is determined according to equations 3.13 and 3.11. $T_{b.t}$ is determined from $T_{b.eq.t}$ for all t according to equation 3.5 in the normal way. Thus $T_{b.t}$ is not necessarily the same as T_{set} which allows for the possibility of T_b varying in response to perturbations. Note also that the width of thermoneutrality is not required *a priori* as an input, but is derived as an output from the modelling. For example, T_{lc} is the solution of equation 3.14 for $K_o = K_{o.\min}$ and $M_{eq.t} = BMR$.

For this echidna let:

(i) $T_{b.0} = 30.2$ °C, $M_0 = 8.9$ W and $K_{o.0} = 0.89$ W.^{o}C^{-1} (as observed),

(ii) T_b be maintained at 30.0 °C (Chapter 2), $\therefore T_{b.eq.t} = 30.0$ for all t,

(iii) $BMR = 2.6$ W (Chapter 2), not dissimilar to BMR from Schmidt-Nielsen *et al.* (1966),

(iv) $0.334 \le K_o \le 1.19$ W.^{o}C^{-1} (Chapter 2),

(v) $V_i = 184$ ml.min^{-1} (Parer & Hodson, 1974) when $M_t = BMR$ and varies in direct proportion to M_t, and

(vi) $h_{22} = 50$ % (as observed).

From the initial value of 30.2 °C modelled $T_{b.t} \rightarrow T_{set}$ over time while $T_e = 22$ °C and M_1 to $M_{370} = 2.86$ W (Figure 3.5). When T_e decreased, both modelled and observed M increased while both modelled and observed T_b remained stable as expected from the classical model of endotherm regulation. Modelled $K_{o.t} = 0.334$ W.^{o}C^{-1} for all t which approximated observed K_o, given that K_o calculated from empirical data are especially subject to experimental error. That modelled $K_{o.t} = K_{o.\min}$ and modelled $M_t > BMR$ indicated that $T_{e.t} < T_{lc}$ for all $t \ge 1$ and that therefore $T_e = 22$ °C $< T_{lc}$, contrary to conclusions by Schmidt- Nielsen *et al.* (1966), but in agreement with those from Chapter 2.

However, the modelling underestimated the observed T_b, the observed M and even the observed E of the echidna (Figure 3.5). Repeating the exercise for a T_{set} of 31.3 ^{o}C (as observed) improves the fit, but only marginally, especially for E_t (not shown), which hints that V_i is underestimated by the values input. The values for V_i input, derived from a study by Parer and Hodson (1974) on the ventilation of echidnas, are low compared to values from other similar studies (Bentley*, et al.*, 1967; Bech*, et al.*, 1992; Frappell*, et al.*, 1994). Higher estimates were provided by Bentley *et al.* (1967) and using their values for V_i instead, provide a much better fit of the modelling to the empirical data (Figure 3.6) for the echidna in this case: although values from Parer and Hodson (1974) produce good fits for most of the other echidna simulations cited here. The high values of Bentley *et al.* (1967) were criticised by Parer and Hodson (1974) and by Bech *et al.* (1992), whose estimates of V_i are less, on the basis that they may have been due to echidnas being stressed by their restraints. This raises

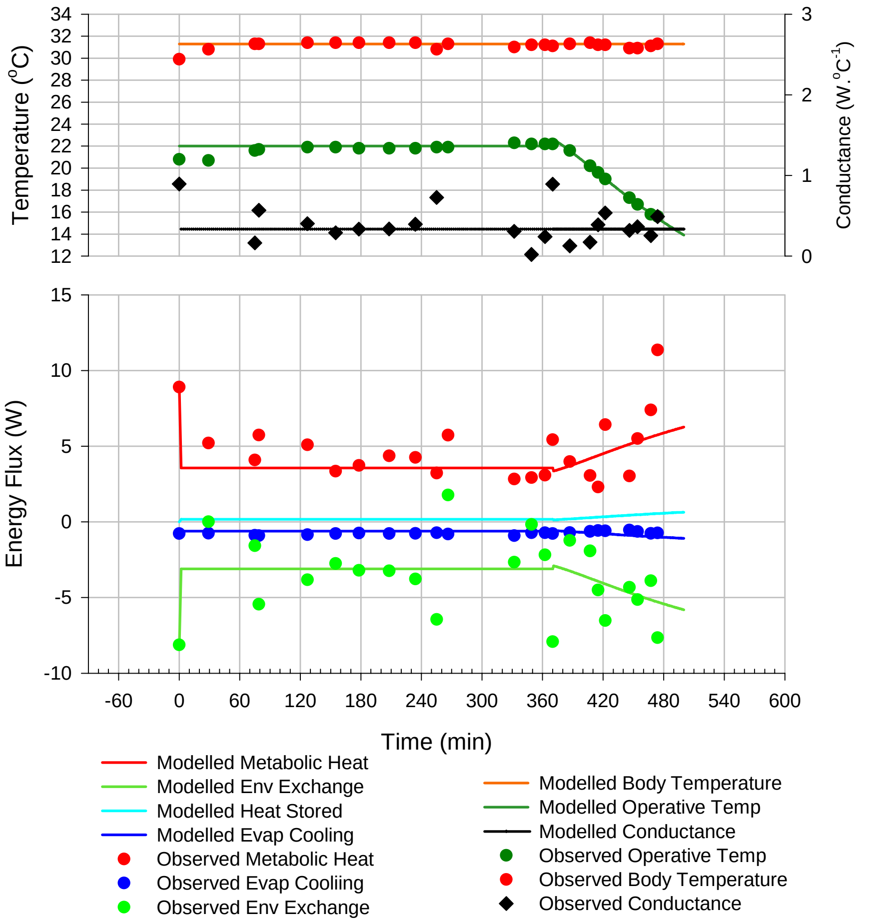


Figure 3.6 T_b, M, E and K_o for a classically thermoregulating echidna compared to initial predictions from modelling using V_i from Bentley *et al.* (1967).

the possibility that in this particular case, the echidna in the metabolic chamber may have been stressed, even though it apparently rested (except for its shivering) throughout most of the experiment. Whatever the case, this shows how modelling might add further insight into various data from various sources.

There still remains a small, but possibly biologically significant discrepancy. Because V_i is linked to M, the modelling predicts an increase in E as M increases when T_e decreases, and this seems not to occur even given the resolution of the data (Figure 3.6). Investigating whether this is just an artifact of the study or whether V_i does increase with M may be a fruitful line of enquiry. It was assumed that during shivering thermogenesis V_i should increase with M because it does in marsupials (Chappell & Dawson, 1994) and in placental mammals (Mortola & Frappell, 2000), but there are exceptions (Mortola & Frappell, 2000). As yet, there are no data specifically relating variations in V_i to variations in M for thermoregulating echidnas: data not easy to obtain from uncooperative echidnas (Nicol & Andersen, 2003). Nevertheless, the modelling has produced testable hypotheses to extend, as well as highlight discrepancies in, understanding of the physiology of echidnas.

Activity Produces More Heat than Shivering

This example quantifies differences in metabolic heat production between an echidna that would be thermoregulating and one that is active. A different echidna (mass 3.10 kg) in the same experimental set up as above reacted to decreasing T_e, not with shivering thermogenesis, but with intensifying attempts at escape. T_e was decreasing during the first 140 minutes (from $T_{e.0}$ of 24.9 ^{o}C at a rate of 1.07 ^{o}C.hr^{-1}), after which the cooling in the metabolic chamber was rapidly increased to a rate as previously determined for any change in the set point of the water bath (Figure 3.7).

While repeating the above modelling exercise with parameters appropriate to this echidna and the decreasing T_e, no amount of simulating classical thermoregulation provided any sort of fit throughout. Figure 3.7 shows the best fit that could be obtained, and it can be seen that when T_e was decreased more rapidly, the echidna increased its M much more than would be expected from one that is quietly shivering. Accordingly, T_b increased substantially throughout the 4 hours of the experiment and more so than predicted for a thermoregulating

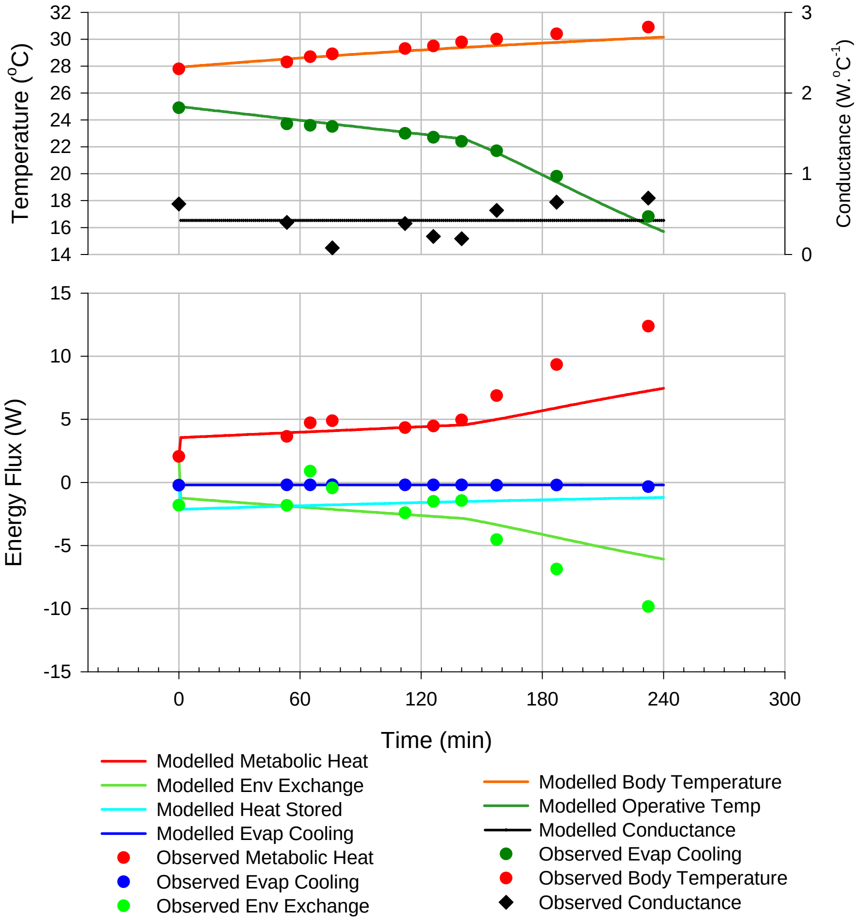


Figure 3.7 Comparing data from an echidna in a dynamic thermal environment to predicted data for a classically thermoregulating echidna in such an environment from modelling to show the lack of fit.

echidna defending (or achieving) a T_b of 33 °C. To replace the simulated thermogenesis by the simulated activity, the dynamics of $M_{eq.t}$ were replaced by:

$$M_t = 2.06 + 0.1t \text{ for } 2.06 + 0.1t \leq 4.5 \text{ and } t \leq 140$$
$$M_t = 4.5 + 0.1(t - 140) \text{ for } 4.5 + 0.1(t - 140) \leq 12.5 \text{ and } 140 < t \leq 240$$

Eq 3.15

because:

(i) $T_{b.eq.t}$ can only be determined by conditions that pertain at t so $M_{eq.t} = M_t$ for all t,

(ii) $M_0 = 2.06$ W which solves for a $T_{b.0}$ of 27.9 °C, assuming equilibrium at the start, which compares to the observed M_0 of 2.06 W and $T_{b.0}$ of 27.8 °C, and

(iii) the echidna apparently increased its activity at a rate of 0.1 W.min^{-1} in response to the stimuli of (a) being placed in the chamber (limited to 4.5 W) and (b) the rate of decrease of T_e being sharply increased (limited to 12.5 W).

and this can be seen to fit observed M_t and observed $T_{b.t}$ well enough (Figure 3.8).

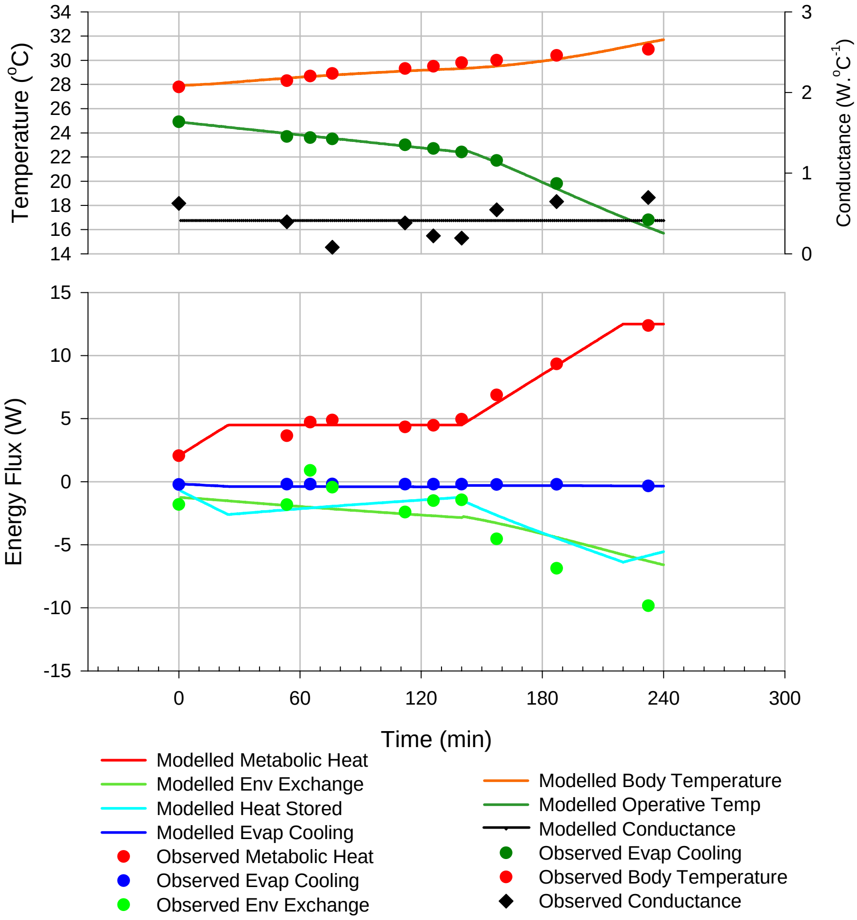


Figure 3.8 Comparing the same empirical data as in Figure 3.7 to a modelled echidna becoming active to show a better fit.

Arousals from Torpor

This example highlights instances in arousing mammals, of several taxa, where only part of the body temperature rises observed are attributable to changes in V_{O2}, changes in T_e and changes in other parameters.

Occasionally, data for a spontaneously arousing echidna are captured fortuitously. On one such occasion, an echidna (mass 2.82 kg) that had been in a constant temperature room set at 12 ^{o}C was gently placed into a metabolic chamber. Its T_b, which was 23.2 ^{o}C initially rose slightly for a while, probably due to its being handled. Nevertheless, it burrowed into some substrate provided within, and T_b declined for a little over an hour (Figure 3.9). After this its M, as measured by V_{O2}, increased rapidly from its previous low levels. The settling of T_b to near previous levels and the delay before arousal helped reassure that the arousal was spontaneous. For various logistical reasons, *TEWL* is not considered in this simulation; it is likely to be low or negligible in such a situation anyway. By using wet thermal conductance, K_{wet}, as a substitute for K_o, any latent heat loss that does occur is lumped in with environmental heat loss. K_o is likely to remain minimal in order to retain the heat gained from increasing M. For the arousal, it was assumed that M_t increase exponentially to some maximum value which is retained until T_b reaches some normothermic value, after which M_t reverts to *RMR* (cf. the Syrian hamster above). T_e remained constant until the echidna started arousing. It then increased slowly as a consequence of the heat lost by the echidna in the confined micro-environment of the metabolic chamber, not as a stimulus to arousal in the echidna. Let:

$$\begin{aligned} T_{e.t} &= 14 \text{ for } t \leq 70 \\ T_{e.t} &= 14 + 0.01539(t - 70) \text{ for } t > 70 \therefore T_{e.200} = 16 \end{aligned}$$

Eq 3.16

For the modelling let:

(i) $TEWL_t = 0$ for all t,

(ii) $K_{o.t}$ = minimum K_{wet} = 0.375 W.^{o}C^{-1} (Chapter 2) for all $t > 0$,

(iii) M_0 = 0.884 W,

(iv) $K_{o.0}$ = 0.087 W.^{o}C^{-1}, and

(v) $T_{b.0}$ = 24.2 ^{o}C.

where assumptions (iii) to (v) reflect the slight disturbance to the echidna that occurred at the start of the experiment. During the arousal, the observed rate of increase in M and the peak M

was mimicked (well) by an initial rate of increase of 10 %.min^{-1} and (approximately) by a peak of 7 W sustained for about 70 minutes (till $T_b > 24.5$ °C): there were no available data to predict these dynamics *a priori* (but for T_b see Anderson & Nicol, 2006). This rate of increase, being three times greater than that of the Syrian hamster (above), raises questions in itself about the mechanisms for generating heat in this prototherian, lacking as it does the brown adipose tissue of the hamster and other therians. Clearly, these rates, chosen arbitrarily because they fit, do not really model the biology of arousal *a priori*, and there is scope for developing the modelling so that they do (e.g. Andersen & Nicol, 2006). Nevertheless, let:

$$\begin{aligned}
&M_{eq.t} = 0.756 \text{ for } t \le 70 \text{ (mean } M \text{ prior to arousal)}\\
&M_{eq.t} = 1.1M_{eq.t-1} \text{ for } t > 70, \text{ for } T_{b.t-1} < 24.5, \text{ for } 1.1M_{eq.t-1} \le 7\\
&M_{eq.t} = 7 \text{ for } t > 70, \text{ for } T_{b.t-1} < 24.5, \text{ for } 1.1M_{eq.t-1} > 7\\
&M_{eq.t} = 5 \text{ for } t > 70, \text{ for } T_{b.t-1} \ge 24.5
\end{aligned}$$

Eq 3.17

The resultant modelling (Figure 3.9) predicted the observed T_b well for the first 150 minutes and depicted the M_t less well throughout. During the first 70 minutes both predicted and observed $T_{b.t}$ dropped in response to the hypometabolism of the echidna at the start. After 70 minutes, the observed spontaneous arousal proceeded as depicted by the model initially. However, for a while the simulation overestimated the observed M_t until both estimated and observed M_t reverted back to about 5 W. Despite this, observed $T_{b.t}$ rose as predicted until the 150th minute. After this, the simulation seriously underestimated the more rapid rise in $T_{b.t}$ towards the end of the experiment.

The most obvious questions from this exercise relate to why observed T_b ends up being some 3 °C higher than predicted, despite the good fit for the first 150 minutes. It may be that there is some internal T_b gradient analogous to that between T_{cp} and T_{rect} in the hamster, and the transmitter is no longer sensing the core average T_b as assumed. However, as the transmitter is implanted posterior to the brain, heart and lungs where warm blood would be directed if this was indeed analogous to the hamster (Lyman, 1948), T_b as measured would be expected to be less than predicted, not more. Perhaps thermogenesis during arousal predominantly occurs elsewhere than the anterior organs in Prototheria. Alternatively, there may be an extra source of heat not modelled: the only heat source in the simulation is metabolic rate which is derived from oxygen consumption.

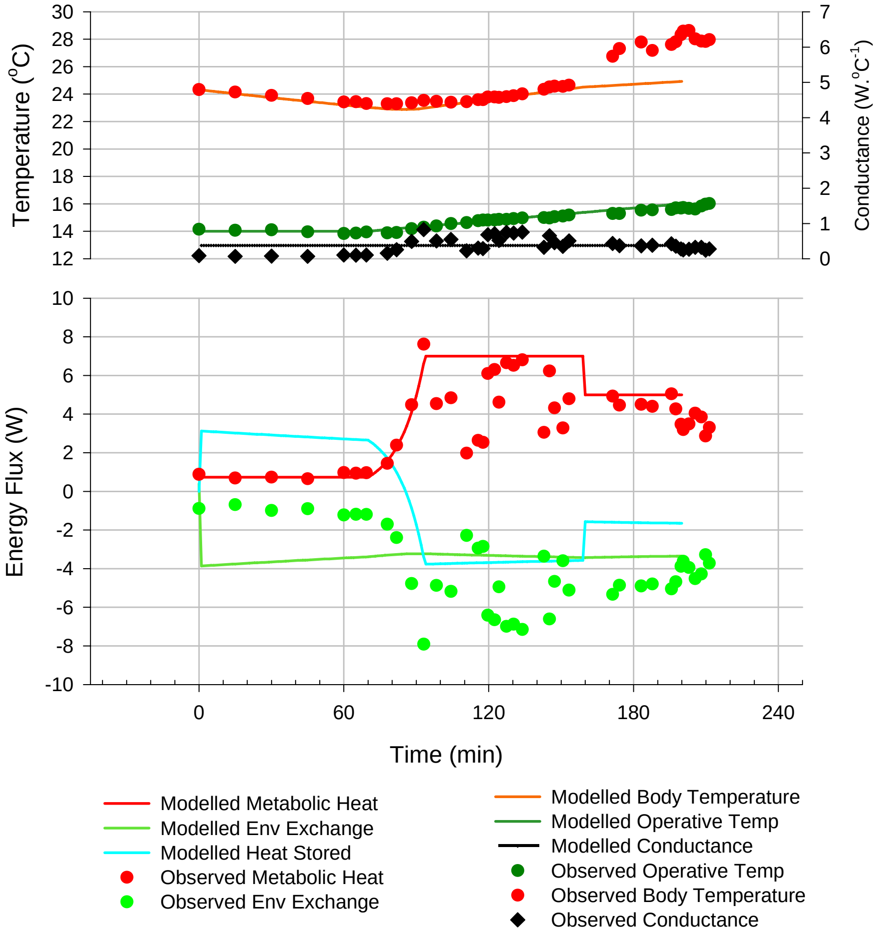


Figure 3.9 Data from an arousing echidna compared to that predicted by modelling showing a discrepancy in final T_b despite an initially good fit.

Fleming (1980) noted that in arousing sugar gliders, *Petaurus breviceps*, aerobic metabolism only accounted for 13.5 °C of an increase of 16.7 °C in T_b. Applying this modelling approach to Fleming's data recreates the arousal in the sugar glider only when the 3 °C discrepancy is taken into account, which seems to support the reality of the discrepancy. The other 3.2 °C Fleming (1980) interpreted as possibly due to anaerobic metabolism. Whatever the case, similar discrepancies may be found comparing modelled T_b to actual T_b in American badgers, *Taxidea taxus*, although the data presented in that study are a little too incomplete for conclusive and robust modelling (Harlow, 1981).

Hypothetical Lizard

This more general example shows the range of thermoregulating behaviours and time courses of $T_{b.t}$ obtained from the modelling. Specifically, this is an example of how the thermoregulation of a large lizard shuttling between two thermal environments might be quantified. Because the modelling inputs include the animal's size and variations in its thermal conductance, perhaps with heating/cooling hysteresis, it automatically accounts for thermal inertia, and T_b is output incorporating the resultant lags. In this way, the modelling inherently addresses concerns about the applicability of measures of thermoregulation (e.g. Hertz*, et al.*, 1993) when used for studies of animals with substantial thermal inertia (see Seebacher & Shine, 2004). Additionally, it incorporates effects of metabolic heat production when applicable, and the resultant Q_{10} effects.

Consider a hypothetical lizard of 5 kg mass, with a choice of two thermal environments in close proximity so that the cost of moving from one to the other is negligible. Assume, for the purposes of demonstration, that the T_e of these environments vary sinusoidally in phase, both with a wave length of 24 hours, and that the amplitude of one ("exposed") is greater than the amplitude of the other ("sheltered"). A thermoregulating lizard can be modelled and its T_b predicted. As this is a relatively large lizard, its M and its $REWL$ may be significant determinants of its T_b. In any case these factors can be included. Assume for now that there is no significant $CEWL$ and also that the animal lacks functional respiratory turbinates.

Let:

(i) T_e vary between 20 ^oC and 50 ^oC in the "exposed" environment,
(ii) T_e vary between 28 ^oC and 32 ^oC in the "sheltered" environment,
(iii) h = 70 % when T_a = 20 ^oC in both environments,
(iv) the lizard be at rest and the activity involved in moving between environments be negligible,
(v) $M_{eq.t}$ = SMR (standard metabolic rate) as predicted from an allometric equation (Withers, 1992a), that is 0.38 W when T_b is 20 ^oC,
(vi) SMR be subject to a Q_{10} of 2.49,
(vii) V_i be 265 ml.min^{-1} when M_t = 0.38 W and T_b = 20 ^oC (Withers, 1992a),

(viii) $V_{i.t}$ be determined by M_t but not in direct proportion (Mortola & Frappell, 2000): the lizard becomes relatively hypoventilated as M increases so $f = 0.8$,

(ix) the lizard have two set point temperatures such that when $T_{b.t\text{-}1} < 35$ °C it chooses the warmer of the two environments at time t, and when $T_{b.t\text{-}1} > 36$ °C it chooses the cooler. As $T_{b.t}$ is unknown at any t until the equations are solved, the lizard can only react to the temperature of the time interval t -1, $T_{b.t\text{-}1}$, and

(x) hysteresis between heating and cooling be apparent in this lizard according to the allometric relations for the thermal time constant as determined by Grigg *et al.* (1979). Therefore, assuming the heat capacity of the lizard's tissues to be 3.35 $J.g^{-1}.^{o}C^{-1}$, $K_o = 6.3$ $W.^{o}C^{-1}$ when T_b is increasing and 4.3 $W.^{o}C^{-1}$ when T_b is decreasing.

Output from the modelling is depicted in Figure 3.10. This modelling predicts:

(i) in "the early morning" the lizard's T_b will equilibrate with the T_e in the sheltered environment,

(ii) the lizard will move to the exposed environment once the T_e in this exceeds that in the sheltered environment,

(iii) as this environment warms so will the lizard but with a noticeable time lag (due to its thermal inertia being a combination of the animal's size and its thermal conductance even though this is maximal),

(iv) that during the "middle" of the day, the lizard will shuttle between the two environments to maintain its T_b within its preferred range (35 °C to 36 °C) as much as possible,

(v) that during "the late afternoon" the lizard will stay in the exposed environment until it becomes cooler than the sheltered one,

(vi) that during "the late afternoon" the animal will retain its heat as long as possible by adjustments to its K_o which increase noticeably the time lag between T_e and T_b,

(vii) that the animal will spend most of "the night" in the sheltered environment and its T_b will slowly equilibrate with that T_e,

(viii) M and E are both minor elements of the thermoregulation, even of this fairly large ectotherm, although no activity was included (though it could be by specifying active metabolic rates in the input),

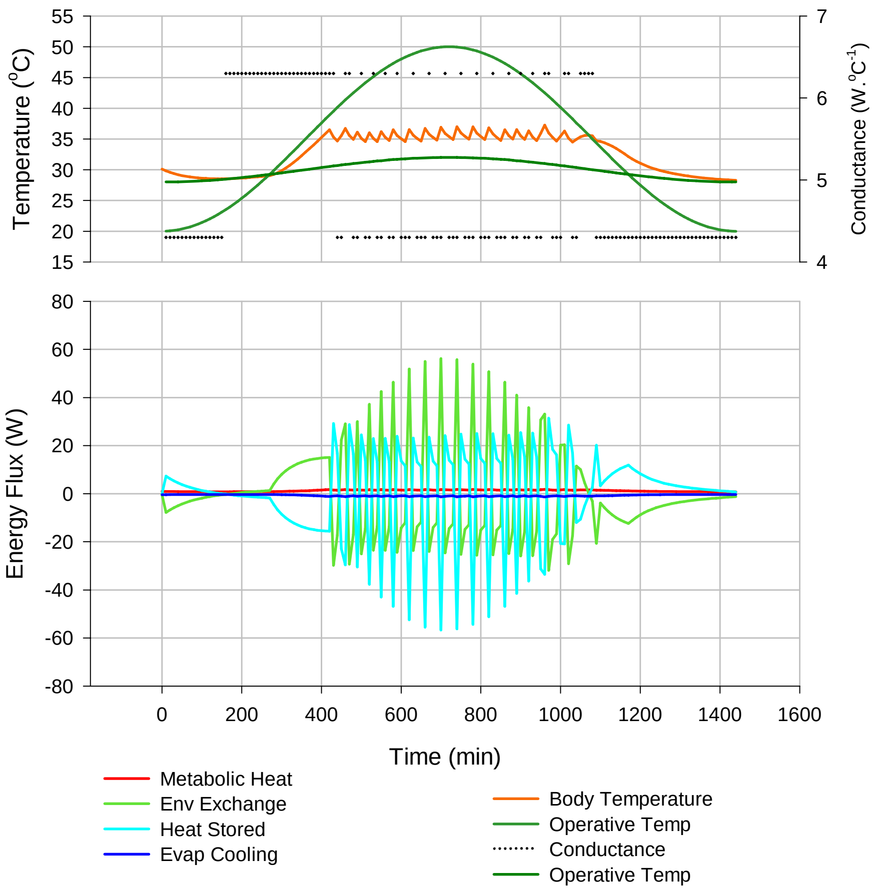


Figure 3.10 Modelled output for a hypothetical thermoregulating 5 kg lizard with two sinusoidally varying thermal environments available to show a suite of predicted thermoregulatory behaviours.

(ix) that nevertheless, both M and E increase during "the heat of the day", M from the effect T_b has on M and E from the effect M has on V_i and T_b has on λ and χ,

(x) that of far more importance than either M or E in the thermoregulation of the ectotherm is the environment and the animal itself; each of which alternates as a heat source and a sink as the animal shuttles, and finally

(xi) the quantities of the various elements in the lizard's thermoregulation at any time t.

Conclusions

This modelling approach has potential in predicting and analysing the thermal biology of a wide range of taxa in complex and dynamic environments, even in mosaics of different environments. It achieves this by solving commonly used equations in thermal biology iteratively for T_b using parameters that may be functions of time or of other parameters, including T_b itself, in a computer program (currently QBASIC). While allowing the incorporation of such interrelationships as the Q_{10} effect, heating/cooling hysteresis and the effect of metabolic rate on respiratory rate, the approach is essentially simple. The equations are simple and intuitive using, as they do, integrated parameters. While this avoids the need to calculate processes such as conduction, convection and radiation separately, it does require values for the parameters T_e and K_o that may not always be easy to acquire (for possible solutions see e.g. Bakken, 1976; 1992; Campbell & Norman, 1998). However, the simplicity of the approach, and the equations on which it is based, does allow focus on the thermal biology of the animal, on the contribution of metabolism and evaporation to the animal's thermal balance and on the dry heat loss and gain to and from its environment, an environment that may be continually changing. For situations where conduction, convection and radiation need to be incorporated, there are many more complex and demanding models available (Bolton*, et al.*, 1996; Florides*, et al.*, 1999; Turnpenny*, et al.*, 2000a; Turnpenny*, et al.*, 2000b for just a few examples). In the situations cited above, the combination of equations used here solved simultaneously and iteratively has predicted T_b well. The hamster arousing from torpor and the echidna entering torpor are examples. In other situations discrepancies appear between predicted solutions and empirical data, for example in an arousing echidna, in arousing sugar gliders and possibly even in badgers. If such discrepancies had not also been noticed by others (and there had not been a long and respected tradition for these equations), then confidence in the modelling might have been undermined. But this is not the case. That the modelling does predict these discrepancies indicates that this approach has utility in analysing and highlighting avenues of further enquiry.

The modelling has potential for investigating, in a range of dynamic environments, the thermal relations of ectotherms, including those with significant activity and/or thermal mass, perhaps including large dinosaurs, and of endotherms, both homeothermic and heterothermic, perhaps including the evolution of endothermy itself. It certainly has potential to analyse data collected from animals in situations made complex, not only by fluctuations in temperature, but also by interactions of physiology and physics imposed by biochemical reaction rates and size. The use of this modelling in Chapter 4 to demonstrate quantitatively the transient storage of heat in a prototherian mammal is but one example of the utility of this modelling in illustrating thermoregulatory strategies in fluctuating thermal environments.

Chapter 4: Heat tolerance of echidnas, *Tachyglossus aculeatus*: combining modelling and experimental approaches in field conditions

Abstract

Lacking effective evaporative cooling, short-beaked echidnas, *Tachyglossus aculeatus*, are considered more competent as behavioural than as physiological thermoregulators in the heat. However, recent data have shown that they tolerate higher temperatures in some day time retreats in the field than they do in laboratories. This study investigated mechanisms whereby echidnas are able to maintain sub-lethal body temperatures when exposed to heat in quasi-field experiments mimicking ambient temperature dynamics similar to those experienced in the wild. Aided by a quantitative modelling analysis of empirical data collected, this study showed that echidnas manage daily potential heat stress through transient storage of heat. Central to this strategy is the ability of echidnas to maintain minimal values of thermal conductance, despite the lack of hair in their pelage and, perhaps more importantly, to maintain minimal values of metabolic heat production. However, while metabolic rates of resting echidnas were near basal throughout, when adjusted for the effect body temperature is likely to have on metabolism, there was no evidence of aestivation.

Introduction

Short-beaked echidnas, *Tachyglossus aculeatus*, are semi-fossorial, prototherian mammals occurring throughout all the major habitats in Australia, including those in the arid and semi-arid zones where environmental temperatures can be extreme (Linacre & Hobbs, 1977; Strahan, 1983). Yet, based on laboratory studies (Martin, 1903; Robinson, 1954; Augee, 1976), many workers have concluded that echidnas are physiologically ill equipped to tolerate heat (Griffiths, 1978). They neither sweat, nor pant. Nor do they spread saliva. When exposed to ambient temperatures above 35 ^{o}C, their body temperatures, T_b, seem to rise inevitably to about 38 ^{o}C (Robinson, 1954; Augee, 1976) which is (frequently) lethal (Augee, 1976).

The ability of echidnas to survive in some of the hottest environments on earth is generally attributed to behavioural, not physiological, thermoregulation (Griffiths, 1978). In hot weather they are nocturnal and seek cooler microenvironments - caves, burrows and the like - during the day (Abensperg-Traun & De Boer, 1992; Brice*, et al.*, 2002b). Frequently echidnas use hollow logs, the remnants of fallen trees lying on the ground exposed to the sun (Abensperg-Traun, 1991a; Wilkinson*, et al.*, 1998; Brice*, et al.*, 2002a). In these microenvironments, air temperatures, T_a, often rise above the levels found to be lethal to echidnas in laboratories. In semi-arid western Queensland, for example, T_a in hollow logs exceed 35 ^{o}C for four to ten hours at a time and may reach at least 42 ^{o}C (Brice*, et al.*, 2002a). Yet echidnas use these logs during hot days even when suitable and cooler caves and burrows are available nearby. And of course, they do so without ill effect.

The key to heat tolerance in echidnas may lie in their “unusual” physiology: “unusual”, that is, for mammals. On the one hand, they lack the usual mammalian cooling mechanisms such as panting and sweating. On the other, they exhibit especially labile T_b. Generally T_b rises during activity, typically to about 32 ^{o}C, while during rest, T_b generally falls, depending on the environmental temperature, typically by 3 ^{o}C to 5 ^{o}C, sometimes as much as 10 ^{o}C (Augee*, et al.*, 1970; Grigg*, et al.*, 1992b; Beard & Grigg, 2000; Nicol & Andersen, 2000; Brice*, et al.*, 2002b; Kuchel, 2003). While echidnas do exhibit torpor over various time frames (Grigg*, et al.*, 1989; Grigg*, et al.*, 1992b; Nicol & Andersen, 1996; Kuchel, 2003), this daily heterothermy is not necessarily an instance. During periods of inactivity, echidnas are rarely lethargic and they are capable of coordinated movement and foraging with T_b as low as 20 ^{o}C (Chapter 2; Brice*, et al.*, 2002b; Kuchel, 2003).

Their capacity for heterothermy coupled with a large thermal inertia, deriving from low mammalian thermal conductance (Chapter 2) and a relatively large size compared to other mammalian heterotherms, may impart to echidnas an ability to cope with the environmental temperatures they encounter in their hollow logs. By allowing T_b to fall while environmental temperatures are at their early morning low and by absorbing heat only slowly from their environment during the heat of the day, echidnas seem to avoid lethally high T_b, and emerge to forage in the late evening with T_b already at approximately “active” levels (Brice*, et al.*, 2002a). In such situations, it seems adaptive to minimise gain from any heat sources: hence the importance of minimising thermal conductance. The other heat source for an echidna is its own metabolism. As prototherians, their metabolic rates are already low by mammalian standards (Dawson*, et al.*, 1979). Perhaps in the heat, echidnas aestivate, that is suppress their metabolism further than expected from a temperature effect on metabolic rate alone. Such a possibility was suggested by Grigg *et al.* (1989) and reiterated by Brice *et al.* (2002a).

This study investigated the mechanisms by which echidnas might tolerate the temperature regimes they have been observed to encounter in the field. This was achieved by quantifying how metabolic rates, M, overall thermal conductances, K_o, body temperatures, T_b, evaporative heat loss, E, air temperature, T_a, and operative temperature, T_e, changed throughout the day for echidnas subjected to quasi-field conditions in which the extremes of temperature experienced were mimicked. In addition, because of the difficulty in quantifying the effect of different mechanisms in such dynamic environments, a modelling approach was used to predict the time course of echidnas' T_b for different strategies, for different levels of metabolic heat production, for variations in thermal inertia and to interpret the empirical data collected.

Methods

Animals

Wild echidnas were caught opportunistically from various locations around Queensland, ranging from Brisbane and Texas in the South East to Idalia National Park 113 km WSW of Blackall inland. Consequently, the echidnas originated from a variety of habitats and climates including mesic and semi-arid areas. Despite this, no distinction was made between echidnas from different locations. All echidnas caught were of the sub-species *Tachyglossus aculeatus acanthion* based on the lack of hair in their dorsal and lateral pelage.

Upon capture, all echidnas were transported to the University of Queensland where temperature-sensitive, two-stage radio transmitters with internal loop antennae (Sirtrack) were implanted into each via a midline laparotomy conducted under halothane anaesthesia. The transmitters, which had been coated with smooth, biologically inert wax (20 % Elvax: Dupont, 80 % paraffin wax), weighed approximately 42 g. Their temperature responses had previously been calibrated against a certified thermometer in water baths ranging from below

8 ^{o}C to above 38 ^{o}C. Echidnas were allowed to recover from surgery before being transported to the University of Queensland Veterinary Farm at Pinjarra Hills just west of Brisbane (S 27^{o} 32', E 152^{o} 55').

Echidnas were maintained in a 30 m by 60 m enclosure in open eucalypt woodland at Pinjarra Hills. Mounds of loose earth and piles of fallen timber were provided for shelter though the echidnas tended to prefer burrows they constructed themselves. Food, an artificial diet adapted from Bellamy (1994), was provided daily except during inclement weather. At different times of the year, feeding was timed to coincide with activity patterns observed for wild echidnas (Brice*, et al.*, 2002b). Throughout the antipodean summer, when experiments were conducted, echidnas were fed at or after dusk. Observations confirmed this allowed the retention in these captive echidnas of the nocturnal activity patterns (Abensperg-Traun & De Boer, 1992; Brice*, et al.*, 2002b) (and their attendant T_b fluctuations) usual in wild echidnas during hot weather.

Echidnas were held in these circumstances for at least four months prior to the commencement of experiments described below. During this time, echidnas became habituated to regular human presence and handling. Up to 12 individuals were kept at any one time although only seven were used in this study.

Experiments

Echidnas readily entered 900 mm lengths of 240 mm diameter unplasticised polyvinylchloride (pvc) tubing left lying horizontally at various locations within the enclosure at Pinjarra Hills and containing small quantities of artificial food.

One piece of piping was modified for respirometry. It was trimmed to 895mm which allowed for vented perspex disks to be clamped firmly at each end. Vents in the end disks allowed the pipe to act as an open-flow respirometer when plumbed into a controlled airflow. When left under a gap in the woodland canopy in the enclosure over a number of days during summer, a datalogger (Onset "Tidbit" temperature logger) suspended within the tubing showed that the daily rise in T_a within matched that within a typical, natural, hollow, wooden log used by sheltering echidnas at Idalia National Park in semi-arid Western Queensland during summer

(Brice*, et al.*, 2002a). Thus, fortuitously, such a length of pvc piping could be used to mimic naturally occurring daily fluctuations of ambient temperature and allow the simultaneous measurement of oxygen consumption, body temperature and other physiological parameters.

After a successful trial on 1 April 2004, experiments were conducted from late October 2004 to late March 2005 coinciding with hot weather in South East Queensland. On each occasion, the experimental apparatus was set up within the echidna enclosure the evening before. A small amount of food was left within the pvc tubing to entice echidnas to enter voluntarily, after which the tube was sealed to become a metabolic chamber. The echidna within was monitored throughout the night. After initial attempts to leave the chamber had failed, the echidna would generally appear to rest within and appear to be at rest before first light the next morning, at which time measurements would begin. Experiments proceeded only while echidnas remained resting, being terminated at any time (after sun-rise) when the echidna within made more than cursory attempts to leave the chamber. At the conclusion of each experiment the echidna was weighed, offered water, more food, and was monitored closely for a further two hours. All echidnas involved were found to behave normally during the hours and weeks after experimentation.

Dry compressed air flow through the chamber was controlled by a needle valve and measured with a calibrated spirometer (AD Instruments) upstream of the chamber. Flow rate was converted to STP as per Hawk *et al.* (1954). Flow rate was varied between 0.5 $l.min^{-1}$ and 5.0 $l.min^{-1}$ to (i) minimise the humidity resultant from the echidnas' respiratory evaporative water loss and (ii) obtain a sufficiently measurable difference in oxygen and carbon dioxide concentrations between the incurrent and the excurrent air (see below). Minimising the humidity within the chamber allowed the maximum respiratory evaporative heat loss for the animal as the temperature within the chamber increased during the day. This approach was considered conservative, especially for a species not known for its physiological cooling mechanisms. Flow rate needed to be varied only infrequently and it was possible to allow the excurrent oxygen concentration to stabilise before taking measurements from resting echidnas.

Humidity in the excurrent air was measured with a relative humidity analyser (Sable Systems RH-100). A subsample of this air was dried by passage through drierite before being directed to oxygen and carbon dioxide analysers (AD Instruments ML205 combined O_2 and CO_2

analysers). As conditions changed during the day, it was necessary to calibrate the gas analysers frequently, sometimes as often as every 15 to 20 minutes. Calibration was performed by replacing the excurrent air to the gas analysers with a N_2/CO_2 mixture devoid of oxygen and containing a certified concentration (~5 %) of CO_2 to set the zero point and span of the O_2 and CO_2 analyser respectively. The span and a near zero point of the O_2 and the CO_2 analyser were determined by introducing the source (dry compressed) air directly to the gas analyser and assuming this dry air to be 20.95 % O_2 and 0.03 % CO_2.

Oxygen consumption and carbon dioxide production were calculated according to Withers (2001). Evaporative water loss was calculated according to Withers (2001) where $F_{E.H2O}$, the fractional concentration of water vapour in the excurrent air, was determined by multiplying the absolute humidity, h_a, (in g.l^{-1}) of the excurrent air by the volume of an ideal gas and dividing by the molecular weight of water (Barrow, 1973). Absolute humidity was determined from the total water vapour pressure of the excurrent air as measured by the humidity analyser and according to the manufacturer's documentation (Sable Systems RH-100).

Air temperature, T_a, within the chamber was measured by a T-type thermocouple introduced through a sealed hole in the roof of the chamber and interpreted by a thermocouple meter (Sable Systems TC-1000). The thermocouple had been calibrated against a certified thermometer by sealing its end within the small airspace in the nozzle of a 0.5 ml syringe with silicon sealant. This nozzle was then immersed in a water bath and allowed to equilibrate with water temperatures between 2 ^{o}C and 40 ^{o}C. Body temperature, T_b, was measured simultaneously by interpreting the transmissions received by a receiver (Telonics TR-2) from the previously calibrated implanted temperature sensitive transmitters.

All raw data, including flow rate, excurrent water vapour, oxygen and carbon dioxide concentration, air temperature and body temperature signal were recorded via an 8 channel data recorder (AD Instruments Powerlab 8 SP) onto a computer (Acer Travelmate 529) using Chart 4.0 software (AD Instruments). Generally, data for analysis were selected according to proximity (in time) to the calibrations of the gas analysers; otherwise data selected were adjusted for analyser drift. Thus data were selected typically every 15 to 30 minutes throughout the day. Selected data were copied into an Excel spreadsheet (Microsoft) where embedded equations performed the necessary calculations.

While the chamber was left on the ground and subject to the day's fluctuations in temperature and insolation, the attached recording and analytical equipment was housed in an adjacent utility vehicle (Ford BA "ute"). The vehicle was parked immediately to the west and within 0.3 m of the chamber which was aligned north-south under a gap in the open woodland canopy. Thus, the chamber was exposed to natural insolation during the morning, but was in the shade of the vehicle during the afternoon. It was found that this allowed T_a to increase at a rate similar to that of wooden logs in hotter climates while still avoiding excessive temperatures (> 50 ^{o}C): tarpaulins and shade cloth were available to shade the chamber on especially hot days.

A 12 volt 75 amp hour deep cycle battery (Exide ED5) and a 300 watt sine wave inverter (Jaycar MI 5082) housed in the vehicle's utility tray allowed the electrical equipment to operate, typically for up to 12 hours. The laptop computer, the humidity analyser and the thermocouple interpreter could run directly from the 12 V DC source while the gas analyser and the powerlab ran from the inverter.

Calculations to Determine Operative Temperature

Because the chamber was located outside and exposed to insolation, there was a distinct possibility that its walls were not isothermal, in which case T_a is not the most appropriate measure of thermal environment for the animal within. Internal surface temperature measurements of a similar length of the pvc tubing left in the sun at Pinjarra Hills confirmed that during the day, the temperature of the roof differed from that of the floor, typically by up to 8 ^{o}C. In such cases operative (environmental) temperature, T_e, *sensu* Bakken (1976) is a more appropriate measure. It accounts for differences in radiation within an enclosure which is not isothermal. Because there was no substantial wind within the chamber (air flow at 5 l.min^{-1} amounted to an average wind speed of < 0.002 m.s^{-1}), it was not necessary to adjust T_e to standard convective conditions and therefore standard operative temperature, T_{es}, was neither necessary nor calculated (Bakken, 1980; Gates, 1980).

Operative temperature was calculated according to Campbell and Norman (1998) and according to Bakken (1981) who includes conduction to/from the substrate. For these calculations, radiation exchange within the chamber was calculated according to the Stefan-Boltzmann equation using surface temperatures measured in the nearby pvc tubing and view factors determined as per Lauzier and Rousse (2003). Further details of the method used to calculate T_e and the assumptions that were necessary to make these calculations can be found in Appendix 2.

Modelling

Using the time course of calculated T_e for each experiment as a driving function, the expected time course of T_b for each echidna was predicted using the modelling described in Chapter 3. This modelling incorporated the observed humidity for each experiment, a reference metabolic rate, M_{ref}, a typical respiratory minute volume, V_i (with V_i assumed to vary in proportion to M), one or more values for overall thermal conductance, K_o, the mass of the animal, and an assumed temperature effect (Q_{10} of 2 or 3). Recognising that in a constantly changing environment, the temperature relations of a heterothermic endotherm of this size will rarely, if ever, be in steady state, this modelling predicted transient changes in T_b over short time intervals in response to continually changing thermal conditions. To do so, a hypothetical reference equilibrium, which the thermal relations would approach in an arbitrary constant environment, needed to be defined for each simulation. While this equilibrium would never be reached in any of the actual, fluctuating T_e regimes, it provided a reference against which transient values of T_b may be calculated given the incorporated temperature effect, thermal inertia and intermediate periods of activity when they occurred (for more details, see Chapter 3). In this study, hypothetical reference equilibria were found for various values of M_{ref}, V_i and K_o for the apparently thermoneutral T_e of 25.5 ^{o}C (Chapter 2). By altering values of M_{ref}, V_i and K_o, equlibria corresponding to differing thermoregulatory strategies and responses could be determined. From these, transient changes in T_b and other parameters resulting from the interactions of these strategies and responses with changes in T_e were predicted. Comparing predicted T_b, M, etc. to observed T_b, M, etc. enabled deductions to be made regarding the implementation and utility of those various strategies and responses. For example, to investigate the predicted effects of different levels of metabolic heat production, different values of M_{ref} were input to define hypothetical reference equilibria from which predicted T_b and M were calculated for given T_e time courses.

Initially, the modelling was used to investigate likely thermoregulatory strategies and responses implicit in the empirical data from the echidnas. Possible values for different parameters were input into the modelling to determine combinations that predicted well the transient changes observed in T_b and other parameters. To retain feasiblity in these simulations, input parameters were varied according to the following guidelines (in order):

1. M_{ref} (in combination with Q_{10}) was varied over the range of M for equivalent sized echidnas as measured in apparent thermoneutrality ($T_e \sim 25.5\ ^{o}$C: Chapter 2).

2. K_o was varied from the mean for equivalent sized echidnas in low T_e (i.e. over all T_e below thermoneutrality as determined in Chapter 2) to the minimum value in low T_e (i.e. the K_o for $T_e \sim 13.5\ ^{o}$C: the lowest T_e used in Chapter 2 which corresponds to the lowest K_o in that study). If the resulting match was poor, calculations of K_o from the empirical data were used as a guide to adjust K_o further, recognising that while the model is especially sensitive to values of K_o (unpub. data), calculated values of K_o are especially subject to experimental error (particularly when $T_e \sim T_b$).

3. Unlike for K_o, the model is not particularly sensitive to variations in V_i, so V_i was varied grossly to achieve final small adjustments to improve the fit of modelled T_b to measured T_b over the time course of the experiments.

For echidnas that were intermittently active, these periods of activity were superimposed onto the above process to incorporate the changes in M such activity implies. In some cases, activity was also associated with increases in K_o, in which cases these changes were also incorporated.

After this initial modelling, the significance of variation in T_e, of low M and of low K_o were investigated for selected echidnas.

To investigate the significance of the variation in T_e, a modelling exercise was undertaken where two echidna experiments (IB13 14 Dec and TB15 16 Feb representing a size range of echidnas) were assumed to each have been placed in constant T_e of 35 ^{o}C and 40 ^{o}C for 720 minutes (from the same starting point as observed) and where the echidnas were assumed to have retained the values of M_{ref}, Q_{10}, K_o and V_i determined from their respective best fit simulations.

To investigate the effects of different rates of metabolism and thermal conductance on the time course of T_b throughout the day, a representative echidna on a representative day (TB15: 23 Mar 05 max T_e = 45.5 °C) was subject to:

(i) an increased M_{ref}, *viz* a basal M for a typical metatherian of this size (2.93 kg) according to equations cited by Withers (1992a), i.e. M_{ref} = 5.26 W with other parameters unchanged,

(ii) an increased M_{ref}, *viz* a basal M for a typical eutherian of this size according to equations cited by Withers (1992a), i.e. a M_{ref} = 7.61 W with other parameters unchanged,

(iii) an increased K_o, *viz* a K_o according to equations cited by Withers (1992a) for a mammal of this size, i.e. a K_o = 0.5559 $W.^{o}C^{-1}$, with other parameters (including M_{ref}) unchanged,

(iv) a combination of increased M_{ref} to 5.26 W and K_o to 0.5559 $W.^{o}C^{-1}$.

Results

Experiments

Seven different echidnas were studied on twelve different days with some echidnas studied more than once. The echidnas sought to leave, and therefore experiments were terminated, at many different times of day between 10:03 hrs and 17:30 hrs (Table 4.1) obviously, on occasion, before maximum T_a had been reached in the metabolic chamber. Maximum T_a measured in the metabolic chamber varied from 30.0 °C to 49.2 °C. For experiments that persisted past the afternoon peak in temperature, maximum T_a ranged from 30.5 °C to 49.2 °C. Minimum T_a ranged from 13.8 °C to 25.2 °C not necessarily in correlation to maximum T_a as some days were overcast when minima were relatively high and maxima low. There was no correlation between time of leaving and minimum T_a (r^2 = 0.0595).

Table 4.1 Summary of parameters measured during metabolic measurements on echidnas subject to quasi-natural variation in temperature in open woodland near Brisbane in south-east Queensland including standard metabolic rate, *SMR*, for these sized echidnas according to Dawson *et al.* (1979).

Date	Echidna	Mass	Finish	Min T_a	Max T_a	Min M	Max M	SMR	Min M	Max M	Min T_b	Time at min T_b	Max T_b	End T_b	T_b range
		kg	hrs	°C	°C	W	W	W	W.kg^{-1}	W.kg^{-1}	°C	hrs	°C	°C	°C
01-Apr-04	IB 13	3.80	17:30	17.8	32.9	1.27	5.66	2.66	0.33	1.49	29.5	09:15	33.0	33.0	3.5
07-Oct-04	IB 13	3.56	14:25	13.8	44.0	2.66	5.77	2.53	0.75	1.63	28.8	08:46	32.9	32.9	4.1
27-Oct-04	IE 25	2.88	16:00	21.9	35.5	1.21	2.62	2.16	0.42	0.91	28.1	08:46	33.0	32.9	4.9
02-Nov-04	IE 24	2.18	10:35	15.9	30.0	0.95	5.00	1.75	0.44	2.30	23.4	06:45	28.7	28.7	5.3
30-Nov-04	RL 28	2.53	10:03	18.2	41.1	1.45	3.79	1.96	0.57	1.50	27.6	06:45	32.4	32.4	4.8
14-Dec-04	IB 13	3.65	11:40	20.0	43.8	1.52	4.86	2.58	0.42	1.33	29.9	06:40	32.8	32.8	2.9
05-Jan-05	IE 26	2.75	10:57	25.2	31.6	1.86	4.53	2.09	0.68	1.65	31.5	09:02	33.1	33.1	1.6
09-Feb-05	IE 26	2.93	10:29	24.8	34.5	2.33	4.67	2.19	0.80	1.60	31.6	07:45	32.5	32.5	0.9
16-Feb-05	TB 15	2.63	14:37	17.8	49.2	1.10	3.41	2.02	0.42	1.30	27.5	07:55	33.6	33.6	6.1
03-Mar-05	VF30	3.18	15:00	18.5	38.4	1.80	3.16	2.33	0.57	1.00	29.7	08:24	33.2	33.0	3.5
17-Mar-05	TB15	2.55	16:00	19.7	30.5	1.69	2.91	1.97	0.66	1.14	27.7	08:15	31.8	31.8	4.1
23-Mar-05	IE24	2.93	12:23	17.1	46.3	1.82	4.90	2.19	0.62	1.68	28.7	08:30	32.3	32.3	3.5
means		2.96	13:18	19.2	38.2	1.64	4.27	2.20	0.56	1.46	28.7	08:04	32.4	32.4	3.8

The most striking pattern was that in 11 out of 12 cases, echidnas sought to leave when their T_b reached between 31.8 °C and 33.6 °C, in 8 of these when T_b was within 0.5 °C of 33.0 °C (Table 4.1). This T_b was almost always close in both time and value to the maximum T_b reached during the experiments.

Minimum T_b varied from 23.4 °C (IE 24 2 Nov 04) to 31.6 °C (IE 26 9 Feb 05) occurring between 06:40 and 09:15 hrs (mean time for min T_b: 08:04 hrs). This gave a daily T_b range (while the echidnas remained in the chamber) varying from 0.9 °C (IE 26 9 Feb 05) to 6.1 °C (TB 15 16 Feb 05). Mean daily T_b range was 3.8 °C for the 12 echidna days of data.

Minimum values of M in these non-steady state echidnas varied from 0.95 W (IE 24 2 Nov 04) to 2.66 W (IB 13 7 Oct 04) with a mean of 1.64 W or, in mass specific units, from 0.33 $W.kg^{-1}$(IB 13 1 Apr 04) to 0.80 $W.kg^{-1}$ (IE 26 9 Feb 05) with a mean of 0.56 $W.kg^{-1}$ (Table 4.1). Maximum M were affected by different levels of activity in some echidnas but nonetheless ranged from 2.62 W (IE 25 27 Oct 04) to 5.77 W (IB 13 7 Oct 04) or from 0.91 $W.kg^{-1}$ (IE 25 27 Oct 04) to 2.30 $W.kg^{-1}$ (IE 24 2 Nov 04).

Measurements for evaporative cooling were not particularly reliable (Figure 4.1 for some examples) affected as they were by (i) build up of humidity overnight when flow rates were low, (ii) slow washout of water vapour when flow rates were increased during the day and (iii) the tendency for active echidnas to insert their snouts into the exhaust vent of the chamber. However, for resting echidnas, available data indicate that evaporative cooling was usually between 0.33 M and 0.50 M, though sometimes it was < 0.10 M. Periodic visual inspections of resting echidnas showed that respiration rates were typically 4 to 5 min^{-1}, rarely if ever > 8 min^{-1}. Thus, there was no evidence of panting at any temperature.

Estimates of K_o calculated from the data are subject to the combined experimental errors of all the data collected. Further, such estimates of K_o are extremely sensitive to the temperature gradient between the animal’s core and the environment: when $T_e = T_b$, K_o is undefined. Despite these limitations, for all T_e where this gradient is high enough, and given the limitations found in determining E (above), K_o was low for a mammal, typically between 0.3 $W.^{o}C^{-1}$ and 0.5 $W.^{o}C^{-1}$, i.e. lower than that found by Schmidt-Nielsen *et al.* (1966) although consistent values as low as about 0.1 $W.^{o}C^{-1}$ were also found.

Operative Temperature Estimates

Operative temperature estimates derived (as per Appendix 2) from days for which pvc tubing surface temperature data were available showed that T_e and T_a within the chamber generally varied by up to 3 ^{o}C throughout the day. However, there were occasions when larger differences in the order of 10 ^{o}C were found. These were attributable to the different situations of the two pvc tubes; it was not always possible to ensure they were in the sun together without one shading the other. Such outliers were eliminated from subsequent T_e calculations. Before 4:40 hrs, T_e exceeded T_a by about 0.6 ^{o}C. This difference increased to about 1.7 ^{o}C by about 6:00 hrs. Even though T_e and T_a both increased sharply during the morning, T_e decreased relative to T_a to be about 3.0 ^{o}C cooler by 14:00 hrs. Thereafter, T_e increased relative to T_a, to be about 0.6 ^{o}C cooler after 15:50 hrs.

A polynomial linear regression of calculated T_e against observed T_a at times of the day between 4:40 hrs and 15:50 hrs showed that:

$$T_e = T_a + \phi \qquad (r^2 = 0.4052)$$

where:

$$\phi = 0.6 \text{ for } d < 4:40$$
$$\phi = -1329.8d^4 + 2308.5d^3 - 1403.1d^2 + 345.08d - 27.685 \text{ for } 4:40 \le d < 15:50$$
$$\phi = -0.6 \text{ for } d \ge 15:50$$

where d is the time of day in hours:minutes. This allowed the more appropriate T_e of the an-isothermal metabolic chamber to be determined from the inappropriate T_a for any time during experiments and used in subsequent analyses.

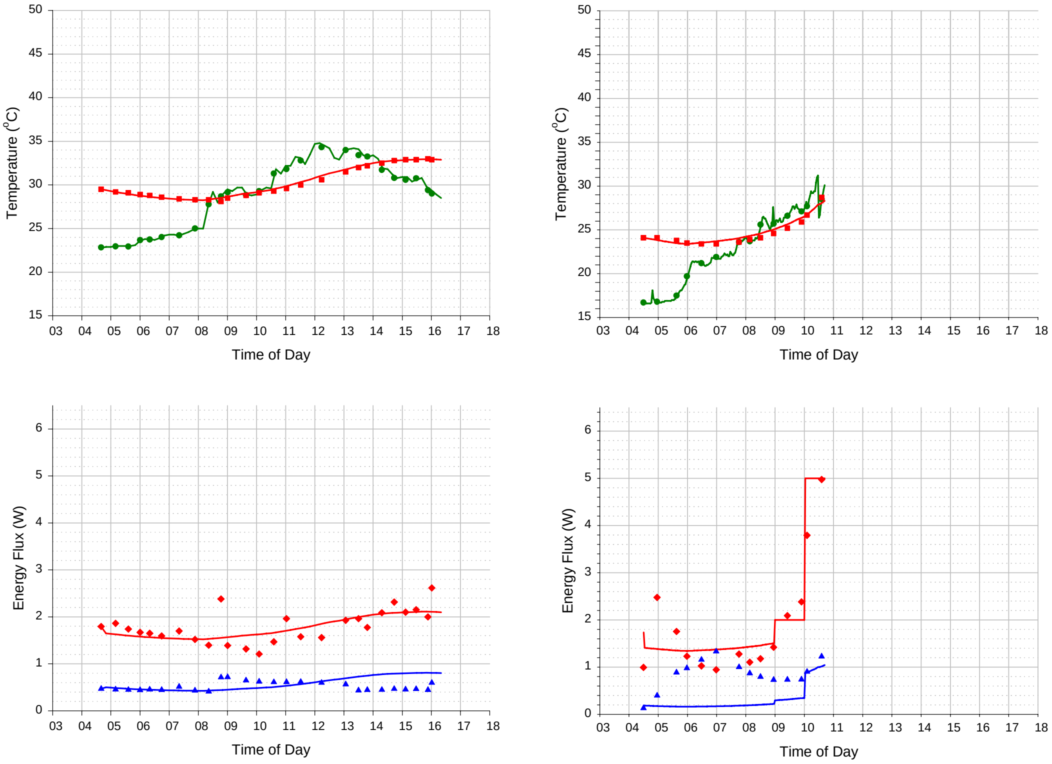
Modelling

In all cases for which modelling data are available for resting echidnas during these experiments (Table 4.2 and see Figure 4.1 for some examples), the best fits to empirical data were achieved where:

(i) M_{ref} was equal to or less than the mean M for an equivalent sized echidna resting at apparent thermoneutrality as measured in Chapter 2 and greater than the minimum M for an equivalent sized echidna resting at apparent thermoneutrality as measured in Chapter 2,

(ii) a Q_{10} of 2 or 3 was applied,

(iii) the input K_o was less than or equal to the mean K_o for T_e below thermoneutrality as determined in Chapter 2, in some cases less than or equal to the minimum K_o



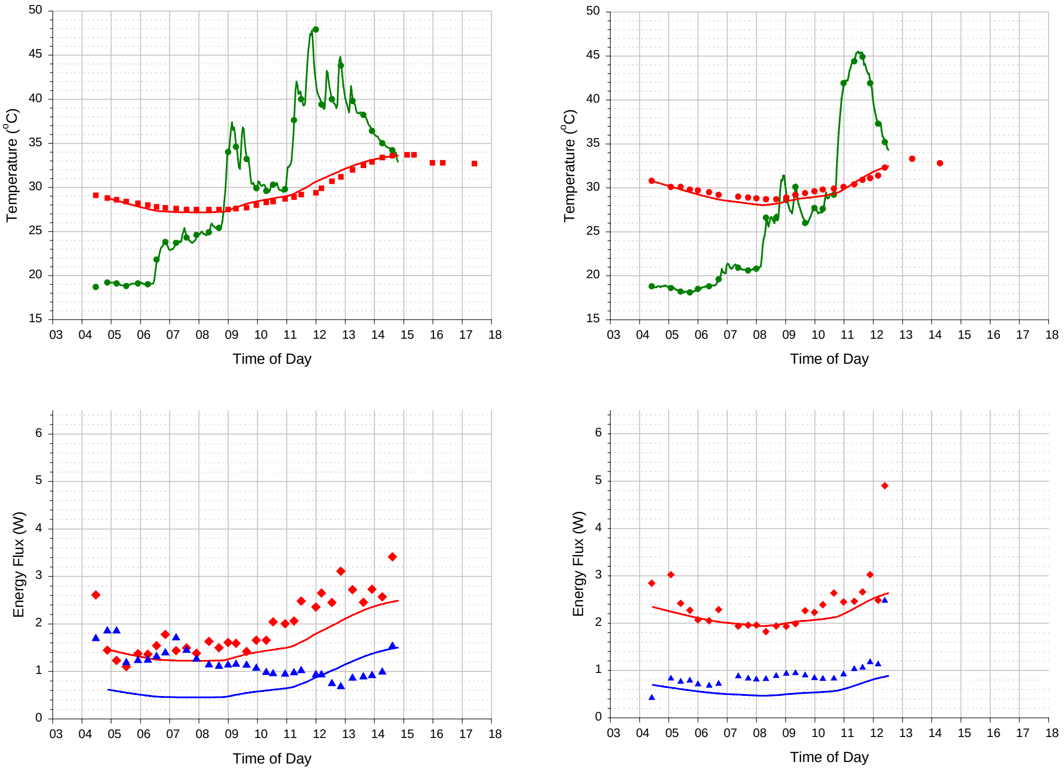
(a) (b)

Figure 4.1 Four examples of empirical data (points) and best fit modelling data (lines) for various of the echidnas subject to quasi-natural operative temperature regimes during experiments conducted in open woodland near Brisbane in south-east Queensland with data from each experiment in two panels, the upper depicting temperature data and the lower energy flux data. For more details on measurements and modelling of parameters see Methods. —●— Green line represents operative temperature to which the echidna was subject in the modelling, green circles represent the operative temperature as calculated from empirical data (of air temperature, surface temperature etc.). —■— Red line represents the body temperature as predicted by the modelling, red squares represent the measured body temperature during the experiments. —◆— Red line represents the metabolic rate of the animal as predicted by the modelling, red diamonds represent the ...

determined in Chapter 2 for equivalent sized echidnas,

(iv) V_i was 750 ml.min^{-1} ± 250 ml.min^{-1} when M equalled M_{ref} and varied in direct proportion to M.

In all cases, modelled T_b was within 1 °C to 2 °C of measured T_b throughout each day but such large errors were relatively rare and often associated with problems with the equipment. Generally, the difference between measured and modelled T_b was < 0.5 °C to 1 °C, often ~ 0.2 °C to 0.5 °C.



(c) (d)

Figure 4.1 (cont) ..actual metabolic rate of the echidna as calculated from oxygen consumption measurements. —▲—Blue line represents the respiratory evaporative cooling as predicted by the modelling, blue triangles represent the actual respiratory evaporative cooling as determined from excurrent air humidity measurements. (a) depicts IE25 on 27 Oct 04 when operative temperature only reached about 35 °C. (b) depicts IE24 on 2 Nov 04 showing increased levels of activity after 09:00 hrs prior to the echidna terminating the experiment during the morning. (c) depicts TB15 on the 16 Feb 05 when operative temperature exceeded 47 °C with the echidna remaining apparently relaxed and at rest until its body temperature reached 33.6 °C. Note that after terminating the experiment, body temperature declined during the rest of the afternoon. (d) depicts IE24 on the 23 Mar 04 when operative temperature exceeded 45 °C again with the echidna remaining apparently relaxed and at rest this time until its body temperature reached 32.3 °C.

Table 4.2 Parameter values for the best model fit for echidnas in a metabolic chamber in open woodland near Brisbane in Queensland compared with parameter values derived in a laboratory study (see Methods for details) showing M_{ref} as equivalent to average or lower values producing the best fit (t_{max} = length of simulation in minutes)

Experimental details				Parameters determined in Chapter 2				Best Fit Model Inputs					Notes
Date	Echidna	mass kg	max T_e °C	M_{av} in TNZ W	$M_{\min}$ in TNZ W	$K_{o.av}$ $T_e<T_{lc}$ W.°C^{-1}	$K_{o.\min}$ $T_e<T_{lc}$ W.°C^{-1}	M_{ref} W	Q_{10}	K_o W.°C^{-1}	V_i ml.m^{-1}	t_{max} min	
01-Apr-04	IB13	3.80	32.4	3.96	2.01	.5341	.4227	2.01	2	.5341	500	637	echidna active to different levels for first 390 minutes
07-Oct-04	IB13	3.55	44.0	3.70	1.88	.4989	.3949	3.70	2	≤.3949	750	592	K_o varied from .3949 to .2000 depending on (T_b–T_e) giving lowest poss T_b
27-Oct-04	IE25	2.88	34.8	3.00	1.52	.4041	.3198	1.52	2	.4041	500	706	
02-Nov-04	IE24	2.18	31.2	2.27	1.15	.3057	.2420	2.27	2	≥.3057	750	372	Increasingly active after 270 minutes (~9 am): M increased accordingly. K_o initially .3057 but increases to 1.2 after 270 minutes.
30-Nov-04	RL28	2.53	41.4	2.63	1.34	.3549	.2809	2.00	2	≥.3549	750	330	Increasingly active after 247 minutes (~9 am): M increased accordingly. K_o initially .3549 but increases to 1.0 after 247 minutes.

Experimental details				Parameters determined in Chapter 2				Best Fit Model Inputs					Notes
Date	Echidna	mass kg	max T_e °C	M_{av} in TNZ W	$M_{\min}$ in TNZ W	$K_{o.av}$ $T_e<T_{lc}$ W.°C^{-1}	$K_{o.\min}$ $T_e<T_{lc}$ W.°C^{-1}	M_{ref} W	Q_{10}	K_o W.°C^{-1}	V_i ml.m^{-1}	t_{max} min	
14-Dec-04	IB13	3.65	43.8	3.80	1.93	.5130	.4060	2.87	2	≤.4060	1000	468	K_o varied from .4060 to .2000 depending on (T_b – T_e) giving lowest poss T_b
05-Jan-05	IE26	2.75	30.9	2.87	1.46	.3865	.3059	1.46	3	.3059	750	399	animal became active after 280 minutes: activity incorporated into modelling
09-Feb-05	IE26	2.93	33.6	3.05	1.55	.4111	.3254	na	na	.4111	750	387	animal active throughout so resting values of M not applicable: modelled with different levels of M to simulate different activity
16-Feb-05	TB15	2.63	47.8	2.74	1.39	.3689	.2920	1.39	3	≤.3689	750	598	K_o varies from .2920 early to .3689 in the opposite sense to IB13 above
03-Mar-05	VF30	3.18	34.7	3.31	1.68	.4462	.3532	2.50	2	.3532	1000	615	
17-Mar-05	TB15												Due to thermocouple problems T_a calculated indirectly and therefore T_e unreliable
23-Mar-05	IE24	2.93	45.5	3.05	1.55	.4111	.3254	2.30	2	.3254	750	486	

For echidnas that were active intermittently at different stages of experiments, incorporating active M as measured into the modelling generally retained the quality of fit for models without changing M_{ref} or the relationship of V_i to M (see Figure 4.1 for an example). However, on some (though not all) occasions, the quality of model fit could only be retained by increasing K_o during activity to higher levels consistent with the dumping of metabolic heat during the periods of activity (Table 4.2).

From modelling in which a constant T_e was maintained and M_{ref}, K_o etc. remained unchanged, IB13's (3.65 kg, $M_{ref} = 2.87$ W and $K_o = 0.406$ W.^{o}C^{-1}) expected T_b reached 38 ^{o}C (lethal levels: Augee, 1976) at about 16:00 hrs (710 minutes) when T_e was 35 ^{o}C and at about 14:00 hrs (590 minutes) when T_e was 40 ^{o}C. For TB15 (2.625 kg, M_{ref}=1.39 W and K_o = .3532 W.^{o}C^{-1}) although smaller, expected T_b only rose to 35.7 ^{o}C during the 720 minute simulation at T_e of 35 ^{o}C. When T_e was 40 ^{o}C expected T_b reached 38 ^{o}C after 720 minutes (16:52 hrs).

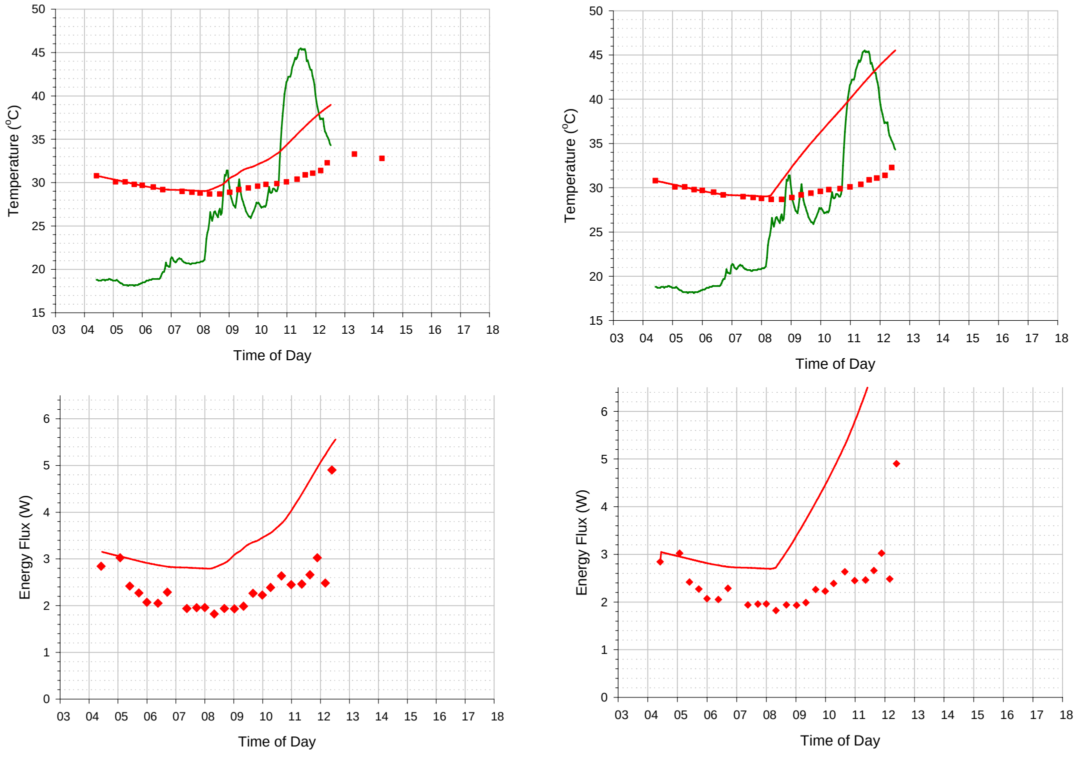
In the particular T_e regime of 23 Mar 05 (max $T_e = 45.5$ ^{o}C) for M_{ref} of 5.26 W (equivalent to a similar sized metatherian) and unchanged K_o, Q_{10} and V_i, expected T_b of IE24 reached 38 ^{o}C after 464 minutes (12:09 hrs: Figure 4.2). For $M_{ref} = 7.61$ W (equivalent to a similar sized eutherian) expected T_b reached 38 ^{o}C 100 minutes earlier at 10:29 hrs. With the original M_{ref} of 2.3 W and a K_o of 0.5559 W.^{o}C^{-1} (as per Withers' (1992a) equation for mammals), expected T_b reduced more rapidly than actual T_b until T_e increased after which expected T_b increased more rapidly to approximate actual T_b once again after 425 minutes (Figure 4.2). Combining $M_{ref} = 5.26$ W and $K_o = 0.5559$ W.^{o}C^{-1} gave a somewhat reduced T_b while T_e was still relatively low, but a rapid rise in T_b once high T_e were experienced (Figure 4.2). Thus a T_b of 38 ^{o}C was reached earlier than when K_o was low; that is after 450 minutes (at 11:55 hrs).

Discussion

Echidnas are clearly able to tolerate environmental temperatures in excess of 40 ^{o}C for at least an hour, even in these artificial or experimental situations. They remain unperturbed by increasing T_e as long as their T_b remain below about 33 ^{o}C; one degree higher than the modal T_b of active echidnas reported elsewhere (Grigg*, et al.*, 1989; Grigg*, et al.*, 1992b; Brice*, et al.*, 2002b; Nicol*, et al.*, 2004). In the absence of any effective evaporative cooling, echidnas in hot environments avoid compromisingly high T_b apparently through the cyclic, transient storage of heat. This study showed that while in the metabolic chamber overnight and into the next day, echidnas allowed their T_b to fall while T_e was still low (Table 4.1). When the environment heated during the day, obviously to different degrees depending on weather, T_b increased until echidnas sought to leave the apparatus. This pattern mirrors that observed in the field in semi-arid western Queensland where echidnas' T_b varied by 3 ^{o}C to 4 ^{o}C while in logs (that lacked any loose substrate) (Brice*, et al.*, 2002a). There, echidnas allowed T_b to reach 34 ^{o}C or even 35 ^{o}C, higher than in these experiments, and they stayed in their natural, hollow logs till after sunset (Brice*, et al.*, 2002a), later than in the pvc tubing. There are important differences between semi-arid western Queensland and mesic south east Queensland. Stevenson screen temperatures are higher and stay higher longer and insolation is more intense in the former.

This study adds to others illustrating the cyclic, transient storage of heat as a thermoregulatory mechanism for mammals subject to potential heat stress, and is the first to experimentally show such a mechanism in Prototheria. Various ground squirrels, including those at high latitudes, shuttle between surface environments where T_e and/or T_{es} exceeds thermoneutrality and burrows where T_e/T_{es} is much cooler (Chappell & Bartholomew, 1981; Long*, et al.*, 2005). They do so at short, sub-24 hour intervals, which is to be expected given their diurnal activity, small size and (therefore) low thermal inertia. Echidnas, being larger, store heat transiently over longer time frames (this study), often all day (Brice*, et al.*, 2002a), presumably conditional upon their maintaining low T_b while at rest. Larger mammals, including diurnally active ones, can clearly utilise the daily fluctuation in T_e/T_{es} to dump at night heat that may then be replaced during the day and such patterns have been described in numerous African artiodactylids (Harthoorn*, et al.*, 1970; Mitchell*, et al.*, 2002) and (classically) the camel, *Camelus dromedarius*, (Schmidt-Nielsen*, et al.*, 1956) as well as, possibly, the metatherian western grey kangaroo, *Macropus fuliginosus* (Maloney*, et al.*, 2002). Assuming it has a role in the water economy of arid zone mammals by reducing their need for evaporative cooling, the significance of such "adaptive heterothermy" has been questioned recently (Mitchell*, et al.*, 2002). Patterns of cyclic, transient heat storage seem to be independent of hydration, at least in arid zone artiodactylids (Mitchell*, et al.*, 2002). Given this as well as the relative insignificance of evaporative cooling in both echidnas (Augee, 1976) and at least some ground squirrels (Long*, et al.*, 2005), it may be argued that transient heat storage is less to do with managing water loss and more to do with managing sensible heat exchange directly. Only when high and possibly compromising T_b are approached is evaporative cooling necessary, and such occasions are presumably rare amongst those mammals that lack the sweating and/or panting response to high T_e. This then encourages speculation that evaporative cooling mechanisms common in mammals are apomorphic to the transient storage of heat as a thermoregulatory mechanism. After all, the resultant patterns of heterothermy are reminiscent of those in ectotherms and the behaviour of ground squirrels (Chappell & Bartholomew, 1981) has obvious parallels with shuttling in many lizards.

It is notable that the cycles of heterothermy extend so long for a mammal as small as an echidna. In the field, echidnas emerge from their hot logs late in the evening and, in this study, at least some echidnas remained at rest within the metabolic chamber till after noon on

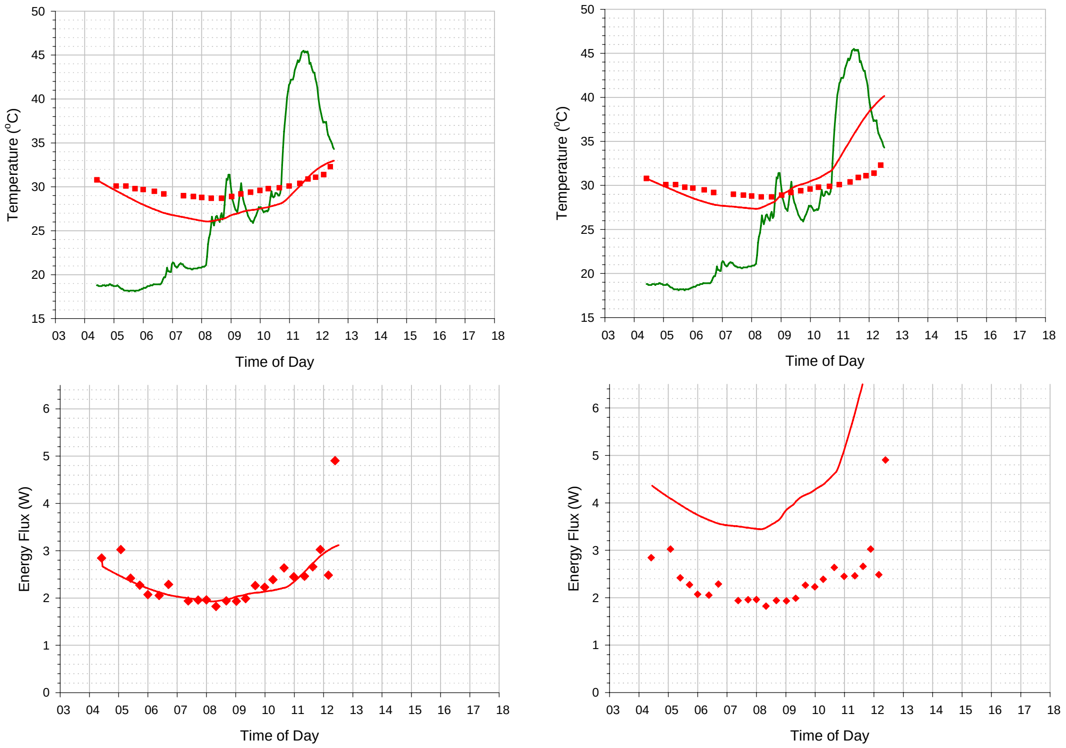


(a)

(b)

Figure 4.2 Alternative models applied to the operative temperature regime of 23 Mar 05 and compared to empirical data for IE24 in that regime (see Figure 4.1d) to show predicted effects of increased metabolic rate and conductance (with layout and legend as per Figure 4.1). (a) lines show predicted body temperature and metabolic rate for an endotherm of the same mass and thermal conductance as IE24 but with a thermoneutral resting metabolic rate typical of a metatherian. (b) lines show predicted body temperature and metabolic rate for an endotherm of the same mass and thermal conductance …

hot days. Due to its effect on thermal inertia, size might be an important factor in whether echidnas utilised potentially hot logs. While there was a tendency for smaller echidnas to seek to leave earlier in this study, such a correlation (a) was weak ($r^2 = 0.17$, P = 0.18) and (b) assumes that each experiment was subject to the same thermal conditions, which was clearly not the case. In fact, one modelling exercise showed that when subject to constant T_e of 35 ^{o}C or 40 ^{o}C a smaller echidna (TB15: 2.63 kg) would take longer to reach lethal levels of T_b than a larger one (IB13: 3.65 kg: see Results). Although this may have been attributable to the smaller animal having a lower M_{ref} or K_o it is more likely due to its lower starting T_b. Starting T_b was found to significantly alter the time course of T_b in determining best fit models when all other factors were equal. Nonetheless, minimum T_b and all other factors being equal, smaller echidnas would be expected to avoid hollow logs on days likely to



(c) (d)

Figure 4.2 (cont) … as IE24 but with a thermoneutral resting metabolic rate typical of a eutherian. (c) lines show predicted body temperature and metabolic rate for an endotherm of the same mass and resting thermoneutral metabolic rate as IE24 but with a minimum thermal conductance typical of mammals in general. (d) lines show predicted body temperature and metabolic rate for an endotherm of the same mass as IE24 but with a thermoneutral resting metabolic rate typical of a metatherian and a minimum thermal conductance typical of mammals in general.

become hot, such as when morning minimum shaded $T_a > 22\ ^{o}C$ at Idalia (Brice*, et al.*, 2002a). At Idalia at least, numerous alternative and cooler retreats are known to be available (Brice*, et al.*, 2002a).

Size is only one factor in an animal's thermal inertia. The other is K_o. Both observed and modelled data showed that minimum values of K_o for echidnas are low despite the scarcity of hair in their pelage. For echidnas at rest, both this study and that reported in Chapter 2 found K_o lower than that predicted for mammals by Withers (1992a), and as low as or perhaps lower than that predicted by Herreid and Kessel (1967). The thermal conductivity of echidna pelage is still unknown but rough estimates may be calculated given the combination of thermal conductivities for the hard keratin that comprises their spines and for the airspaces both within and between the spines. Such estimates vary between 0.150 $W.m^{-1}.^{o}C^{-1}$ or fractionally higher than that cited for the pelage of cattle with the highest conductivities (Cena & Clark, 1978 citing Gonzalez-Jimenez & Blaxter, 1962 and Bennett, 1964) and 0.055 $W.m^{-1}.^{o}C^{-1}$ or approximately equal to that of a kangaroo (Cena & Clark, 1978 citing Dawson & Brown, 1970) depending on the proportion of spines that are hollow. That the actual minimum K_o of echidnas is lower than the values implied by these conductivities indicates the probable importance of the subcutaneous tissues in the thermal conductance of echidnas. Comprising the *panniculus adiposus* underlying the *panniculus carnosus*, these tissues extend an estimated mean of 18 mm below the dorsal epidermis in an averaged sized echidna for this study (data from Augee, 1969 scaled to a 2.96 kg echidna). Presumably as these tissues may be perfused to different degrees, this supports conjecture by Grigg *et al.* (2004) about the role of by-passable insulation in echidnas and echidna-like proto-endotherms.

Despite the errors and uncertainties in calculating K_o from experimental data, it seems that the echidnas in this study maintained low values ($< 0.5\ W.^{o}C^{-1}$) at all T_e (between 15.1 ^{o}C and 47.8 ^{o}C) when resting. This is supported by model predictions incorporating such values as inputs (Table 4.2). The maintenance of low K_o above, as well as below thermoneutrality, is in contrast, not only to the classical model of endotherm thermoregulation (Scholander*, et al.*, 1950a), but also to a study of echidnas in the laboratory. There, in steady state conditions, mean K_o varied between 0.329 $W.^{o}C^{-1}$ and 1.065 $W.^{o}C^{-1}$ (for an average sized echidna) as T_e varied between 13 ^{o}C and 31 ^{o}C, although there was some evidence of variations within T_e occurring (Chapter 2). Minimising K_o retards heat absorption from the hot environment and is therefore expected in non steady state conditions while $T_b < T_e$. On the other hand, thermal conductance was also low early in the day when $T_e < T_b$. If echidnas anticipated later hot conditions, then it might be expected that echidnas maximise K_o to lose as much heat as possible while conditions are still cool. However there is no robust evidence of this. Modelling such maximum values produces relatively poor fits for observed T_b and other parameters, while empirical data from resting echidnas entering torpor (Chapter 3; unpub. data) also show they maintain K_o at low levels. In the field, echidnas avoid logs on warm mornings (Brice*, et al.*, 2002a). While this may be in anticipation of particularly high T_e later it may also be due to the limited opportunity to allow heat dissipation early in the day. One explanation for the low K_o early here, and for the low K_o on entry into torpor, may relate to an unlikely combination of a low M and a high heart rate. Cutaneous perfusion necessary for high K_o is facilitated by high heart rates (Bartholomew & Tucker, 1963; Heatwole, 1976; Kamau & Maloiy, 1982; Withers, 1992a) and high heart rates often correlate with high M (Morhardt & Morhardt, 1971; McNairn & Fairall, 1979; Kamau & Maloiy, 1982; Boyd*, et al.*, 1995). Given the importance of minimising M in situations that become hot later (below), the benefit of losing heat maximally to the environment may not be sufficient to justify the metabolic cost of increased K_o. Increasing K_o to equal the average for all mammals has less of an effect on T_b than increasing M_{ref} to metatherian levels. The only variations required to K_o in producing best fit models for resting echidnas involved changes between low values ($\leq$ 0.406 $W.^oC^{-1}$) and very low values (0.200 $W.^oC^{-1}$). Scope to fine tune K_o without compromising low M, through changes in piloerection and posture is to be expected.

Apparently of greater importance than adjustments in K_o are adjustments in M. In Chapter 2, the resting metabolic rate of echidnas at thermoneutrality was found to vary between 0.53 $W.kg^{-1}$ and 1.37 $W.kg^{-1}$. Modelling showed that the metabolic rates of resting echidnas in this study were consistent with these values, particularly values at the lower end of this range, once adjusted for temperature effect (Q_{10} of 2 or 3) and accounting for the thermal inertia of the variously sized echidnas responding to variations in T_e (Table 4.2). This suggests that metabolic rate was basal or near basal (as adjusted for different temperatures) throughout and that there was no metabolic depression that might be expected for aestivating animals. Further modelling shows that these near basal metabolic rates may be key to retaining low T_b throughout the different T_e. Metatherian M are generally higher than prototherian M and using expected metatherian M for these sized animals with the same Q_{10} and K_o shows that in these T_e regimes, T_b would be expected to rise rapidly to what for echidnas (though not necessarily other mammals) would be lethal levels (Figure 4.2). Using an even higher eutherian basal M and the same Q_{10} and K_o in the same environment, T_b might well rise to what would be lethal for most mammals of any "sub-class". Adjusting M to therian levels had a more significant effect on T_b than adjusting K_o to average mammalian levels (above). Given that variations in M potentially have a significant effect on the ability of echidnas to avoid compromisingly high T_b, it is to be expected that increases in M due to activity or stress should be avoided. In all the experimental days in this study, the four earliest end times (when the echidna sought to leave) all involved echidnas that were intermittently active throughout the morning. On only one day did an echidna that was active "settle" to rest within the chamber well into the afternoon but that was on a mildly warm, not a hot day. Similarly, elevated metabolic rates due to stress would compromise the echidnas' abilities to survive heat tolerance experiments. In this study, echidnas were well habituated to their environment and to the experimental apparatus, and the occurrence of low metabolic rates, often below those measured in thermoneutrality in other experimental studies, supports the conclusion that in this study echidnas were relaxed. Sadly, this may not have been the case in some previous studies where some echidnas failed their heat tolerance trials.

Chapter 5: General Discussion

The primary aim of this project was achieved by demonstrating, for the first time in Prototheria, the utility of cyclic, transient storage of heat as a strategy for thermoregulation during daily exposure to potential heat stress (Chapter 4). During hot weather, when echidnas are nocturnal, they are able to take the opportunity afforded by the early morning cool to lose the heat they accumulated while foraging during the previous night. This then allows scope to absorb heat during the day, which becomes especially important if they choose a log as a retreat. Although echidnas do tend to avoid them on especially warm mornings, logs get hotter than other retreats during the day, frequently reaching temperatures that have proved lethal to echidnas in laboratory experiments, and echidnas are now known to tolerate these high temperatures at least for a while (Brice*, et al.*, 2002a). Echidnas absorb heat from these microenvironments only slowly, so that by evening when the environment inside and outside the log is cooling, echidnas still have not reached lethal body temperatures. In the wild echidnas emerge from these retreats in the evenings to forage with body temperatures up to about 35 ^{o}C or 3 ^{o}C above their normal active levels (Brice*, et al.*, 2002a). They then forage through the night till around dawn when they select another retreat for the next day. In experiments, they tend to emerge earlier when their body temperatures reach 33 ^{o}C (Chapter 4).

Three factors underlie the echidnas' ability to utilise transient storage of heat as a thermoregulatory strategy. The first and most fundamental is their heterothermy; the up to 10 ^{o}C range in body temperature experienced daily by echidnas (Augee*, et al.*, 1970; Grigg*, et al.*, 1989; Grigg*, et al.*, 1992b; Nicol & Andersen, 2000; Kuchel, 2003). The second is their thermal inertia which ensures echidnas warm only slowly during the day so that even when their daytime retreats get hot, echidnas survive into the cool of the evening. This thermal inertia is a result of their relatively large size - only a few mammalian daily heterotherms exceed 1 kg (Prothero & Jürgens, 1986; Geiser, 2004) - and their low thermal conductance - lower than many other mammals of their size (Chapter 4). Cyclic, transient storage of heat has been reported in Theria ranging in size from ground squirrels (Chappell & Bartholomew, 1981; Long*, et al.*, 2005) to camels (Schmidt-Nielsen*, et al.*, 1956). The small ground squirrels need to visit burrows regularly during the day to lose the heat they gain from being active in the open in high operative temperatures (Chappell & Bartholomew, 1981). In this their behaviour is reminiscent of shuttling ectotherms. For the large camels, thermal inertia is so high that lasting the day is not an issue. Thus when black-globe temperatures (a surrogate for operative temperatures) reach 50 °C to 60 °C on a daily basis, camels' T_b vary by 6 °C: less (2 °C) when there is enough water available to drive their evaporative cooling mechanisms (Schmidt-Nielsen*, et al.*, 1956).

Of greater importance than their thermal inertia is the low metabolic rate of echidnas (Chapter 4); the third factor underlying echidnas' transient storage of heat. Tachyglossid metabolic rates are less than half those of metatherian and less than a third those of eutherian mammals of the same size (Dawson*, et al.*, 1979). This is sufficiently low to ensure that metabolic heat gained by an echidna resting all day in its retreat does not compromise the rate at which T_b rises, even though that rise in T_b itself causes an increase in metabolic heat production (Chapter 4). However, even small further increases of metabolic heat production are enough to threaten the animal's survival in its hot microenvironment. Had the echidna a metatherian metabolic rate, then it would be unable to withstand the heat in logs long enough (Chapter 4). It is likely that elevated metabolic rates compromised echidnas' ability to tolerate heat in previous laboratory experiments. During "short" exposure to high T_a, reported heart rates of 50 min^{-1} to > 100 min^{-1} (Augee, 1976) are equivalent to those for echidnas active in the field (unpub. data) suggesting elevated metabolism during Augee's experiments. In Robinson's (1954) trials, echidnas had mean heart rates of 96 min^{-1} and respiration rates of 11 min^{-1} (double that in the quasi-field experiments: Chapter 4) even before exposure to heat commenced. On the other hand, given that tachyglossid metabolic rates are low enough to ensure tolerance of heat, there is no need for metabolic depression. No evidence of aestivation has been found in echidnas (Chapter 4; Brice*, et al.*, 2002a; Brice*, et al.*, 2002b) despite their widespread use of hibernation, often from late summer (Grigg*, et al.*, 1989; Grigg*, et al.*, 1992b; Nicol & Andersen, 2000; Kuchel, 2003) including in arid (Grigg & Beard, pers. com.) and semi-arid areas (unpub. data).

Quantification of factors underlying the transient storage of heat was possible only through the application of modelling to echidnas in their experimental situations. In this, the project achieved its second aim. Quantitative modelling demonstrated the likely effect of both increased thermal conductance and increased metabolic rate for echidnas resting in thermally dynamic environments. However, the modelling has greater utility than this because it was developed *a priori* (Chapter 3). It is intuitive and simple because it integrates conduction, convection and radiation. It is applicable to animals of a wide size range, whether ectothermic or endothermic. It allows for animals with any thermal conductance. It allows for animals with widely varying metabolic rates and for animals with or without heat storage. It incorporates van't Hoff effects of temperature on metabolic rate for a wider range of situations then previously considered feasible (Chaui-Berlinck*, et al.*, 2002). But what really sets it apart from all other models reviewed is the combination of these attributes with the ability to incorporate dynamically fluctuating environments; the sorts of environments commonly experienced by animals in the field.

To be of use, the modelling required realistic inputs for parameter values and to this end - the third aim of this project - additional (laboratory) experiments were conducted on echidnas. As well as providing data for modelling input, these experiments revealed new insights into echidna thermoregulation. Common responses of mammals to cold include both thermogenesis, which more or less maintains a relatively high and stable T_b, and hypometabolism, which does not, and echidnas demonstrate both of these strategies (Chapter 2). Unusually for mammals though, echidnas show responses intermediate to these, a partial thermogenesis which only partially maintains T_b. Fundamental to this, as to their heat tolerance, is their heterothermy. To coin a phrase, they are "relaxed about maintaining a body temperature" (Grigg) allowing their T_b to drop below active levels (32 ^{o}C) when inactive and to rise again during activity. More than being merely heterothermic though, echidnas are constitutional eurytherms in that they are capable of coordinated and vigorous activity even with body temperatures as low as 20 ^{o}C. This allows them to forage without warming up first (although details of their eurythermic capabilities are still to be determined) and this has implications for their energy budget. Any mammal that need not stay warm, need not warm up before foraging, has a low metabolic rate anyway and hibernates for substantial parts of the year to boot must have significant advantages over those that must fuel constant and higher body temperatures. If this is the case, and if declining productivity is a feature of European impacts on much of the Australian landscape (Burbidge & McKenzie, 1989), then perhaps echidnas with their physiological attributes are relatively insensitive to those impacts. With this idea in mind, it would be instructive to analyse energy supplies available to echidnas in the field (ants and termites) and compare these with those available to other Australian mammals.

On the other hand, even before European arrival, ecosystems in Australia were characterised by low productivity compared with those elsewhere (Donald, 1967; Dragovich, 1987). More than this, Australian ecosystems, especially arid zone ones, are characterised by low predictability of resource availability (Stafford Smith & Morton, 1990). Presumably the Australian Theria as well as the Prototheria are adapted to these characteristics. Many, though by no means all Australian mammals, are Metatheria with metabolic rates intermediate to some of the Prototheria and Eutheria. Many are heterotherms becoming hypometabolic on a daily or seasonal basis (Geiser, 2004). Thus they share, to a lesser degree, the energetic economy of the echidna. Further, at least some bask in direct sun (at the entrance to burrows) while arousing (Warnecke, *et al.*, 2006). Although presumably this saves on the cost of rewarming to active T_b, this seems likely to increase their vulnerability to predation; and increased (feral) predation is the other often cited cause for recent mammalian decline and extinction in arid Australia (Morton, 1990; Johnson, 2006). However, when basking, many of these Metatheria are alert and readily escape attempts to catch them (Geiser and Warnecke, pers. com.), so perhaps constitutional eurythermy is more widespread than currently realised. In any case these heterotherms are small compared to the echidna, by far the largest mammalian heterotherm known in Australia (Geiser, 2004). Perhaps larger Metatheria, like larger Eutheria (Prothero & Jürgens, 1986), cannot afford the cost of regular arousals. Such a question might be addressed by combining information about resource availability with the modelling presented in Chapter 3 (further integrating ecology and physiology). What is known though, is that when exposed to cool ambient temperatures in a laboratory, hare-wallabies, bandicoots and bilbies (consistently?) increase their metabolic rate in a classical mammalian fashion (Table 2.1; Hulbert & Dawson, 1974) whereas echidnas, being more "relaxed about maintaining a body temperature" may not (Chapter 2). And it is the hare-wallabies, bandicoots and bilbies that have declined in, or disappeared from, the arid zone of Australia, not the echidna (Burbidge & McKenzie, 1989). Evidently there is further scope to combine physiological and ecological approaches (and modelling) to understand better the dynamics of all the mammals (Prototheria, Metatheria and, of course, Eutheria) in the changing Australian environments.

It should come as no surprise that a sister clade which diverged from the rest of the Mammalia before the Cretaceous has evolved its own adaptations to the environment. Indeed such a group should be a combination of plesiomorphies and apomorphies (Chapter 1). The thermoregulatory physiology of the echidna is certainly unusual (Chapter 2) and likely flexible enough in unpredictable and impoverished environments to allow the echidna to persist (above). It is also an attractive model for at least one stage in the evolution of endothermy; classically understood to mean homeothermic endothermy *sensu* Scholander *et al.* (1950a). As a heterothermic animal, with a wide range of thermal conductance, for which thermogenesis is often optional and variable, the echidna fits well the model proposed by Grigg *et al.* (2004). However this particular type of physiology may or may not be plesiomorphic. It is certainly not a synapomorphy for the Prototheria. When exposed to low ambient temperatures, the platypus defends its body temperature both in the laboratory (Grant & Dawson, 1978) and in the field (Grigg*, et al.*, 1992a). Given that "platypuses are almost certainly Cretaceous in origin while echidnas ... appear to be a mid-Tertiary group" (Musser, 2003) this might imply that heterothermy is apomorphic in echidnas. This is especially so if it is accepted that the tachyglossids split from the ornithorhynchids. However, this is by no means certain or even likely (Musser, 2003), and clearly these two prototherian families have evolved differences in thermoregulatory strategies, either from a common as yet unknown ancestor, or one from the other. If heterothermy is plesiomorphic, this would imply that homeothermic endothermy is apomorphic in platypus, having evolved independently from the other mammals. Homeothermic endothermy has evolved independently at least two (birds and mammals) or three (pterosaurs (?): Colbert, 1969; Benton, 1997) times and presumably it is not beyond the bounds of possibility that it would have evolved more often. For homeothermy to be an evolutionarily stable strategy, there must be enough reliable resources in the environment to fuel its energy requirements. A comparative analysis of the energy available to both platypuses and echidnas in their respective habitats might aid in such speculation and would probably have to suffice until more fossil material is found. Sadly few clues as to physiology of ancestral species are recorded in fossils and progress into understanding the evolution of endothermy might have to rely on supposition, or perhaps on modelling (Brice, in prep.).

To really understand biology it is important to understand how animals and plants interact with their environments in the field. For obvious logistical reasons so much biology has been conducted in laboratories and, because of various technical restraints, it has been difficult to apply laboratory derived insights to the field. For example, early studies into the thermoregulation of echidnas were conducted prior to the realisation that echidna T_b were so labile, a realisation dependent upon the availability of implantable temperature sensitive transmitters and loggers. Early heat tolerance trials in laboratories did not allow for the initial loss of heat that is a pre-requisite for its subsequent storage as a thermoregulatory strategy should the environment become hot. Neither, probably, was the importance of a low metabolic rate, the correlate of a stress-free and habituated echidna, probably fully realised. Only after heart rate data from echidnas ranging free from continual human interference were collected (unpub. data) was it realised that heart rates reported in the literature (above) were probably too high to come from animals truly at rest. One set of experiments in this project (Chapter 4) was conducted in field conditions. This was to quantify echidnas' thermoregulatory responses to the high, but fluctuating temperatures, they experience in the wild. In so doing, this set of experiments was the first to successfully demonstrate echidnas' tolerance to ambient temperatures above 35 °C. Based on data from a number of laboratory studies, this was previously thought impossible for echidnas (Griffiths, 1978). Conversely, a disadvantage of field based experiments is the difficulty of controlling environmental and other variables. Laboratory studies are attractive because they offer the opportunity to control precisely, and in isolation, a single variable. In the field study reported above (Chapter 4), the complexity of dynamic thermal environments varying within and between days could be controlled only in the most rudimentary of ways (selecting appropriate weather for experiments, placing the chamber under a suitable gap in the woodland canopy, etc.). The resultant dynamic, complex and interacting physiological responses (of metabolic rate, thermal conductance, temperature effect) could only be analysed by applying a modelling technique. But it was this integration of modelling and (quasi-) field data that allowed conclusions to be drawn. Thus modelling is a powerful tool, especially if developed *a priori* from current biological (or physical) concepts, in analysing factors in complex situations. Modelling is frequently used throughout science, including ecology and environmental science, because of these strengths. Modelling is likely a powerful tool in quantitatively analysing aspects of the biology of extinct species, the evolution of physiology, of endothermy and so on. Nonetheless, traditional experiments (Chapter 2) were also used in this project - obviously they have their place. Importantly though, as well as providing quantitative data, these were related to the field biology of echidnas to provide insights into their energy economy and ecology.

In summary, this project combined laboratory data, modelling and (quasi-) field data to demonstrate in echidnas their tolerance to the heat and their indifference to the cold; a flexibility in physiology quite unlike other Mammalia. It is this flexibility that exonerates echidnas from the need for thermogenesis, except when incubating, and from the need for evaporative cooling. Underlying these attributes is their heterothermy, at least, and their constitutional eurythermy, probably: aspects of mammalian physiology not yet well recognised, that may well provide models for evolution in endotherms.

References

Abbott, P.F. & Tabony, R.C., (1985), "The estimation of humidity parameters", *Meteorological Magazine,* **114**: 49-56.

Abensperg-Traun, M., (1991a), "A study of home-range, movements and shelter use in adult and juvenile echidnas, *Tachyglossus aculeatus* (Monotremata: Tachyglossidae), in Western Australian wheatbelt reserves", *Australian Mammalogy,* **14**: 13-21.

Abensperg-Traun, M., (1991b), "Survival strategies of the echidna *Tachyglossus aculeatus* Shaw 1792 (Monotremata: Tachyglossidae)", *Biological Conservation,* **58**: 317-328.

Abensperg-Traun, M. & De Boer, E.S., (1992), "The foraging ecology of a termite- and ant-eating specialist, the echidna *Tachyglossus aculeatus* (Monotremata: Tachyglossidae)", *Journal of Zoology, London,* **226**: 243-257.

Almirall, H., (1997), "Modeling the body temperature throughout the day with a two-term function", *Behavior Research Methods Instruments and Computers,* **29**: 595-599.

Andersen, N.A. & Nicol, S. (2006) "Comparing apples with apples, how to compare arousal rates in different species" in *Australian and New Zealand Society for Comparative Physiology and Biochemistry: 23rd. Annual Conference*, p 61.

Argall, T.W., (1987), *A feasibility study of the use of heart rate as an index of energy expenditure and activity in free-ranging echidnas*, Bachelor of Science (Honours) Thesis, University of Sydney, Sydney.

Armitage, K.B., Melcher, J.C. & Ward, J.M.J., (1990), "Oxygen consumption and body temperature in yellow-bellied marmot populations from montane-mesic and lowland-xeric environments", *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology,* **160**: 491-502.

Arnold, J. & Shield, J., (1970), "Oxygen consumption and body temperature of the chuditch (*Dasyurus geoffroii*)", *Journal of Zoology (London),* **160**: 391-404.

Augee, M.L., (1969), *Temperature Regulation and Adrenal Function in the Echidna*, PhD Thesis, Monash University, Melbourne.

Augee, M.L., (1976), "Heat tolerance of Monotremes", *Journal of Thermal Biology,* **1**: 181-184.

Augee, M.L., (1978), "Monotremes and the evolution of homeothermy", *Australian Zoologist,* **20**: 111-119.

Augee, M.L., Ealey, E.H.M. & Price, I.P., (1975), "Movements of echidnas, *Tachyglossus aculeatus*, determined by marking-recapture and radio-tracking", *Australian Wildlife Research,* **2**: 93-101.

Augee, M.L., Ealey, E.H.M. & Spencer, H., (1970), "Biotelemetric studies of temperature regulation and torpor in the echidna, *Tachyglossus aculeatus*", *Journal of Mammalogy,* **51**: 561-570.

Augee, M.L. & Grant, T.R., (1976), "Heat tolerance of echidnas", *Bulletin: Australian Mammal Society,* **3**: 18.

Bakken, G.S., (1976), "A heat transfer analysis of animals: unifying concepts and the application of metabolism chamber data", *Journal of Theoretical Biology,* **60**: 337-384.

Bakken, G.S., (1980), "The use of standard operative temperature in the study of the thermal energetics of birds", *Physiological Zoology,* **53**: 108-119.

Bakken, G.S., (1981), "A two-dimensional operative-temperature model for thermal energy management by animals", *Journal of Thermal Biology,* **6**: 23-30.

Bakken, G.S., (1992), "Measurement and application of operative and standard operative temperatures in ecology", *American Zoologist,* **32**: 194-216.

Bakken, G.S., Erskine, D.J. & Santee, W.R., (1983), "Construction and operation of heated taxidermic mounts used to measure standard operative temperature", *Ecology,* **64**: 1658-1662.

Bakken, G.S., Kenow, K.P., Korschgen, C.E. & Boysen, A.F., (2000), "Statistical and procedural issues in the use of heated taxidermic mounts", *Journal of Thermal Biology,* **25**: 317-321.

Bakken, G.S., Reynolds, P.S., Kenow, K.P., Korschgen, C.E. & Boysen, A.F., (1999), "Standardization and calibration of heated mounts illustrated with day-old mallard ducklings", *Physiological and Biochemical Zoology,* **72**: 502-506.

Bakken, G.S., Santee, W.R. & Erskine, D.J., (1985), "Operative and standard operative temperature: tools for thermal energetics studies", *American Zoologist,* **25**: 933-943.

Bakken, G.S., Williams, J.B. & Ricklefs, R.E., (2002), "Metabolic response to wind of downy chicks of Arctic-breeding shorebirds (Scolopacidae)", *Journal of Experimental Biology,* **205**: 3435-3443.

Barrow, G.M., (1973), *Physical Chemistry.* 3rd. ed. Tokyo: McGraw-Hill Kogakusha.

Bartholomew, G.A. & Tucker, V.A., (1963), "Control of changes in body temperature, metabolism, and circulation by the agamid lizard, *Amphibolurus barbatus*", *Physiological Zoology,* **36**: 199-218.

Bartholomew, G.A. & Tucker, V.A., (1964), "Size, body temperature, thermal conductance, oxygen consumption, and heart rate in Australian varanid lizards", *Physiological Zoology,* **37**: 341-354.

Baxter, S., (1945), "The thermal conductivity of textiles", *Proceedings of the Physics Society,* **58**: 105-118.

Beard, L.A. & Grigg, G.C., (2000), "Reproduction in the short-beaked echidna, *Tachyglossus aculeatus*: field observations at an elevated site in south-east Queensland", *Proceedings of the Linnean Society of New South Wales,* **122**: 89-99.

Beard, L.A., Grigg, G.C. & Augee, M.L., (1992). "Reproduction by echidnas in a cold climate", pp. 93-100 in Augee, M.L. (ed.), *Platypus and Echidnas.* Sydney: Royal Zoological Society of N.S.W

Bech, C., Nicol, S. & Anderson, N., (1992). "Ventilation in the echidna (*Tachyglossus aculeatus*)", pp. 134-139 in Augee, M.L. (ed.), *Platypus and Echidnas.* Sydney: Royal Zoological Society of N.S.W

Bech, C. & Nicol, S.C., (1999), "Thermoregulation and ventilation in the tawny frogmouth, *Podargus strigoides*: A low-metabolic avian species", *Australian Journal of Zoology,* **47**: 143-153.

Beer, T., (1990), *Applied Environmetrics Meteorological Tables* Software, Melbourne: Applied Environmetrics

Bellamy, T.A. (1994) "Handrearing native animals" in *Wildlife: The T.G. Hungerford Refresher Course for Veterinarians*

Bentley, P.J., Clyde, F.H.I. & Schmidt-Nielsen, K., (1967), "Respiration in a monotreme, the echidna, *Tachyglossus aculeatus*", *American Journal of Physiology,* **212**: 957-961.

Benton, M.J., (1997), *Vertebrate Palaeontology.* 2nd. ed. London: Chapman & Hall.

Blackadar, A.K., (1983), "Using home computers to study the weather", *Weatherwise,* **36**: 193-195.

Blackburn, T.M., Lawton, J.H. & Perry, J.N., (1992), "A method of estimating the slope of upper bounds of plots of body size and abundance in natural animal assemblages", *Oikos,* **65**: 107-112.

Block, R.J., (1939), "The composition of keratins: the amino acid composition of hair, wool, horn and other eukeratins." *Journal of Biological Chemistry,* **128**: 181-186.

Bolton, D.P.G., Nelson, E.A.S., Taylor, B.J. & Weatherall, I.L., (1996), "Thermal balance in infants", *Journal of Applied Physiology,* **80**: 2234-2242.

Booth, D.T., (1984), "Thermoregulation in neonate mallee fowl *Leipoa ocellata*", *Physiological Zoology,* **57**: 251-260.

Boyd, I.L., Woakes, A.J., Butler, P.J., Davis, R.W. & Williams, T.M., (1995), "Validation of heart rate and doubly labelled water as measures of metabolic rate during swimming in California sea lions", *Functional Ecology,* **9**: 151-160.

Brice, P.H., (in prep.), "Quantifying aspects of the evolution of endothermy", *Australian Zoologist*.

Brice, P.H., Grigg, G.C., Beard, L.A. & Donovan, J.A., (2002a), "Heat tolerance of short-beaked echidnas (*Tachyglossus aculeatus*) in the field." *Journal of Thermal Biology,* **27**: 449-457.

Brice, P.H., Grigg, G.C., Beard, L.A. & Donovan, J.A., (2002b), "Patterns of activity and inactivity in echidnas (*Tachyglossus aculeatus*) free-ranging in a hot dry climate: correlates with ambient temperature, time of day and season." *Australian Journal of Zoology,* **50**: 461-475.

Britton, S.W. & Atkinson, W.E., (1938), "Poikilothermism in the sloth", *Journal of Mammalogy,* **19**: 94-99.

Brown, G.D., (1984). "The biology of marsupials of the Australian arid zone", pp. 253-262 in Cameron, E.E. (ed.), *Arid Australia.* Sydney: Australian Museum

Brown, R.D. & Gillespie, T.J., (1986), "Estimating outdoor thermal comfort using a cylindrical radiation thermometer and an energy budget model", *International Journal of Biometeorology,* **30**: 43-52.

Buck, A.L., (1981), "New equations for computing vapor pressure and enhancement factor", *Journal of Applied Meteorology,* **20**: 1527-1532.

Buffenstein, R. & Yahav, S., (1991), "Is the naked mole-rat *Heterocephalus glaber* an endothermic yet poikilothermic mammal?" *Journal of Thermal Biology,* **16**: 227-232.

Burbidge, A.A. & McKenzie, N.L., (1989), "Patterns in the modern decline of Western Australia's vertebrate fauna: causes and conservation implications", *Biological Conservation,* **50**: 143-198.

Buse, M. & Werner, J., (1985), "Heat balance of the human body: influence of variations of locally distributed parameters", *Journal of Theoretical Biology,* **114**: 34-52.

Buttemer, W.A., Hayworth, A.M., Weathers, W.W. & Nagy, K.A., (1986), "Time-budget estimates of avian energy expenditure: physiological and meteorological considerations", *Physiological Zoology,* **59**: 131-149.

Campbell, G.S. & Norman, J.M., (1998), *An Introduction to Environmental Biophysics.* 2nd. ed. New York: Springer.

Cartar, R.V. & Morrison, R.I.G., (1997), "Estimating metabolic costs for homeotherms from weather data and morphology: an example using calidridine sandpipers", *Canadian Journal of Zoology,* **75**: 94-101.

Casey, T.M. & Casey, K.K., (1979), "Thermoregulation of Arctic weasels", *Physiological Zoology,* **52**: 153-164.

Cena, K. & Clark, J.A., (1978), "Thermal insulation of animal coats and human clothing", *Physics in Medicine and Biology,* **23**: 565-591.

Chappell, M.A. & Bartholomew, G.A., (1981), "Activity and thermoregulation of the antelope ground squirrel *Ammospermophilus leucurus* in winter and summer", *Physiological Zoology,* **54**: 215-223.

Chappell, M.A. & Dawson, T.J., (1994), "Ventilatory accommodation of changing oxygen consumption in dasyurid marsupials", *Physiological Zoology,* **67**: 418-437.

Chappell, M.A. & Roverud, R.C., (1990), "Temperature effects on metabolism ventilation and oxygen extraction in a neotropical bat", *Respiration Physiology,* **81**: 401-412.

Chaui-Berlinck, J.G., Monteiro, L.H.A., Navas, C.A. & Bicudo, J., (2002), "Temperature effects on energy metabolism: a dynamic system analysis", *Proceedings of the Royal Society of London Series B-Biological Sciences,* **269**: 15-19.

Colbert, E.H., (1969), *Evolution of the Vertebrates: a History of the Backboned Animals Through Time.* 2nd. ed. New York: John Wiley & Sons.

Collins, B.G., (1973), "Physiological responses to temperature stress by an Australian murid, *Rattus lutreolus*", *Journal of Mammalogy,* **54**: 356-368.

Cooper, C.E. & Withers, P.C., (2002), "Metabolic physiology of the numbat (*Myrmecobius fasciatus*)", *Journal of Comparative Physiology B,* **172**: 669-675.

Cooper, S.J., (1999), "The thermal and energetic significance of cavity roosting in mountain chickadees and juniper jitmice", *Condor,* **101**: 863-866.

Daniels, H.L., (1984), "Oxygen consumption in *Lemur fulvus*: deviation from the ideal model", *Journal of Mammalogy,* **65**: 584-592.

Davis, D.E., (1976), "Hibernation and circannual rhythms of food consumption in marmots and ground squirrels", *Quarterly Review of Biology,* **51**: 477-514.

Dawson, T.J., (1969), "Temperature regulation and evaporative water loss in the brush-tailed possum *Trichosurus vulpecula*", *Comparative Biochemistry and Physiology,* **28**: 401-407.

Dawson, T.J., Blaney, C.E., Munn, A.J., Krockenberger, A. & Maloney, S.K., (2000a), "Thermoregulation by kangaroos from mesic and arid habitats: Influence of temperature on routes of heat loss in eastern grey kangaroos (*Macropus giganteus*) and red kangaroos (*Macropus rufus*)", *Physiological and Biochemical Zoology,* **73**: 374-381.

Dawson, T.J., Fanning, D. & Bergin, T.J., (1978), "Metabolism and temperature regulation in the New Guinea monotreme *Zaglossus bruijni*", *Australian Zoologist,* **20**: 99-103.

Dawson, T.J. & Fanning, F.D., (1981), "Thermal and energetic problems of semiaquatic mammals: a study of the Australian water rat, including comparisons with the platypus", *Physiological Zoology,* **54**: 285-296.

Dawson, T.J., Grant, T.R. & Fanning, D., (1979), "Standard metabolism of monotremes and the evolution of homeothermy", *Australian Journal of Zoology,* **27**: 511-515.

Dawson, T.J., Munn, A.J., Blaney, C.E., Krockenberger, A. & Maloney, S.K., (2000b), "Ventilatory accommodation of oxygen demand and respiratory water loss in kangaroos from mesic and arid environments, the eastern grey kangaroo (*Macropus giganteus*) and the red kangaroo (*Macropus rufus*)", *Physiological and Biochemical Zoology,* **73**: 382-388.

Dawson, W.R. & Bartholomew, G.A., (1958), "Metabolic and cardiac responses to temperature in the lizard, *Dipsosaurus dorsalis*", *Physiological Zoology,* **31**: 100-111.

Dawson, W.R. & Bennett, A.F., (1978), "Energy metabolism and thermoregulation of the spectacled hare wallaby (*Lagorchestes conspicillatus*)", *Physiological Zoology,* **51**: 114-130.

De Jong, P.W., Gussekloo, S.W.S. & Brakefield, P.M., (1996), "Differences in thermal balance, body temperature and activity between non-melanic and melanic two-spot ladybird beetles (*Adalia bipunctata*) under controlled conditions", *Journal of Experimental Biology,* **199**: 2655-2666.

de Miklouho Maclay, N., (1883), "Temperature of the body of *Echidna hystrix* Cuv." *Proceedings of the Linnean Society of New South Wales,* **8**: 425-426.

Donald, C.M., (1967). "Innovation in agriculture", pp. 57-86 in Williams, D.B. (ed.), *Agriculture in the Australian Economy.* Sydney: University Press

Dragovich, D., (1987). "Climate and agricultural production", pp. 237-258 in Jeans, D.N. (ed.), *Australia - a Geography. Volume 2: Space and Society.* 2nd. ed., Sydney: Sydney University Press

Dreisig, H., (1984), "Control of body temperature in shuttling ectotherms", *Journal of Thermal Biology,* **9**: 229-234.

Dreisig, H., (1985), "A time budget model of thermoregulation in shuttling ectotherms", *Journal of Arid Environments,* **8**: 191-206.

Dzialowski, E.M. & O'Connor, M.P., (2001), "Thermal time constant estimation in warming and cooling ectotherms", *Journal of Thermal Biology,* **26**: 231-245.

Eisentraut, M., (1960), "Heat regulation in primitive mammals and tropical species", *Bulletin of the Museum of Comparative Zoology at Harvard College,* **124**: 31-43.

Eppley, Z.A., (1994), "A mathematical model of heat flux applied to developing endotherms", *Physiological Zoology,* **67**: 829-854.

Fielden, L.J., Waggoner, J.P., Perrin, M.R. & Hickman, G.C., (1990), "Thermoregulation in the Namib Desert golden mole, *Eremitalpa granti namibensis* (Chrysochloridae)." *Journal of Arid Environments,* **18**: 221-237.

Fleming, M.R., (1980), "Thermoregulation and torpor in the sugar glider, *Petaurus breviceps* (Marsupialia: Petauridae)", *Australian Journal of Zoology,* **28**: 521-534.

Fleming, M.R., (1985), "The thermal physiology of the feathertail glider, *Acrobates pygmaeus* (Marsupialia: Burramyidae)", *Australian Journal of Zoology,* **33**: 667-682.

Florides, G.A., Wrobel, L.C., Kalogirou, S.A. & Tassou, S.A., (1999), "A thermal model for reptiles and pelycosaurs", *Journal of Thermal Biology,* **24**: 1-13.

Fox, B.J., (1989). "Community ecology of macropodoids", pp. 89-104 in Grigg, G., Jarman, P. & Hume, I. (ed.), *Kangaroos, Wallabies and Rat-Kangaroos.* Chipping Norton, N.S.W.: Surrey Beatty and Sons

Frappell, P.B., Franklin, C.E. & Grigg, G.C., (1994), "Ventilatory and metabolic responses to hypoxia in the echidna, *Tachyglossus aculeatus*", *American Journal of Physiology,* **267**: R1510-R1515.

Friend, J.A., (1990), "The numbat *Myrmecobius fasciatus* (Myrmecobiidae): history of decline and potential for recovery", *Proceedings of the Ecological Society of Australia,* **16**: 369-377.

Gates, D.M., (1980), *Biophysical Ecology.* New York: Springer-Verlag.

Geiser, F., (1986), "Thermoregulation and torpor in the kultarr, *Antechinomys laniger* (Marsupialia: Dasyuridae)", *Journal of Comparative Physiology B,* **156**: 751-757.

Geiser, F., (2004), "Metabolic rate and body temperature reduction during hibernation and daily torpor", *Annual Review of Physiology,* **66**: 239-274.

Geiser, F. & Baudinette, R.V., (1987), "Seasonality of torpor and thermoregulation in three dasyurid marsupials", *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology,* **157**: 335-344.

Geiser, F. & Baudinette, R.V., (1988), "Daily torpor and thermoregulation in the small dasyurid marsupials *Planigale gilesi* and *Ningaui yvonneae*", *Australian Journal of Zoology,* **36**: 473-481.

Geiser, F. & Brigham, R.M., (2000), "Torpor, thermal biology, and energetics in Australian long-eared bats (*Nyctophilus*)", *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology,* **170**: 153-162.

Geiser, F., Coburn, D.K., Kortner, G. & Law, B.S., (1996), "Thermoregulation, energy metabolism, and torpor in blossom-bats, *Syconycteris australis* (Megachiroptera)", *Journal of Zoology,* **239**: 583-590.

Geiser, F. & Ruf, T., (1995), "Invited perspectives in physiological zoology: hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns", *Physiological Zoology,* **68**: 935-966.

Gentilli, J., (1986). "Climate", pp. in Jeans, D.N. (ed.), *Australia - a Geography. Volume 1. The Natural Environment.* Sydney: Sydney University Press

Golightly, R.T., Jr. & Ohmart, R.D., (1983), "Metabolism and body temperature of two desert canids: coyotes and kit foxes", *Journal of Mammalogy,* **64**: 624-635.

Goodfellow, (2007), *Polyvinylchloride Unplasticized (UPVC): Material Information.* http://www.goodfellow.com/csp/active/static/A/Polyvinylchloride_-_Unplasticized.html visited 31st March 2007

Grant, B.W. & Porter, W.P., (1992), "Modeling global macroclimatic constraints on ectotherm energy budgets", *American Zoologist,* **32**: 154-178.

Grant, T.R. & Dawson, T.J., (1978), "Temperature regulation in the platypus, *Ornithorhynchus anatinus*: production and loss of metabolic heat in air and water", *Physiological Zoology,* **51**: 315-332.

Green, B., Griffiths, M. & Newgrain, K., (1992), "Seasonal patterns in water, sodium and energy turnover in free-living echidnas, *Tachyglossus aculeatus* (Mammalia: Monotremata)", *Journal of Zoology, London,* **227**: 351-365.

Griffiths, M., (1968), *Echidnas.* Oxford: Pergamon Press.

Griffiths, M., (1978), *The Biology of Monotremes.* New York: Academic Press.

Grigg, G. & Beard, L., (2000). "Hibernation by echidnas in mild climates: hints about the evolution of endothermy?" pp. 5-19 in Heldmaier, G. & Klingenspor, M. (ed.), *Life in the Cold: Eleventh International Hibernation Symposium.* Berlin: Springer-Verlag

Grigg, G., Beard, L., Grant, T. & Augee, M., (1992a), "Body temperature and diurnal activity patterns in the platypus (*Ornithorhynchus anatinus*) during winter", *Australian Journal of Zoology,* **40**: 135-142.

Grigg, G.C., Augee, M.L. & Beard, L.A., (1992b). "Thermal relations of free-living echidnas during activity and in hibernation in a cold climate", pp. 160-173 in Augee, M.L. (ed.), *Platypus and Echidnas.* Sydney: Royal Zoological Society of N.S.W.

Grigg, G.C., Beard, L.A. & Augee, M.L., (1989), "Hibernation in a monotreme, the echidna (*Tachyglossus aculeatus*)", *Comparative Biochemistry and Physiology A Comparative Physiology,* **92A**: 609-612.

Grigg, G.C., Beard, L.A. & Augee, M.L., (2004), "The evolution of endothermy and its diversity in mammals and birds", *Physiological and Biochemical Zoology,* **77**: 982-997.

Grigg, G.C., Drane, C.R. & Courtice, G.P., (1979), "Time constants of heating and cooling in the eastern water dragon, *Physignathus lesueruii* and some generalizations about heating and cooling in reptiles", *Journal of Thermal Biology,* **4**: 95-103.

Haim, A., (1987), "Thermoregulation and metabolism of Wagner's gerbil *Gerbillus dasyurus* a rock dwelling rodent adapted to arid and mesic environments", *Journal of Thermal Biology,* **12**: 45-48.

Haim, A., Van Aarde, R.J. & Skinner, J.D., (1990), "Metabolism and thermoregulation in the Cape porcupine, *Hystrix africaeaustralis*", *Physiological Zoology,* **63**: 795-802.

Hammel, H.T., (1955), "Thermal properties of fur", *American Journal of Physiology,* **182**: 369-376.

Hammel, H.T., (1966), *The regulator of body temperature.* Columbia: University of Missouri.

Harlow, H.J., (1981), "Torpor and other physiological adaptations of the badger (*Taxidea taxus*) to cold environments", *Physiological Zoology,* **54**: 267-275.

Harthoorn, A.M., Finch, V., Hopcroft, D. & McGommis, S.M., (1970), "Adaptation to solar radiation by African large herbivores: a preliminary report", *Journal of the South African Veterinary Medical Association,* **41**: 17-24.

Hawk, P.B., Oser, B.L. & Summerson, W.H., (1954), *Practical Physiological Chemistry.* 13th. ed. New York: Blakiston.

Heatwole, H., (1976), *Reptile Ecology.* St. Lucia: University of Queensland Press.

Heldmaier, G. & Steinlechner, S., (1981), "Seasonal pattern and energetics of short daily torpor in the Djungarian hamster, *Phodopus sungorus*", *Oecologia,* **48**: 265-270.

Herreid, C.F. & Kessel, B., (1967), "Thermal conductance in birds and mammals", *Comparative Biochemistry and Physiology,* **21**: 405-414.

Hertz, P.E., Huey, R.B. & Stevenson, R.D., (1993), "Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question", *American Naturalist,* **142**: 796-818.

Hicks, J.W. & Bennett, A.F., (2004), "Eat and run: prioritization of oxygen delivery during elevated metabolic states", *Respiratory Physiology & Neurobiology,* **144**: 215-224.

Hillman, P.E. & Scott, N.R., (1989), "Energy budget of the chicken foot", *Journal of Thermal Biology,* **14**: 205-218.

Hokkanen, J.E.I., (1990), "Temperature regulation of marine mammals", *Journal of Theoretical Biology,* **145**: 465-486.

Holman, J.P., (1992), *Heat Transfer.* 7th. ed. London: McGraw-Hill.

Hosken, D.J. & Withers, P.C., (1999), "Metabolic physiology of euthermic and torpid lesser long-eared bats, *Nyctophilus geoffroyi* (Chiroptera : Vespertilionidae)", *Journal of Mammalogy,* **80**: 42-52.

Howell, J.R., (2001), *A Catalogue of Radiation Heat Transfer Configuration Factors.* http://www.me.utexas.edu/~howell/ visited 31st March 2007

Hudson, J.W., (1978). "Shallow daily torpor: a thermoregulatory adaptation", pp. 67-108 in Wang, L.C.H. & Hudson, J.W. (ed.), *Strategies in the Cold: Natural Torpidity and Thermogenesis.* New York: Academic Press

Hudson, J.W. & Dawson, T.J., (1975), "Role of the sweating from the tail in the thermal balance of the rat-kangaroo *Potorous tridactylus*", *Australian Journal of Zoology,* **23**: 453-461.

Hulbert, A.J. & Dawson, T.J., (1974), "Thermoregulation in perameloid marsupials from different environments", *Comparative Biochemistry and Physiology - Part A: Physiology,* **47**: 591-616.

Ikonomopoulou, P. & Rose, R.W., (2003), "The metabolic rate and thermal conductance of the eastern barred bandicoot (*Perameles gunnii*) at different ambient temperatures", *Australian Journal of Zoology,* **51**: 603-610.

Incropera, F.P. & DeWitt, D.P., (1990), *Fundamentals of Heat and Mass Transfer.* 3rd. ed. New York: John Wiley & Sons.

IUPS Thermal Commision, (2003), "Glossary of terms for thermal physiology", *Journal of Thermal Biology,* **28**: 75-106.

Ivlev, Y.F. & Chernova, O.F., (2005), "The insulating properties of the pelage of the North-American porcupine (*Erethizon dorsatum*): the influence of quill-like structures on heat transfer", *Doklady Akademii Nauk,* **403**: 706-709.

Jardine, D.S., (1992), "A mathematical model of life-threatening hyperthermia during infancy", *Journal of Applied Physiology,* **73**: 329-339.

Johnson, C., (2006), *Australia's Mammal Extinctions: a 50 000 year history.* Cambridge: Cambridge University Press.

Kamau, J.M.Z. & Maloiy, G.M.O., (1982), "The relationship between rate of oxygen consumption, heart rate and thermal conductance of the dik-dik antelope (*Rhynchotragus kirkii*) at various ambient temperatures." *Comparative Biochemistry and Physiology,* **73A**: 21-24.

Kanoh, S. & Nakajima, T., (1998), "Determining specific gravity, specific heat, and surface area of rabbits for a possible thermodynamic approach to body temperature change", *Biological & Pharmaceutical Bulletin,* **21**: 565-568.

Katzner, T.E., Parker, K.L. & Harlow, H.H., (1997), "Metabolism and thermal response in winter-acclimatized pygmy rabbits (*Brachylagus idahoensis*)", *Journal of Mammalogy,* **78**: 1053-1062.

Kettlewell, P.J. & Moran, P., (1992), "A study of heat production and heat loss in crated broiler chickens: a mathematical model for a single bird", *British Poultry Science,* **33**: 239-252.

Kingsolver, J., (1983), "Thermoregulation and flight in *Colias* butterflies: elevational patterns and mechanistic limitations", *Ecology,* **64**: 534-545.

Kingsolver, J. & Watt, W.B., (1983), "Thermoregulatory strategies in *Colias* butterflies: thermal stress and the limits to adaptation in thermally varying environments", *American Naturalist,* **121**: 32-55.

Kleiber, M., (1961), *The fire of life: an introduction to animal energetics.* New York: Wiley.

Kreith, F., (1965), *Principles of Heat Transfer.* 2nd. ed. Scranton, Pennsylvania: International Textbook.

Kuchel, L.J., (2003), *The energetics and patterns of torpor in free-ranging Tachyglossus aculeatus from a warm-temperate climate*, PhD Thesis, University of Queensland, Brisbane.

Lactin, D.J. & Johnson, D.L., (1997), "Response of body temperature to solar radiation in restrained nymphal migratory grasshoppers (Orthoptera: Acrididae): Influences of orientation and body size", *Physiological Entomology,* **22**: 131-139.

Larcombe, A., (2002), "Effects of temperature on metabolism, ventilation, and oxygen extraction in the southern brown bandicoot *Isoodon obesulus* (Marsupialia: Peramelidae)", *Physiological and Biochemical Zoology,* **75**: 405-411.

Larochelle, J., (1998), "Comments on a negative appraisal of taxidermic mounts as tools for studies of ecological energetics", *Physiological Zoology,* **71**: 596-598.

Lauzier, N. & Rousse, D., (2003), *View Factors with GUI* Software, Quebec: Université Laval

Leitner, P. & Nelson, J.E., (1967), "Body temperature oxygen consumption and heart rate in Australian false vampire bat, *Macroderma gigas*", *Comparative Biochemistry and Physiology,* **21**: 65-74.

Linacre, E. & Hobbs, H., (1977), *The Australian Climatic Environment.* Milton: John Wiley and Sons.

Liu, J., (2001), "Uncertainty analysis for temperature prediction of biological bodies subject to randomly spatial heating", *Journal of Biomechanics,* **34**: 1637-1642.

Long, R.A., Martin, T.J. & Barnes, B.M., (2005), "Body temperature and activity patterns in free-living Arctic ground squirrels", *Journal of Mammalogy,* **86**: 314-322.

Lovegrove, B.G., Heldmaier, G. & Knight, M., (1991), "Seasonal and circadian energetic patterns in an arboreal rodent *Thallomys paedulcus* and a burrow-dwelling rodent *Aethomys namaquensis* from the Kalahari Desert South Africa", *Journal of Thermal Biology,* **16**: 199-210.

Lyman, C.P., (1948), "The oxygen consumption and temperature regulation of hibernating hamsters", *Journal of Experimental Zoology,* **109**: 55-78.

Maloney, S.K. & Dawson, T.J., (1994a), "Thermoregulation in a large bird, the emu (*Dromaius novaehollandiae*)", *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology,* **164**: 464-472.

Maloney, S.K. & Dawson, T.J., (1994b), "Ventilatory accommodation of oxygen demand and respiratory water loss in a large bird, the emu (*Dromaius novaehollandiae*), and a re-examination of ventilatory allometry for birds", *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology,* **164**: 473-481.

Maloney, S.K., Fuller, A., Kammerman, P.R. & Mitchell, D. (2002) "Carotid blood and brain temperatures of free ranging western grey kangaroos (*Macropus fuliginosus*)" in *Animal Function in Arid Environments: Scientific Meeting at Fowlers Gap Research Station, 13-16 December 2002*

Marhold, S. & Nagel, A., (1995), "The energetics of the common mole rat *Cryptomys*, a subterranean eusocial rodent from Zambia", *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology,* **164**: 636-645.

Martin, C.J., (1903), "Thermal Adjustment and Respiratory Exchange in Monotremes and Marsupials - A Study in the Development of Homeothermism." *Philosophical Transactions of the Royal Society of London. Series B.,* **195**: 1-37.

McArthur, A.J., (1987), "Thermal interaction between animal and microclimate: A comprehensive model", *Journal of Theoretical Biology,* **126**: 203-238.

McArthur, A.J., (1990), "Thermal Interaction between animal and microclimate: specification of a "standard environmental temperature" for animals outdoors", *Journal of Theoretical Biology,* **148**: 331-344.

McCafferty, D.J., Moncrieff, J.B. & Taylor, I.R., (2001), "How much energy do barn owls (*Tyto alba*) save by roosting?" *Journal of Thermal Biology,* **26**: 193-203.

McCarron, H.C.K. & Dawson, T.J., (1989). "Thermal relations of Macropodoidea in hot environments", pp. 255-263 in Hume, I. (ed.), *Kangaroos, Wallabies and Rat-Kangaroos.* Chipping Norton, N.S.W.: Surrey Beatty and Sons

McDevitt, R. & Andrews, J.F., (1995), "Seasonal variation in the metabolic rate of the pygmy shrew, *Sorex minutus*: can resting metabolic rate be measured in post-absorptive shrews?" *Journal of Thermal Biology,* **20**: 255-261.

McNab, B.K., (1966), "Metabolism of fossorial rodents - a study of convergence", *Ecology,* **47**: 712-733.

McNab, B.K., (1980), "On estimating thermal conductance in endotherms", *Physiological Zoology,* **53**: 145-156.

McNab, B.K., (1988), "Energy conservation in a tree kangaroo (*Dendrolagus matschiei*) and the red panda (*Ailurus fulgens*)", *Physiological Zoology,* **61**: 280-292.

McNairn, I.S. & Fairall, N., (1979), "Relationship between heart rate and metabolism in the hyrax (*Procavia capensis*) and guineapig (*Cavia porcellus*)", *South African Journal of Zoology,* **14**: 230-232.

McNairn, I.S. & Fairall, N., (1984), "Metabolic rate and body temperature of adult and juvenile hyrax (*Procavia capensis*)", *Comparative Biochemistry and Physiology a Comparative Physiology,* **79**: 539-546.

Milsom, W.K., Zimmer, M.B. & Harris, M.B., (1999), "Regulation of cardiac rhythm in hibernating mammals", *Comparative Biochemistry and Physiology Part A Molecular and Integrative Physiology,* **124**: 383-391.

Mitchell, D., Maloney, S.K., Jessen, C., Laburn, H.P., Kammerman, P.R., Mitchell, G. & Fuller, A., (2002), "Adaptive heterothermy and selective brain cooling in arid-zone mammals", *Comparative Biochemistry and Physiology B. Biochemistry and Molecular Biology,* **131**: 571-585.

Monteith, J.L. & Unsworth, M.H., (1990), *Principles of Environmental Physics.* 2nd. ed. London: Edward Arnold.

Morhardt, J.E. & Morhardt, S.S., (1971), "Correlations between heart rate and oxygen consumption in rodents", *American Journal of Physiology,* **221**: 1580-1586.

Mortola, J.P. & Frappell, P.B., (2000), "Ventilatory responses to changes in temperature in mammals and other vertebrates", *Annual Review of Physiology,* **62**: 847-874.

Morton, S.R., (1990), "The impact of European settlement on the vertebrate animals of arid Australia: a conceptual model", *Proceedings of the Ecological Society of Australia,* **16**: 201-213.

Musser, A.M., (2003), "Review of the monotreme fossil record and comparison of paleontological and molecular data", *Comparative Biochemistry and Physiology - Part A,* **136**: 927-942.

Nicol, S. & Andersen, N.A., (1996). "Hibernation in the echidna: not an adaptation to the cold?" pp. 7-12 in Geiser, F., Hubert, A.J. & Nicol, S.C. (ed.), *Adaptations to the Cold: Tenth International Hibernation Symposium.* Armidale: University of New England Press

Nicol, S. & Andersen, N.A., (2003), "Control of breathing in the echidna (*Tachyglossus aculeatus*) during hibernation", *Comparative Biochemistry and Physiology Part A Molecular & Integrative Physiology,* **136A**: 917-925.

Nicol, S., Anderson, N.A. & Mesch, U., (1992). "Metabolic rate and ventilatory pattern in the echidna during hibernation and arousal", pp. 150-159 in Augee, M.L. (ed.), *Platypus and Echidnas.* Sydney: Royal Zoological Society of N.S.W.

Nicol, S. & Maskrey, M., (1980), "Thermoregulation and sleep in the Tasmanian devil, *Sarcophilus harrisii* (Marsupialia: Dasyuridae)", *Journal of Comparative Physiology,* **140**: 241-248.

Nicol, S., Vedel-Smith, C. & Andersen, N.A., (2004). "Behaviour, body temperature, and hibernation in Tasmanian echidnas (*Tachyglossus aculeatus*)", pp. 149-159 in Barnes, B.M. & Carey, H.V. (ed.), *Life in the Cold: Evolution, Mechanisms, Adaptation, and Application.* Fairbanks: Institute of Arctic Biology, University of Alaska Fairbanks

Nicol, S.C. & Andersen, N.A., (2000). "Patterns of hibernation of echidnas in Tasmania", pp. 21-28 in Heldmaier, G. & Klingenspor, M. (ed.), *Life in the Cold: Eleventh International Hibernation Symposium.* Berlin: Springer-Verlag

Norbury, G.L., Sanson, G.D. & Lee, A.K., (1989). "Feeding ecology of the Macropdoidea", pp. 169-178 in Grigg, G., Jarman, P. & Hume, I. (ed.), *Kangaroos, Wallabies and Rat-Kangaroos.* Chipping Norton, N.S.W.: Surrey Beatty and Sons

O'Connor, M.P., (1999), "Physiological and ecological implications of a simple model of heating and cooling in reptiles", *Journal of Thermal Biology,* **24**: 113-136.

O'Connor, M.P. & Dodson, P., (1999), "Biophysical constraints on the thermal ecology of dinosaurs", *Paleobiology,* **25**: 341-368.

Pal, D.S. & Pal, S., (1990), "Prediction of temperature profiles in the human skin and subcutaneous tissues", *Journal of Mathematical Biology,* **28**: 355-364.

Pal, D.S. & Pal, S., (1993), "Effects of blood flow, curved boundary and environmental conditions on temperature distribution in a two dimensional model of human skin and subcutaneous tissues", *Journal of Mathematical Biology,* **32**: 17-32.

Parer, J.T. & Hodson, W.A., (1974), "Respiratory studies of monotremes. IV. Normal respiratory functions of echidnas and ventilatory response to inspired oxygen and carbon dioxide", *Respiration Physiology,* **21**: 307-316.

Parsons, K.C., (1995), "Computer models as tools for evaluating clothing risks and controls", *Annals of Occupational Hygiene,* **39**: 827-839.

Phillips, P.K. & Heath, J.E., (1992), "Heat exchange by the pinna of the African elephant *Loxodonta africana*", *Comparative Biochemistry and Physiology A,* **101**: 693-699.

Phillips, P.K. & Heath, J.E., (2001), "Heat loss in Dumbo: a theoretical approach", *Journal of Thermal Biology,* **26**: 117-120.

Porter, W.P. & Gates, D.M., (1969), "Thermodynamic equilibria of animals with environment", *Ecological Monographs,* **39**: 227-244.

Porter, W.P., Mitchell, J.W., Beckman, W.A. & DeWitt, D.C.B., (1973), "Behavioral implications of mechanistic ecology: thermal and behavioural modelling of desert ectotherms and their microenvironment", *Oecologia,* **13**: 1-54.

Porter, W.P., Munger, J.C., Stewart, W.E., Budaraju, S. & Jaeger, J., (1994), "Endotherm energetics: from a scalable individual-based model to ecological applications", *Australian Journal of Zoology,* **42**: 125-162.

Prothero, J. & Jürgens, K.D., (1986), "An energetic model of daily torpor in endotherms", *Journal of Theoretical Biology,* **121**: 403-416.

Pulawa, L.K. & Florant, G.L., (2000), "The effects of caloric restriction on the body composition and hibernation of the golden-mantled ground squirrel (*Spermophilus lateralis*)", *Physiological and Biochemical Zoology,* **73**: 538-546.

Refinetti, R., (1999), "Amplitude of the daily rhythm of body temperature in eleven mammalian species", *Journal of Thermal Biology,* **24**: 477-481.

Refinetti, R. & Menaker, M., (1992), "The circadian rhythm of body temperature", *Physiology & Behavior,* **51**: 613-637.

Rismiller, P.D. & McKelvey, (1996). "Sex, torpor and activity in temperate climate echidnas", pp. in Nicol, S.C. (ed.), *Adaptations to the Cold: Tenth International Symposium.* Armidale: University of New England Press

Rismiller, P.D. & McKelvey, M.W., (2000), "Frequency of breeding and recruitment in the short-beaked echidna, *Tachyglossus aculeatus*", *Journal of Mammalogy,* **81**: 1-17.

Robinson, D.E., Campbell, G.S. & King, J.R., (1976), "An evaluation of heat exchange in small birds", *Journal of Comparative Physiology,* **105**: 153-166.

Robinson, K.W., (1954), "Heat tolerances of Australian monotremes and marsupials", *Australian Journal of Biological Sciences,* **7**: 348-360.

Rogowitz, G.L., (1990), "Seasonal energetics of the white-tailed jackrabbit (*Lepus townsendii*)", *Journal of Mammalogy,* **71**: 277-285.

Schleucher, E., Prinzinger, R. & Withers, P.C., (1991), "Life in extreme environments: investigations on the ecophysiology of a desert bird, the Australian diamond dove (*Geopelia cuneata* Latham)", *Oecologia,* **88**: 72-76.

Schmidt-Nielsen, K., (1990), *Animal Physiology: Adaptation and Environment.* 4th. ed. Cambridge: University Press.

Schmidt-Nielsen, K., Dawson, T.J. & Crawford Jn., E.C., (1966), "Temperature regulation in the echidna (*Tachyglossus aculeatus*)", *Journal of Cellular Physiology,* **67**: 63-72.

Schmidt-Nielsen, K., Schmidt-Nielsen, B., Jarnum, S.A. & Houpt, T.R., (1956), "Body temperature of the camel and its relation to water economy", *American Journal of Physiology,* **188**: 103-112.

Scholander, P.F., Hock, R., Walters, V., Johnson, F. & Irving, L., (1950a), "Heat regulation in some arctic and tropical mammals and birds", *Biological Bulletin,* **99**: 237-258.

Scholander, P.F., Walters, V., Hock, R. & Irving, L., (1950b), "Body insulation of some arctic and tropical mammals and birds", *Biological Bulletin,* **99**: 225-236.

Seebacher, F. & Grigg, G.C., (1997), "Patterns of body temperature in wild freshwater crocodiles, *Crocodylus johnston*i: Thermoregulation versus thermoconformity, seasonal acclimatization, and the effect of social interactions", *Copeia*: 549-557.

Seebacher, F. & Shine, R., (2004), "Evaluating thermoregulation in reptiles: the fallacy of the inappropriately applied method", *Physiological and Biochemical Zoology,* **77**: 688-695.

Shkolnik, A. & Schmidt-Nielsen, K., (1976), "Temperature regulation in hedgehogs from temperate and desert environments", *Physiological Zoology,* **49**: 56-64.

Siegel, R. & Howell, J.R., (1981), *Thermal Radiation Heat Transfer.* 2nd. ed. Washington: Hemisphere Publishing Corporation.

Smith, A.P., Wellham, G.S. & Green, S.W., (1989), "Seasonal foraging activity and microhabitat selection by echidnas (*Tachyglossus aculeatus*) on the New England Tablelands (Australia)", *Australian Journal of Ecology,* **14**: 457-466.

Smyth, D.M., (1973), "Temperature regulation in platypus, *Ornithorhynchus anatinus* (Shaw)", *Comparative Biochemistry and Physiology,* **45**: 705-715.

Snapp, B.D. & Heller, H.C., (1981), "Suppression of metabolism during hibernation in ground squirrels (*Citellus lateralis*)", *Physiological Zoology,* **54**: 297-307.

Song, G., Kortner, G. & Geiser, F., (1995), "Reduction of metabolic rate and thermoregulation during daily torpor", *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology,* **165**: 291-297.

Song, X., Körtner, G. & Geiser, F., (1997), "Thermal relations of metabolic rate reduction in a hibernating marsupial", *American Journal of Physiology,* **273**: R2097-R2104.

Spotila, J.R., Lomman, P.W., Bakken, G.S. & Gates, D.M., (1973), "A mathematical model for body temperatures of large reptiles: implications for dinosaur ecology", *The American Naturalist,* **107**: 391-404.

Spotila, J.R., Soule, O.H. & Gates, D.M., (1972), "The biophysical ecology of the alligator: heat energy budgets and climate spaces", *Ecology,* **53**: 1094-1102.

Stafford Smith, D.M. & Morton, S.R., (1990), "A framework for the ecology of arid Australia", *Journal of Arid Environments,* **18**: 255-278.

Stahl, W.R., (1967), "Scaling of respiratory variables in mammals", *Journal of Applied Physiology,* **22**: 453-460.

Stewart, C.A., Owens, J. & McClure, P.A., (1990), "Air flow and convective heat loss from small mammals in laboratory metabolism chambers", *Journal of Thermal Biology,* **15**: 313-316.

Strahan, R., (ed.), (1983), *The Australian Museum Complete Book of Australian Mammals.* Sydney: Angus and Robertson.

Strunk, T.H., (1971), "Heat Loss from a Newtonian animal", *Journal of Theoretical Biology,* **33**: 35-61.

Templeton, J.R., (1970). "Reptiles", pp. in Whittow, G.C. (ed.), *Comparative Physiology of Thermoregulation.* London: Academic Press

Thorkelson, J. & Maxwell, R.K., (1974), "Design and testing a heat transfer model of a raccoon (*Procyon lotor*) in a closed tree den", *Ecology,* **55**: 29-39.

Tikuisis, P., (1989), "Prediction of the thermoregulatory response for clothed immersion in cold water", *European Journal of Applied Physiology and Occupational Physiology,* **59**: 334-341.

Tikuisis, P., (2003), "Heat balance precedes stabilization of body temperatures during cold water immersion", *Journal of Applied Physiology,* **95**: 89-96.

Tikuisis, P., Gonzalez, R.R. & Pandolf, K.B., (1988), "Thermoregulatory model for immersion of humans in cold water", *Journal of Applied Physiology,* **64**: 719-727.

Tracy, C.R., (1976), "A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment", *Ecological Monographs,* **46**: 293-326.

Tracy, C.R., (1982). "Biophysical modelling in reptilian physiology and ecology", pp. 275-321 in Gans, C. & Pough, F.H. (ed.), *Biology of the Reptilia.* New York: Academic Press

Trites, A.W., (1990), "Thermal budgets and climate spaces the impact of weather on the survival of Galapagos *Arctocephalus galapagoensis* Heller and Northern Fur Seal Pups *Callorhinus ursinus* L", *Functional Ecology,* **4**: 753-768.

Turnpenny, J.R., McArthur, A.J., Clark, J.A. & Wathes, C.M., (2000a), "Thermal balance of livestock: 1. A parsimonious model", *Agricultural and Forest Meteorology,* **101**: 15-27.

Turnpenny, J.R., Wathes, C.M., Clark, J.A. & McArthur, A.J., (2000b), "Thermal balance of livestock: 2. Applications of a parsimonious model", *Agricultural and Forest Meteorology,* **101**: 29-52.

Vispo, C.R. & Bakken, G.S., (1993), "The influence of thermal conditions on the surface activity of thirteen-lined ground squirrels", *Ecology,* **74**: 377-389.

Visser, G.H. & Ricklefs, R.E., (1993), "Temperature regulation in neonates of shorebirds", *Auk,* **110**: 445-457.

Walsberg, G.E. & Wolf, B.O., (1996a), "An appraisal of operative temperature mounts as tools for studies of ecological energetics", *Physiological Zoology,* **69**: 658-681.

Walsberg, G.E. & Wolf, B.O., (1996b), "A test of the accuracy of operative temperature thermometers for studies of small ectotherms", *Journal of Thermal Biology,* **21**: 275-281.

Wardlaw, H.S.H., (1915), "The temperature of *Echidna aculeata*." *Proceedings of the Linnean Society of New South Wales,* **40**: 231-258.

Warnecke, L., Turner, J.M. & Geiser, F. (2006) "Basking behaviour in small heterothermic arid zone marsupials" in *Australian and New Zealand Society for Comparative Physiology and Biochemistry: 23rd. Annual Conference*, p 11.

Watmough, J. & Camazine, S., (1995), "Self-organized thermoregulation of honeybee clusters", *Journal of Theoretical Biology,* **176**: 391-402.

Watts, P., Hansen, S. & Lavigne, D.M., (1993), "Models of heat loss by marine mammals: thermoregulation below the zone of irrelevance", *Journal of Theoretical Biology,* **163**: 505-525.

Weathers, W.W. & Sullivan, K.A., (1989), "Juvenile foraging proficiency, parental effort, and avian reproductive success", *Ecological Monographs,* **59**: 223-246.

Webb, P.I., Speakman, J.R. & Racey, P.A., (1993), "The implication of small reductions in body temperature for radiant and convective heat loss in resting endothermic brown long-eared bats (*Plecotus auritus*)", *Journal of Thermal Biology,* **18**: 131-135.

Werner, J., Buse, M. & Foegen, A., (1989), "Lumped versus distributed thermoregulatory control: results from a three-dimensional dynamic model", *Biological Cybernetics,* **62**: 63-74.

Werner, J. & Webb, P., (1993), "A six-cylinder model of human thermoregulation for general use on personal computers", *Annals of Physiological Anthropology,* **12**: 123-134.

Wiersma, P., Bruinzeel, L. & Piersma, T., (1993), "Energy savings in waders: studies on the insulation of knots", *Limosa,* **66**: 41-52.

Wiersma, P. & Piersma, T., (1994), "Effects of microhabitat, flocking, climate and migratory goal on energy expenditure in the annual cycle of red knots", *Condor,* **96**: 257-279.

Wilkinson, D.A., Grigg, G.C. & Beard, L.A., (1998), "Shelter selection and home range of echidnas, *Tachyglossus aculeatus*, in the highlands of south-east Queensland", *Wildlife Research,* **25**: 219-232.

Wilson, K.J. & Lee, A.K., (1970), "Changes in oxygen consumption and heart-rate with activity and body temperature in tuatara, *Sphenodon punctatum*", *Comparative Biochemistry and Physiology,* **33**: 311-322.

Wilz, M. & Heldmaier, G., (2000), "Comparison of hibernation, estivation and daily torpor in the edible dormouse, *Glis glis*", *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology,* **170**: 511-521.

Withers, P.C., (1977), "Measurement of V_{O2}, V_{CO2}, and evaporative water loss with a flow-through mask", *Journal of Applied Physiology,* **42**: 120-123.

Withers, P.C., (1992a), *Comparative Animal Physiology.* Fort Worth: Saunders College Publishing.

Withers, P.C., (1992b), "Metabolism, water-balance and temperature regulation in the golden bandicoot (*Isoodon auratus*)", *Australian Journal of Zoology,* **40**: 523-531.

Withers, P.C., (2001), "Design, calibration and calculation for flow-through respirometry systems", *Australian Journal of Zoology,* **49**: 445-461.

Withers, P.C., Thompson, G.G. & Seymour, R.S., (2000), "Metabolic physiology of the north-western marsupial mole, *Notoryctes caurinus* (Marsupialia: Notoryctidae)", *Australian Journal of Zoology,* **48**: 241-258.

Withers, P.C. & Williams, J.B., (1990), "Metabolic and respiratory physiology of an arid-adapted Australian bird, the Spinifex pigeon", *Condor,* **92**: 961-969.

Wohlsen, A.L., (1997), *Heart rate as an indicator of oxygen consumption in free-ranging echidnas,* Tachyglossus aculeatus, Bachelor of Science (Honours) Thesis, University of Queensland, Brisbane.

Wooden, K.M. & Walsberg, G.E., (2002), "Effect of environmental temperature on body temperature and metabolic heat production in a heterothermic rodent, *Spermophilus tereticaudus*", *Journal of Experimental Biology,* **205**: 2099-2105.

Xu, X. & Werner, J., (1997), "A dynamic model of the human/clothing/environment-system", *Applied Human Science,* **16**: 61-75.

Appendix 1: Parameter Equations and Functions

Below is a list of the major functions currently incorporated in the modelling in Chapter 3. While some minor variations have been omitted, this list is accurate at the time of printing, but is likely to change and expand as the modelling is refined and developed. Refinements may make some of the following functions redundant or even erroneous if those refinements are found to cause conflicts. Therefore, some of the following functions may be deleted in time as others are added.

Latent Heat of Evaporation

in J.g^{-1}H$_2$O

$$\begin{aligned}\lambda_{Tb} &= 3016.425295 - 0.747321528(T_b + 273.15)\\ &- 0.006807852(T_b + 273.15)^2 + 9.70358721\times 10^{-6}(T_b + 273.15)^3\end{aligned}$$

Absolute Humidity

of air saturated at $T_a = T$ in g H$_2$O.m^{-3}

$$\begin{aligned}\gamma_T &= 2994.107829057 - 14.394881496(T + 273.15) - 0.1973654193(T + 273.15)^2\\ &+ 0.001882855747(T + 273.15)^3 - 0.000005725808021(T + 273.15)^4\\ &+ 6.030746587\times 10^{-9}(T + 273.15)^5\end{aligned}$$

and in g H$_2$O.ml^{-1}

$$\chi_T = \frac{\gamma_T}{10^6}$$

Scaling and Interval Length

The default interval length between recalculations of equilibria in stage (iii) simulations is one minute. However, this exceeds the limits of (some versions of) QBASIC, so for long simulations where $t_{max} > 360$, interval lengths, i (in min) are scaled according to:

$i = 1$ for $t_{max} < 360$

$i = 2$ for $360 \leq t_{max} < 600$

$i = 10$ for $600 \leq t_{max} < 3600$

$i = 60$ for $3600 \leq t_{max}$

This scaling is relevant for some of the functions below (see subscripts)

Operative Temperature

in ^{o}C

Constant k for all time t

$$T_{e.t} = k + 273.15$$

Step function from k_1 to k_2 at time m

$$T_{e.t} = k_1 + 273.15 \text{ for all } t < m$$

$$T_{e.t} = k_2 + 273.15 \text{ for all t} \geq m$$

Ramp function change from k at rate m (^{o}C.min^{-1})

$$\begin{aligned} T_{e.0} &= k + 273.15 \\ T_{e.t} &= T_{e.\left(t/i\right)-1} + mi \end{aligned}$$

Sinusoidal function change from ($T_{e.\mathrm{min}}$ + 273.15) to ($T_{e.\mathrm{max}}$ + 273.15) with wavelength 1440 minutes (24 hours)

$$T_{e.t} = T_{e.av} + T_{e.amp} \cos\left(\frac{2\pi t}{1440}\right)$$

where $T_{e.av} = \dfrac{T_{e.\min} + T_{e.\max}}{2}$ and $T_{e.amp} = \dfrac{T_{e.\min} - T_{e.\max}}{2}$ if T_e starts the simulation at its minimum or $T_{e.amp} = \dfrac{T_{e.\max} - T_{e.\min}}{2}$ if T_e starts the simulation at its maximum.

Two thermal environments each varying sinusoidally, one with a larger range/amplitude than the other. Let the thermal environment with the larger amplitude nominally be defined as "exposed", and the other as "sheltered". Relevant minima and maxima given by:

$$T_{e.exd.\min} = k_1 + 273.15$$
$$T_{e.exd.\max} = k_2 + 273.15$$
$$T_{e.shel.\min} = k_3 + 273.15$$
$$T_{e.shel.\max} = k_4 + 273.15$$

where k_1, k_2, k_3, and k_4 are constants. So,

$$T_{e.exd.av} = \frac{T_{e.exd.\min} + T_{e.exd.\max}}{2}$$
$$T_{e.shel.av} = \frac{T_{e.shel.\min} + T_{e.shel.\max}}{2}$$

Where T_e starts the simulation at their minima, the amplitude of the T_e waves are:

$$T_{e.exd.amp} = \frac{T_{e.exd.\min} - T_{e.exd.\max}}{2}$$
$$T_{e.shel.amp} = \frac{T_{e.shel.\min} - T_{e.shel.\max}}{2}$$

and where T_e starts the simulation at their maxima, the amplitude of the T_e waves are:

$$T_{e.exd.amp} = \frac{T_{e.exd.\max} - T_{e.exd.\min}}{2}$$
$$T_{e.shel.amp} = \frac{T_{e.shel.\max} - T_{e.shel.\min}}{2}$$

Thus:

$$T_{e.exd.t} = T_{e.exd.av} + T_{e.exd.amp} \cos\left(\frac{2\pi t}{1440}\right)$$
$$T_{e.shel.t} = T_{e.shel.av} + T_{e.shel.amp} \cos\left(\frac{2\pi t}{1440}\right)$$

Further inputs are required to define how the virtual animal chooses between the two environments. Let $T_{b.usp}$ (upper set point) be the T_b above which the animal seeks the cooler environment and $T_{b.lsp}$ (lower set point) be the T_b below which the animal seeks the warmer environment. Then:

$$T_{b.usp} = l_1 + 273.15$$
$$T_{b.lsp} = l_2 + 273.15$$

where l_1 and l_2 are constants and:

$$T_{e.t} = T_{e.shel.t} \text{ for } T_{b.\left(t/_i\right)-1} > T_{b.usp} \text{ and } T_{e.exd.t} > T_{e.shel.t}$$

$$T_{e.t} = T_{e.exd.t} \text{ for } T_{b.\left(t/_i\right)-1} > T_{b.usp} \text{ and } T_{e.exd.t} < T_{e.shel.t}$$

$$T_{e.t} = T_{e.exd.t} \text{ for } T_{b.\left(t/_i\right)-1} < T_{b.lsp} \text{ and } T_{e.exd.t} > T_{e.shel.t}$$

$$T_{e.t} = T_{e.shel.t} \text{ for } T_{b.\left(t/_i\right)-1} < T_{b.lsp} \text{ and } T_{e.exd.t} < T_{e.shel.t}$$

For situations where body temperature is between the two set points, pick the environment that continues the pre-existing trend. That is:
for all

$$T_{b.lsp} \leq T_{b\left(t/_i\right)-1} < T_{b.usp}$$

either

$$T_{e.t} = T_{e.exd.t} \text{ for } T_{b\left(t/_i\right)-1} < T_{b.eq\left(t/_i\right)-1} \text{ and } T_{e.exd.t} > T_{e.shel.t}$$

$$T_{e.t} = T_{e.shel.t} \text{ for } T_{b\left(t/_i\right)-1} < T_{b.eq\left(t/_i\right)-1} \text{ and } T_{e.exd.t} < T_{e.shel.t}$$

which is for an increasing T_b or,

$$T_{e.t} = T_{e.shel.t} \text{ for } T_{b\left(t/_i\right)-1} > T_{b.eq\left(t/_i\right)-1} \text{ and } T_{e.exd.t} > T_{e.shel.t}$$

$$T_{e.t} = T_{e.exd.t} \text{ for } T_{b\left(t/_i\right)-1} > T_{b.eq\left(t/_i\right)-1} \text{ and } T_{e.exd.t} < T_{e.shel.t}$$

for a decreasing T_b. In the first interval before a T_b history is established, the trend in T_b is determined by comparing starting values with the $T_{b.eq.1}$.

Turbinate Effects

in ^{o}C

Constant effect, k

$$T_{ue} = k$$

Separate constant effects, k_1 and k_2 above and below a threshold l in T_e

$$T_{ue} = k_1 \text{ for all } T_{e.t} > l$$

$$T_{ue} = k_2 \text{ for all } T_{e.t} \leq l$$

Effect increasing at a rate m with T_e from a minimum k_1 to a maximum k_2 below a threshold l in T_e

$$T_{eu} = k_1 \text{ for all } T_{e.t} > l$$

$$T_{ue} = k_1 + mT_{e.t} \text{ for } T_{e.t} \leq l \text{ and } k_1 + mT_{e.t} \leq k_2$$

$$T_{ue} = k_2 \text{ for } T_{e.t} \leq l \text{ and } k_1 + mT_{e.t} > k_2$$

Thermal Conductance

in W.^{o}C^{-1}

Constant, k

$$K_{o.t} = k \text{ for all } t$$

Heating/cooling hysteresis as seen in many ectotherms where K_o varies between $K_{o.\max}$ (heating) and $K_{o.\min}$ (cooling)

$$\text{For } t = i$$

$$K_{o.t} = K_{o.\max} \text{ for } T_{b.(t/i)\text{-}1} > T_{b.1}$$

$$K_{o.t} = K_{o.\min} \text{ for } T_{b.(t/i)\text{-}1} \leq T_{b.1}$$

$$\text{For } t > i$$

$$K_{o.t} = K_{o.\max} \text{ for } T_{b.(t/i)\text{-}2} > T_{b.(t/i)\text{-}1}$$

$$K_{o.t} = K_{o.\min} \text{ for } T_{b.(t/i)\text{-}2} \leq T_{b.(t/i)\text{-}1}$$

Hysteresis reversed above a set point T_b, $T_{b.swap}$

For $T_{b.(t/i)\text{-}1} < T_{b.swap}$

For $t = i$

$$K_{o.t} = K_{o.\max} \text{ for } T_{b.(t/i)\text{-}1} > T_{b.0}$$

$$K_{o.t} = K_{o.\min} \text{ for } T_{b.(t/i)\text{-}1} \leq T_{b.0}$$

For $t > i$

$$K_{o.t} = K_{o.\max} \text{ for } T_{b.(t/i)\text{-}2} > T_{b.(t/i)\text{-}1}$$

$$K_{o.t} = K_{o.\min} \text{ for } T_{b.(t/i)\text{-}2} \leq T_{b.(t/i)\text{-}1}$$

For $T_{b.(t/i)\text{-}1} \geq T_{b.swap}$

For $t = i$

$$K_{o.t} = K_{o.\min} \text{ for } T_{b.(t/i)\text{-}1} > T_{b.ss}$$

$$K_{o.t} = K_{o.\max} \text{ for } T_{b.(t/i)\text{-}1} \leq T_{b.ss}$$

For $t > i$

$$K_{o.t} = K_{o.\min} \text{ for } T_{b.(t/i)\text{-}2} > T_{b.(t/i)\text{-}1}$$

$$K_{o.t} = K_{o.\max} \text{ for } T_{b.(t/i)\text{-}2} \leq T_{b.(t/i)\text{-}1}$$

Facilitate approach to preferred T_b by varying K_o within limits

$$K_{o.t} = \frac{M_{\left(t/i\right)-1} - E_{\left(t/i\right)-1} - S_{\left(t/i\right)-1}}{T_{set} - T_{e.\left(t/i\right)-1}}$$

which uses the previous interval's values for M etc. to calculate a K_o that balances for T_{set}. If $K_{o.t}$ falls outside the permitted range, then it is defined as the appropriate limit (analogous to above).

Reduce T_b above an upper set point and increase T_b below a lower set point with K_o constrained to a maximum, a minimum or a given value, l, in between.

$$K_{o.t} = l \text{ for } T_{b.lsp} \leq T_{b.(t/i)\text{-}1} \leq T_{b.usp}$$

For $T_{b.(t/i)\text{-}1} > T_{b.usp}$

$$K_{o.t} = K_{o.\max} \text{ for } K_{o.t}(T_{b.(t/i)\text{-}1} - T_{e.t}) > 0$$

$$K_{o.t} = K_{o.\min} \text{ for } K_{o.t}(T_{b.(t/i)\text{-}1} - T_{e.t}) < 0$$

$$\text{For } T_{b.(t/i)\text{-}1} < T_{b.lsp}$$

$$K_{o.t} = K_{o.\min} \text{ for } K_{o.t}(T_{b.(t/i)\text{-}1} - T_{e.t}) > 0$$

$$K_{o.t} = K_{o.\max} \text{ for } K_{o.t}(T_{b.(t/i)\text{-}1} - T_{e.t}) < 0$$

Alternative algorithm to facilitate approach and retention of T_b to a preferred range.

$$\text{For } T_{b.(t/i)\text{-}1} < T_{e.t}$$

$$K_{o.t} = K_{o.max} \text{ for } T_{b.(t/i)\text{-}1} < T_{b.lsp}$$

$$K_{o.t} = K_{o.min} \text{ for } T_{b.(t/i)\text{-}1} > T_{b.usp}$$

$$K_{o.t} = \frac{M_{\left(t/i\right)-1} - E_{\left(t/i\right)-1} - S_{\left(t/i\right)-1}}{T_{set} - T_{e.\left(t/i\right)-1}} \text{ for } T_{b.lsp} > T_{b.(t/i)\text{-}1} > T_{b.usp}$$

$$\text{For } T_{b.(t/i)\text{-}1} > T_{e.t}$$

$$K_{o.t} = K_{o.\max} \text{ for } T_{b.(t/i)\text{-}1} > T_{b.usp}$$

$$K_{o.t} = K_{o.\min} \text{ for } T_{b.(t/i)\text{-}1} < T_{b.lsp}$$

$$K_{o.t} = \frac{M_{\left(t/i\right)-1} - E_{\left(t/i\right)-1} - S_{\left(t/i\right)-1}}{T_{set} - T_{e.\left(t/i\right)-1}} \text{ for } T_{b.lsp} > T_{b.(t/i)\text{-}1} > T_{b.usp}$$

which combines salient features of the above two options excepting that whether the environment is hotter or cooler than the animal is given preference over where $T_{b.t}$ is in relation to set points.

Homeothermic endothermy is simulated by combining similar algorithms with functions simulating variation in metabolic heat production (below).

Metabolic Heat Production

in W

The importance of the distinction between $M_{eq.t}$ and M_t should be recognised. In many cases M_t is output determined by the time course of $T_{b.t}$ towards an equilibrium $T_{b.eq.t}$. This equilibrium is determined, in part, by $M_{eq.t}$ which may actually be input. For example, in the first function below, $T_{b.t}$ may approach an equilibrium defined by a given $M_{eq.t}$ specified as a constant for all t and, due to effect $T_{b.t}$ has on M_t, M_t may vary as T_b varies while that equilibrium is being approached. This flow-on effect of changes in T_b may be particularly apparent in step function changes of, say T_e. For situations where $M_{eq.t}$ is specified as input without prior knowledge of M_t, M_t may be calculated according to a Q_{10} effect calculated automatically from M_1, $M_{t.max}$, $T_{b.1}$ and $T_{b.t.max}$.

Constant equilibrium *M* specified

$$M_{eq.t} = k \text{ for all } t$$

$$M_{t/i} = M_{(t/i)-1} Q_{10}^{\frac{T_{b(t/i)-1} - T_{b(t/i)}}{10}}$$

One equilibrium *M* replaced by another

$$M_{eq.t} = k_1 \text{ for all } t < m$$

$$M_{eq.t} = k_2 \text{ for all } t \geq m$$

$$M_{t/i} = M_{(t/i)-1} Q_{10}^{\frac{T_{b(t/i)-1} - T_{b(t/i)}}{10}}$$

***Q*₁₀ specified** (see also Chapter 3)

$$M_{eq.t} = M_{eq.0} Q_{10}^{\frac{\left(Tb_{eq.t} - Tb_{eq.0}\right)}{10}}$$

$$M_{eq} = K_{o.eq}\left(T_{b.eq} - T_{e.eq}\right) + E_{eq}$$

solved simultaneously for $T_{b.eq}$ and $M_{eq.t}$, as Chapter 3 (stage (iii) simulation).

Q_{10} interrupted by activity where there are k interruptions to the Q_{10} determinations of $T_{b.t}$, each starting at $interrupt_{start.j}$, ending at $interrupt_{fin.j}$ and having a metabolic rate of $M_{interrupt.j}$ where $j = 1, 2, \ldots k$. Previous solutions from the Q_{10} algorithms are overwritten by:

For j = 1,2,...,k:

For $interrupt_{start.j} \leq t \leq interrupt_{fin.j}$

$$M_{eq.t} = M_{interrupt.j}$$

$$M_t = M_{interrupt.j}$$

In this case $M_t = M_{eq.t}$ because M_t is determined by the activity level of the animal at time t and $T_{b.eq.t}$ responds to this actual value of M_t thus making both metabolic rates equivalent. $M_{interrupt.j}$ may be specified as multiples of $M_{eq.t}$ as solved by the Q_{10} algorithms. $M_{interrupt.j}$ may also be constrained so as not fall below the values solved by the Q_{10} algorithms.

Themogenesis below a set point T_{set}

If the Q_{10} algorithm solves for less than the following, then

$$M_{eq.t/i} = K_{o.t}\left(T_{set} - T_{e.t/i}\right) + E_{\left(t/i\right)-1} + \frac{\frac{C}{60}\left(T_{set} - T_{b.\left(t/i\right)-1}\right)}{i}$$

but only if the aerobic scope implied by $M_{\max}$ is not exceeded. This just states that the M required to get T_b above T_{set} is equal to that currently lost to the environment as sensible heat plus that (recently) lost as latent heat (as the current E is calculated only after M) plus that required to overcome any shortfall in the current heat stored in the tissues. This last term was found to be useful as a capacitor to smooth out the fluctuations in M that otherwise appeared. For homeothermic animals, this would not be necessary as T_b never drops below T_{set}. For heterothermic endotherms when thermogenesis is initiated, then M responds to both T_e and T_b fluctuations as per the above equation. In these conditions, thermogenesis is initiated when T_b drops below the set point but only if, given current T_e, warming does not exceed the aerobic scope of the animal. Otherwise the default Q_{10} algorithms pertain and the animal remains in/returns to its effectively ectothermic state; its T_b being driven predominantly by T_e. There is a *caveat* though. In T_e increasing from particularly low levels (where the thermogenesis would exceed the scope) to higher levels (where thermogenesis becomes feasible) this algorithm delays “arousal” or onset of thermogenesis because of the storage term until the difference between T_{set} and $T_{b.t}$ is small, very small. For this reason, the conditions favouring thermogenesis (that is the criteria when thermogenesis is initiated) include:

$$M_{eq.t/i} \langle K_{o.t}\left(T_{set} - T_{e.t/i}\right) + E_{\left(t/i\right)-1} + \frac{\frac{C}{60}\left(T_{set} - T_{b.\left(t/i\right)-1}\right)}{i}$$

plus either:

$$T_{e.t/i} \rangle T_{e.\left(t/i\right)-1} \text{ and } K_{o.t}\left(T_{set} - T_{e.t/i}\right) + E_{\left(t/i\right)-1} \le M_{\max}$$

or:

$$T_{e.t/i} \langle T_{e.(t/i)-1} \text{ and } K_{o.t}\left(T_{set} - T_{e(t/i)}\right) + E_{(t/i)-1} + \frac{\frac{C}{60}\left(T_{set} - T_{b(t/i)-1}\right)}{i} \le M_{\max}$$

This allows thermogenesis to initiate at lower increasing T_e without curtailing it at higher decreasing T_e.

Fixed or regulated *M* independently of other parameters where, because M_t is fixed, $M_{eq.t}$ must be M_t throughout: T_b can only respond to the fixed value of M. Thus:

$$M_{eq.t} = k \text{ for all } t$$
$$M_{t/i} = k \text{ for all } t$$

where k is a constant. Alternatively, for one constant M rate to be followed by another constant M.

$$M_{eq.t} = k_1 \text{ for all } t < l$$
$$M_{t/i} = k_1 \text{ for all } t < l$$
$$M_{eq.t} = k_2 \text{ for all } t \ge l$$
$$M_{t/i} = k_2 \text{ for all } t \ge l$$

or for when T_b reaches some value l:

$$M_{eq.t} = k_1 \text{ for all } T_{b(t/i)-1} < l$$
$$M_{t/i} = k_1 \text{ for all } T_{b(t/i)-1} < l$$
$$M_{eq.t} = k_2 \text{ for all } T_{b(t/i)-1} \ge l$$
$$M_{t/i} = k_2 \text{ for all } T_{b(t/i)-1} \ge l$$

or alternatively, these constants can be replaced by linear functions of t to represent steadily increasing or decreasing M.

Other simpler arousal algorithms than above can be represented by examples such as the following, where M increases exponentially till a given T_b is reached:

$$M_{eq.0} = M_0$$

$$M_{eq.t} = M_{t/i} = M_{eq.(t/i)-1} r^i \text{ for all } T_{b(t/i)-1} < l$$

$$M_{eq.t} = M_{t/i} = k \text{ for all } T_{b(t/i)-1} \geq l$$

or where M increases to an asymptote:

$$M_{eq.t} = \frac{M_{eq.t-1} r}{1 + aM_{eq.t-1}}$$

where $a = \frac{r-1}{M_{\max}}$, $M_{\max}$ is the user chosen maximum value for M, r is the nominal rate of increase (the base of the exponential function) and $M_{eq.t}$ is the equilibrium M at time t. However, because calculations are not performed in the program for every t (minute), but for every interval, i where i is the length of the interval (in minutes, e.g. 1,2,10 etc), $M_{eq.t}$ often must be calculated on the basis of $M_{eq.(t/i)-1}$ some i minutes before.

Thus:

$$M_{eq.0} = M_0$$

$$M_{eq.t} = M_{t/i} = \frac{M_{eq.(t/i)-1} r^i}{1 + aM_{eq.(t/i)-1}\left(r^0 + r^1 + r^2 + \ldots + r^{i-1}\right)} \text{ for all } T_{b(t/i)-1} < l$$

$$M_{eq.t} = M_{t/i} = k \text{ for all } T_{b(t/i)-1} \geq l$$

where a is as above (a computer calculated parameter based on $M_{\max}$, a maximum metabolic rate obtained during the arousal), r is the nominal or intrinsic rate of increase of M and l is the T_b threshold at which this arousal function ceases and at which $M_{eq.t}$ reverts back to k.

Evaporative Cooling

in W

Respiratory minute volume, $V_{i.t}$, may be specified as a function of M_t according to functions described in Chapter 3. Either $V_{i.t}$ or $TEWL_t$ can be overridden by any constant for any $T_{b.t} >$

some given threshold. By specifying a value large enough, significant evaporative cooling such as panting and/or sweating can be included (simplistically) while T_b is high.

Appendix 2: Operative Temperature inside the Field Metabolic Chamber

Definitions and Equations

Because the metabolic chamber used in experiments described in Chapter 4 was in the open and exposed to direct sunlight, it cannot be assumed that the interior was isothermal. Therefore, air temperature, T_a, was not the most appropriate measure of the thermal conditions within and should be replaced by operative (environmental) temperature, T_e. T_e may be defined as:

> the temperature of an isothermal blackbody enclosure with the same convection (wind) conditions as the actual environment [in this case within the metabolic chamber], which would result in the same net sensible heat flow to or from the same animal with the same surface or body core temperature.

(Bakken, *et al.*, 1985)

For practical purposes, T_e may be considered as T_a adjusted for sensible heat exchanged via radiation, convection (Campbell & Norman, 1998) and, if necessary, conduction. During experiments, echidnas were observed to be sprawled across the floor of the chamber, especially during hot times of the day. With so much surface area in contact with the floor of the chamber, itself on the ground, and given that the thermal properties of pvc approximate those of sand (Baxter, 1945; Incropera & DeWitt, 1990; Goodfellow, 2007), conductive exchange between the echidna and the ground could not be ignored. Therefore, a two dimensional model was used where the echidna was considered to be two elements, (i) ventral in contact with the floor and (ii) dorsal; the rest including the lateral and end surfaces. T_e was calculated as:

$$T_e = \frac{GT_g + K_{od}T_{ed}}{K_o}$$

(Bakken, 1981)

where G is the conductive conductance (see Thermal Conductances below) through element (i), T_g is the temperature of the chamber floor, K_{od} is the overall thermal conductance of element (ii) and T_{ed} is the operative temperature for element (ii) alone. T_{ed} is calculated as:

$$T_{ed} = T_a + \frac{Q_a - A_{ed}\varepsilon\sigma(T_a + 273.15)^4}{H_d + R_d}$$

(Bakken, 1981)

Here Q_a is the radiation absorbed by element (ii) of the echidna and A_{ed} is its surface area. H_d (calculated according to Campbell & Norman, 1998) and R_d (calculated according to Bakken, 1981) are the convective and radiative thermal conductances of this element respectively. This equation (10b in Bakken, 1981) provides an estimate less accurate than Bakken's (1981) more difficult equation 10a. However, equation 10a was found to alter results by < 0.01 °C, so was not used here.

Additionally:

$$K_o = G + K_{od}$$

and

$$K_{od} = \frac{K_{sfd}(R_d + H_d)}{K_{sfd} + R_d + H_d}$$

(Bakken, 1981)

where K_{sfd} is the thermal conductance of the shell of element (ii), i.e. the pelage and subcutaneous tissues of element (ii) in series, as commonly explained (Bakken, 1976; 1981; Campbell & Norman, 1998). Consequently, to calculate the operative temperature of the echidna within the metabolic chamber required calculation of (i) the radiation exchanged between the environment and the echidna, (ii) the thermal conductances (G & K_{sfd}) of various tissues and regions of the echidna and (iii) dimensions and other characteristics of echidnas (mainly from first principles).

Radiation and View Factors

While direct sunlight illuminated the outside of the metabolic chamber in the field, only a small amount of diffuse light entered the interior through the somewhat opaque perspex ends.

Therefore, all radiation within the chamber was assumed to be long wave and dependent upon the surface temperatures of various elements within. To estimate the distribution of internal surface temperatures in the chamber throughout experiment days (without interfering with the experiments themselves), a similarly aligned identical length of pvc tubing was placed nearby such that it was subject to the same insolation and other conditions as much as possible, without impact (such as by casting a shadow) on the main experimental chamber, for five out of the twelve experiment days. The internal cylindrical surface of the tubing was divided conceptually into four equal (presumed isothermal) sectors, east, west, roof and floor. At intervals throughout the day, the surface temperature of each of these sectors was recorded with a radiometric thermometer (Raytek Minitemp FS). Thus the radiation emitted by each of the sectors could be calculated according to the Stefan-Boltzmann equation (Incropera & DeWitt, 1990) in which the long-wave emissivity of the pvc surface was assumed to be 0.97 (Campbell & Norman, 1998).

To calculate the proportion of this radiation intercepted by the echidna, view factors for the intercepting surfaces (echidna) with respect to the emitting surfaces were required (Incropera & DeWitt, 1990). View factors for some geometries of plane surfaces are readily available (Siegel & Howell, 1981; Holman, 1992; Howell, 2001), but for animals other than pigs and humans (Howell, 2001 citing Minowa and Dunkle), less so. For the system under investigation here, it was necessary to simplify the geometry of the emitting surfaces and the receiving surfaces. For the emitting surfaces, the sectors of the cylinders, the radiation passing through imaginary rectangles defined by the sector edges and the ends of the chamber was calculated according to the principles outlined in Holman (1992) and Incropera and DeWitt (1990). Thus, each emitting surface can be considered as a plane surface (895mm by 170mm) emitting known quantities of diffuse long wave radiation. When fully relaxed (under anaesthesia) and prone, an echidna's shape is surprisingly reminiscent of a cuboid in that each flank is roughly parallel for as much as 85% of its total length and its height is relatively constant for a similar proportion. In any case, subsequent calculations demonstrated that variations in height (that is distance from the top emitting surface) had little effect on the resultant calculated T_e. Similarly, the calculations are relatively insensitive to distance from the end of the chamber so complexities at both ends of the echidna (the rostrum and head region, and the caudal region) were simplified to flat surfaces. Measurements of dimensions from echidnas under anaesthesia indicated that a fully relaxed echidna of average body mass in this study might be represented (at least initially) by a cuboid 330 mm long by 170 mm wide by 98 mm high. Therefore, this radiation exchange system was represented by a solid cuboid of these dimensions and emissivity of 0.97 intercepting radiation within an emitting cuboid 895 mm long by 170 mm wide by 170 mm high with emissivity 0.97 and walls of different temperatures. The surface temperatures of the ends of the chamber were assumed to equal the average of the surface temperatures of each of the other four adjacent surfaces. The intercepting cuboid (echidna) was assumed to be midway along the emitting surfaces (pvc tubing) with its ventral surface common to part of the base of the emitting cuboid. View factors were calculated according to Lauzier and Rousse (2003) which allowed the radiation intercepted by the echidna cuboid to be determined.

Thermal Conductances

This method of calculating T_e assumes the animal has an isothermal core at body temperature, T_b, and a shell consisting of peripheral tissues and pelage outside of which the environment is at T_e. Therefore, this method of calculating T_e requires thermal conductances of the shell (G & K_{sfd}), which in turn depend on the thicknesses and thermal conductivities of the various peripheral tissues. Based on observations during implant surgery and on measurements taken from an echidna transversely dissected at the pectoral girdle (Augee, 1969), the thickness of the ventral skin and subcutaneous tissue (element (i)) was assumed to average 2.8 mm and based on measurements from the dissected echidna (Augee, 1969), the thickness of the dorsal skin and subcutaneous tissue (element (ii)) was assumed to average 18.0 mm. In echidnas the thickness of the sub-cutaneous tissue is due to two layers, a predominantly muscular layer, the *panniculus carnosus*, and a fat layer, the *panniculus adiposus* (Augee, 1969). Given the thickness and the conductivity of each of these and the skin (Incropera & DeWitt, 1990; Schmidt-Nielsen, 1990; Withers, 1992a), the conductivity of the peripheral dorsal tissues is approximately 0.211 $W.m^{-1}.K^{-1}$ and is relatively insensitive to the proportions of muscle and fat tissue in the fatty *panniculus carnosus*. This conductivity is within the range of 0.204 $W.m^{-1}.K^{-1}$ to 0.621 $W.m^{-1}.K^{-1}$, depending on degree of perfusion, cited by Bakken (1981) for subcutaneous tissues. For this study, the conductivities for both the ventral and the dorsal elements of the virtual echidna were varied between 0.21 $W.m^{-1}.K^{-1}$ and 0.62 $W.m^{-1}.K^{-1}$ to simulate the effect of vasomotor control on the thermal conductance of the peripheral tissues.

Mammals are characterised by hair which contributes significantly to their insulation. Echidnas used in this study typically had fine hairs up to 22 mm long on their ventral surfaces. Their orientation was such that the echidnas typically had a coat up to 11 mm thick on their ventral surfaces when not compressed against a substrate. The conductivity of hair, fur and wool have variously been determined (e.g. Hammel, 1955; Cena & Clark, 1978; Schmidt-Nielsen, 1990; Holman, 1992; Withers, 1992a) and here a value of 0.036 $W.m^{-1}.K^{-1}$ is used. (Had a value of 0.040 $W.m^{-1}.K^{-1}$ been used (Bakken, 1981) resultant T_e would have been reduced by about 0.05 ^{o}C.) The dorsal and lateral pelage of these echidnas was dominated by spines, typically up to 55 mm long and of 3 mm in diameter; dorsal and lateral hair was virtually absent. Although the conductivity of the pelage of the porcupine, *Erethizon dorsatum*, has been characterised (Ivlev & Chernova, 2005), echidna spines are harder than porcupine quills and their amino acid composition is more akin to the keratin in rhinoceros horn, for which conductivity is known (Baxter, 1945), than to either quills or other forms of mammalian pelage (Block, 1939). Therefore, an estimate of the conductivity of echidna pelage was determined by assuming that it consists of a combination of rhinoceros horn type keratin and air in parallel; the proportional area of keratin being assumed to be due to 9 spines per square centimetre each with an average diameter of 3mm (pers. obs.). This gave an estimated conductivity of 0.150 $W.m^{-1}.K^{-1}$ for echidna dorsal (and lateral) pelage. The thickness of the pelage was assumed to average 2 mm for the ventral element (hair compressed between the substrate and the echidna) and to vary between 10 mm and 50 mm for the dorsal element (to simulate the effect of piloerection). From these values of thicknesses and thermal conductivities, thermal conductances for each element (i: G & ii: K_{sfd}) may be determined by considering the pelage and each cutaneous and subcutaneous tissue as conductors in series (Bakken, 1981; Campbell & Norman, 1998).

Echidna Surface Temperatures and Dimensions

As mean airflow amounted to a maximum of 0.002 $m.s^{-1}$, even for high flow rates (Chapter 4), convection was assumed to be free and minimal (Gates, 1980; Stewart*, et al.*, 1990). Because convection was assumed to be free, estimates of echidna surface temperature were required to calculate H_d (Campbell & Norman, 1998). Echidna surface temperatures for free roaming echidnas were measured opportunistically and found to be between air temperature (as measured in a nearby Stevenson screen) and body temperature. Thus, echidna surface temperatures were assumed to be midway between T_b and T_a during experiments. T_e estimates were found to be very insensitive to variations in the value of surface temperature input.

Calculation of H_d also required the characteristic dimension of the animal (Campbell & Norman, 1998). Characteristic dimension was determined by assuming the volume of the animal can be determined from its mass for a specific gravity of 1 (Werner*, et al.*, 1989; Kanoh & Nakajima, 1998; Liu, 2001).

The surface area of the different elements (i) and (ii) were also required to calculate T_e according to Bakken (1981) and above. The total surface area was determined from the animal's volume using the Meeh's formula as cited for echidnas by Schmidt-Nielsen *et al.* (1966). The surface area of the ventral element was determined by drawing outlines of relaxed, prone echidnas (under anaesthesia) onto graph paper. Thus the maximum surface area in contact with the substrate was determined to be about 26.9% of total surface area, which is nearly equal to the area of the base, as a proportion to total surface area, of the cuboid used in the radiation view factor estimates. It was assumed that the ventral surface area of the echidna was 26.9% of its total surface area for all T_e calculations for all echidnas of different mass.