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RESEARCH ARTICLE

Flexibility in thermoregulatory physiology of two dunnarts, *Sminthopsis macroura* and *Sminthopsis ooldea* (Marsupialia; Dasyuridae)

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SUMMARY

Stripe-faced dunnarts (*Sminthopsis macroura*) and Ooldea dunnarts (*S. ooldea*) were acclimated for 2 weeks to ambient temperature (T_a) regimes of 12–22°C, 18–28°C and 25–35°C, and then measured for standard, basal (BMR) and maximum (MMR) metabolic rate using flow-through respirometry. *Sminthopsis macroura* maintained a stable body temperature under all experimental T_a and acclimation regimes. Although its BMR was not statistically different between the three acclimation regimes, the lower end of the thermoneutral zone (TNZ) shifted from 30°C under the 18–28°C and 12–22°C acclimation regimes to 35°C under the 25–35°C acclimation regime. MMR increased significantly at the cooler acclimation regimes. EWL increased at T_a=35°C, compared with lower T_a, in all acclimation regimes, but an increase in evaporative water loss (EWL) at T_a=10°C observed in cool acclimations did not occur at the 25–35°C regime. In contrast, *S. ooldea* had variable body temperature between experimental T_a in all acclimation regimes, but no acclimational shift in TNZ, which was between 30 and 35°C. Neither BMR nor MMR was affected by exposure to the three acclimation regimes. EWL did not change across T_a or with acclimation regime. *Sminthopsis macroura* was flexible in many aspects of its thermoregulation (involving energy and water balance) in response to thermal acclimation, presumably allowing it to balance its energy and water requirements over a broad range of climatic conditions. *Sminthopsis ooldea* seems to have an inflexible energetic and water balance in response to thermal acclimation, but has low nominal expenditure of either resource on thermoregulation because it thermoregulates less precisely than *S. macroura*. It seems that *S. ooldea* is adapted to a more narrow, stable climate.

Key words: thermoregulation, metabolic rate, evaporative water loss, physiological flexibility, acclimation, *Sminthopsis macroura*, *Sminthopsis ooldea.*

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INTRODUCTION

Phenotypic plasticity [or flexibility *sensu* Piersma and Drent (Piersma and Drent, 2003)] occurs when organisms respond to environmental variability on an ecological scale (Nespolo et al., 2001), whereby aspects of physiology, for example, are altered in a way that optimises the phenotype for certain environmental conditions (Lewontin, 1969; Feder, 1987). For example, birds and mammals enhance heat or cold resistance seasonally (acclimatisation) or in response to short-term, experimental thermal challenges (acclimation) (Nespolo et al., 2001; Soobramoney et al., 2003). Dawson (Dawson, 2003) suggested that higher ambient temperatures (either in the laboratory or as a result of seasonal warming) can lead to downregulation of metabolic capacity in birds, including changes in thermogenic capacity measured as basal metabolic rate (BMR) or maximum metabolic rate (MMR) (Bozinovic et al., 1990; Nespolo et al., 2001). Nespolo et al. (Nespolo et al., 2001) suggested, however, that MMR provides the most accurate assessment of acclimation and thermoregulatory flexibility because it incorporates BMR together with shivering and non-shivering thermogenesis, all of which can be enhanced in response to continued exposure to low temperatures. As such, MMR provides an omnibus measure of thermoregulatory response capacity.

Although it has been shown for several species that metabolic rate is flexible in response to environmental changes, less is known about the flexibility of evaporative water loss (EWL). Although evaporative heat loss (EHL) is a major thermoregulatory effector during high temperature exposure (Angilletta et al., 2010), elevated EHL at low temperature exposures, resulting from poor recuperative heat and water exchange in the respiratory tract, can be a significant contributor to thermal balance when ventilation increases to satisfy increased oxygen demand. Therefore, thermal acclimation may not only influence metabolic rate but also impact the water budget. Recent studies have shown a flexibility of EWL in birds (Williams and Tieleman, 2000) and large mammals (Ostrowski et al., 2006). This has implications for the water budget [e.g. relative water economy (RWE), the amount of water lost relative to levels of metabolic water production, as discussed by Cooper and others (Cooper and Withers, 2008; Cooper and Withers, 2009; Cooper et al., 2009; Withers and Cooper, 2009)], especially in species adapted to arid habitats with variable and unpredictable conditions (Lee and Schmidt-Nielsen, 1971; Menon et al., 1989; Williams and Tieleman, 2000). The current paucity of data on the interaction between water loss and energetics makes prediction of the effects of thermal acclimation upon these aspects of physiology difficult, but the importance of these data to the basic physiology suggests that they should be incorporated into acclimational studies.

Enhancement or contraction of metabolism can impart acclimatory responses in homeothermic mammals, but heterothermic mammals are intrinsically more flexible in their metabolic responses to acute changes in their environment (Geiser and Turbill, 2009).

Although there has been interest in the acclimation of heterotherms to chronic changes in temperature, these have most often focused upon biochemical aspects of thermogenesis (Klingenspor et al., 2000; Nespolo et al., 2002) and the increased energy efficiency of re-warming after torpor (Opazo et al., 1999). At a coarser scale, however, energetic plasticity may include changes in the propensity towards torpor, the length and depth of bouts, or the intensity of torpor use in response to environmental conditions, optimising torpor use as an evasive strategy at low ambient temperature (T_a) (Geiser, 1994; Geiser et al., 2003). Although some species enter torpor only under thermal challenges of low temperature, food restriction or both (Hudson, 1978; Wang, 1989; Withers et al., 1990; Malan, 1996), the propensity of a species to enter torpor following acclimation to different environmental conditions has been investigated only recently when Munn et al. (Munn et al., 2010) showed that bouts of torpor occurred more often when food was available stochastically than when the same amount of food was available consistently. Given that torpor is viewed as an adaptation that reduces energy expenditure that is normally associated with thermoregulation (Nicol and Anderson, 1996; Grigg and Beard, 2000), a heterotherm acclimated to low temperatures might not utilise torpor as much if it has acclimatory compensation mechanisms to low temperature, other than torpor, that result in lower overall energy use (i.e. torpor use may be reduced as a response to cold in cold-acclimated heterotherms).

Sminthopsis macroura (Gould 1845) and *S. ooldea* Troughton 1965 represent two distinct clades of dunnart, the *S. macroura* and *S. psammophila* species groups, respectively (Archer, 1981; Blacket et al., 1999). *Sminthopsis macroura* has an extensive geographical distribution across Australia (McKenzie et al., 2006), whereas *S. ooldea* is limited to the central Australian arid environments of Western Australia, South Australia and the Northern Territory (Aslin, 1983). The considerable environmental variation across the geographic distribution of *S. macroura* would suggest that this species is highly adaptable, whereas *S. ooldea* may be more specifically adapted to, and therefore limited to, the 'hyper-arid' environments of the Australian centre (*sensu* Archer, 1981; Withers and Cooper, 2009). The two species are similar in ecology, habit and even size and mass range (Ewer, 1968; Aslin, 1983; Morton, 1983a; Morton, 1983b), but have substantially different basic energetic patterns. *Sminthopsis macroura* appears to be a strong thermoregulator and a classical heterotherm (i.e. spontaneous, regulated daily torpor), but *S. ooldea* is more thermolabile while also using torpor (Tomlinson, 2012). Where two closely related species diverge in their distributions, especially where many aspects of their biology are very similar, differences in their thermogenic plasticity may be an important driver of this divergence.

The focus of the present study was to determine the capacity of *S. macroura* and *S. ooldea* to acclimate to changes in ambient temperatures (i.e. to respond over a period of weeks rather than tolerate over a period of hours in a respirometry trial), and establish whether any differences were related to their differing distributions and thermal physiology. 'Phenotypic plasticity' is taken to be variability in BMR, metabolic heat production (MHP), MMR and propensity for torpor, as well as evaporative water loss (EWL), EHL and RWE following acclimation to different ambient temperature regimes. We predict that both aspects of metabolism (BMR and MMR) of the two dunnarts studied here will show plasticity resulting from acclimation, but we expect this plasticity to differ between the species. As a contributor to thermoregulation, EWL (through EHL) should be reduced at low T_a following chronic cold acclimation, reducing respiratory heat and energy loss and thus increasing RWE. *Sminthopsis macroura*, having a broad distribution covering a range of climatic conditions, and being a strong thermoregulator (Tomlinson, 2012), is expected to show phenotypic flexibility, essentially tailoring the thermoregulatory energy budget to chronic T_a regimes. *Sminthopsis ooldea* is expected to be less variable in response to acclimation to a range of chronic T_a regimes because of the lower climatic variation within its geographic distribution.

MATERIALS AND METHODS

Animal housing and acclimation regimes

Stripe-faced dunnarts (*S. macroura*) were captured from various locations within the natural distribution of the species in Western Australia, and Ooldea dunnarts (*S. ooldea*) were captured at Lorna Glen Station (26.227°S, 121.5597°E). The dunnarts were transferred to the University of Western Australia (Crawley campus) within 1 week of capture. The dunnarts were maintained on a *per diem* diet of approximately 1 g of minced red meat (generally kangaroo), a similar portion of canned cat food and three mealworms (*Tenebrio molitor* larvae). For the duration of acclimation, the dunnarts were maintained in a social arena in which they could socialise freely, but during the period of respirometry experiments the individuals were housed separately (in containers approximately 40×50×30 cm length×width×depth) so that they could be food deprived for ≥12 h prior to measurement. The dunnarts were acclimated to several T_a regimes for 2 weeks each prior to experimental measurements of their metabolic rates under these different regimes. Temperature regimes consisted of 12 h low nocturnal T_a, followed by 10°C elevation to a higher diurnal T_a. These acclimation regimes were 12–22°C, 18–28°C and 25–35°C. The 18–28°C regime was selected because it overlaps the maintenance conditions described by other studies with which these data can be compared (Geiser and Baudinette, 1985; Song et al., 1995; Song and Geiser, 1997; Song et al., 1998; Cooper et al., 2005; Withers and Cooper, 2009), whereas the higher and lower regimes encompass almost the full breadth of average temperatures experienced within the geographical distributions of the species (Australian Bureau of Meteorology, unpublished data).

All animal procedures conformed to guidelines of the National Health and Medical Research Council and were approved by the Animal Ethics Committee of The University of Western Australia under permits RA/3/100/654, RA/3/100/704 and RA/3/100/868.

Respirometry

Two flow-through respirometry systems (see Withers, 2001) consisted of airflow through a cylindrical PVC metabolic chamber (270×250 mm) that was regulated at approximately 437 ml min^{-1} (standard temperature and pressure) by an Aalborg GFC-17 (Aalborg, New York, NY, USA) or a Brooks 5871-A (Brooks Instrument, Hatfield, PA, USA) mass flow controller. Relative humidity of the excurrent air stream was measured by Vaisala HMP 35B and HMI 33 probes (Vaisala Oyj, Helsinki, Finland), which also measured T_a within the temperature-controlled cabinet. Excurrent air was then dried by a Drierite column (anhydrous calcium sulfate; W. A. Hammond Drierite, Xenia, OH, USA), and passed through a David Bishop 280 Combo gas analyser (David Bishop Instruments, Warwickshire, UK) which measured both [O_2] and [CO_2]. The analysers were interfaced to a PC using a PICO ADC-11 A/D converter (Pico Technology, St Neots, Cambridgeshire, UK) and recorded using custom-written Visual Basic v6.0 software (Microsoft, Redmond, WA, USA). Ambient temperatures for the acute 8-h measurement trials were 10, 25, 30

and 35°C (in random order), with one trial being conducted per day. Individual dunnarts were only tested once in any three consecutive days.

Baseline readings of background O_2 and CO_2 were established for 60 min before and after metabolic trials. Metabolic rate was measured for at least 8 h until a 20 min steady recording was obtained. Body temperature (T_b) was measured to ±0.1°C at the end of the trials using a pre-calibrated Radiospares 611-234 thermocouple reader (Wetherill Park, NSW, Australia) by inserting a thermocouple 1.5 cm into the rectum within 1 min of removing the dunnart from the chamber. These methods precluded the measurement of T_b during normothermia or torpor prior to the end of the experiment. Estimation of basic physiological parameters was made using custom-written Visual Basic v6.0 software that calculated $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and EWL according to Withers (Withers, 2001), by averaging the lowest and most stable 20 min period. The point where these rates were minimal for euthermic dunnarts was considered to be BMR, and was otherwise referred to as standard metabolic rate (SMR). Wet thermal conductance (C_{wet}) was calculated as $C_{wet}=\dot{V}_{O_2}/(T_b-T_a)$, measured in ml O_2 g^{-1} h^{-1} °C^{-1}, and converted to J g^{-1} h^{-1} °C^{-1} by multiplying $\dot{V}_{O_2}$ by 20.1 J ml^{-1} O_2. Dry thermal conductance (C_{dry}) was subsequently calculated as $C_{dry}=\{(\dot{V}_{O_2}\times 20.1)-[(EWL\times 10^{-3})\times$latent heat of evaporation$]\}/(T_b-T_a)$. Metabolic water production (MWP; mg H_2O ml^{-1} O_2), calculated using the measured RER by the equation MWP=(0.326×RER)+0.337 after Withers (Withers, 1992) and Cooper and Withers (Cooper and Withers, 2009), was used to estimate the relative water economy (RWE; MWP/EWL) and the point of relative water economy (PRWE), which is the T_a where RWE=1. All allometric corrections for the effects of body mass (M) are made using scaling exponents reported by Withers et al. (Withers et al., 2006) of $M^{0.75}$ for metabolic rate, $M^{0.68}$ for EWL and $M^{0.57}$ for thermal conductance.

Maximum metabolism was measured by flow-through respirometry in a helox atmosphere (19.1% O_2 in He; BOC, Perth, WA, Australia) at T_a=10°C (Rosenmann and Morrison, 1974; Smith and Dawson, 1985; Geiser et al., 1996; Thomas et al., 1998; Holloway and Geiser, 2001; Geiser et al., 2003). Baseline readings of air and helox were taken for 20 min before and after the trial. The dunnarts were exposed to helox for 20 min, or until their metabolism declined from a peak, reflecting incipient hypothermia, at which time they were removed and T_b was measured. MMR was estimated using custom-written Visual Basic v6.0 software by measuring the peak $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ following the introduction of a dunnart to the system, and also by averaging the metabolic rate over the first two and five minutes of the trial.

Statistical analysis

The effect of T_a on physiological variables was examined by linear regression and ANOVA, with Student–Newman–Keuls (SNK) *post hoc* tests. Initially a repeated-measures design was intended; however, natural attrition of individuals during the study period precluded this. Given that a repeated-measures design accounts for individual variation and so has a lower error term, the use of full-factorial ANOVA is generally more conservative than repeated-measures ANOVA (see Cohen, 2008). The maintenance of normothermic T_b was tested by linear regression against T_a, and was additionally analysed by testing the equality of variance of the T_b residuals between the T_a treatments using a Bartlett's test. Torpor data were compared with normothermia data using ANOVA. The occurrence of torpor appeared to be random amongst individuals, as not all individuals had the same responses at all experimental T_a values. Comparisons of normothermic metabolism between acclimation regimes were made within species by comparing the slope and intercept of the regressions of metabolic rate ($\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$) below the thermoneutral zone (TNZ). Normothermic responses of each species were examined by comparing regressions below the TNZ. Comparisons of maximum metabolism, propensity towards torpor, and torpid metabolism between acclimation regimes were made by ANOVA. All statistical analyses with the exception of Bartlett's test of variance were conducted using statistiXL v.1.7 (statistiXL, www.statistixl.com). Bartlett's test of variance was conducted by hand, following Zar (Zar, 1999). Following discussion by Felsenstein (Felsenstein, 1985) and Garland and Adolph (Garland and Adolph, 1994) suggesting that two-species comparisons are less informative than phylogenetically informed analyses, these data would benefit from phylogenetic correction. Such strategies were, however, precluded by the paucity of data on metabolic physiology and acclimation responses in the Sminthopsini. Values are presented as means ± s.e.m.; sample sizes are given as n=the number of individuals and N=total sample size (i.e. the total number of measurements).

RESULTS

Body mass and acclimation

Body mass across all respirometry trials and all acclimation regimes was 17.1±0.29 g for *S. macroura* (n=6, N=24) and 11.1±0.13 g for *S. ooldea* (n=8, N=32). There was no significant change in body mass of *S. macroura* at the different acute T_a treatments within the 12–22°C regime ($F_{4,25}$=0.190, P=0.942), the 18–28°C regime ($F_{4,24}$=0.500, P=0.735) or the 25–35°C regime ($F_{4,24}$=1.16, P=0.355). However, body mass was significantly higher during the 25–35°C regime than during the other two acclimation regimes ($F_{2,87}$=17.8, $P=3.36\times10^{-7}$; Table 1). There were no significant changes in the body mass of *S. ooldea* within the 18–28°C regime ($F_{3,28}$=0.390, P=0.761), the 12–22°C regime ($F_{3,28}$=2.04, P=0.131) or the 25–35°C regime ($F_{3,28}$=0.480, P=0.698), but they were heavier for the 12–22°C regime than the other two regimes ($F_{2,93}$=4.87, P=0.00980; Table 1).

Body temperature and basal metabolic rate

The T_b of *S. macroura* was consistent between acclimation regimes (33.8±0.2°C; two-way ANOVA, T_a, $F_{2,55}$=0.593, P=0.556; Table 2), and under all acute ambient temperature exposures between acclimation regimes (acclimation, $F_{6,55}$=1.07, P=0.391). There was no difference in the variance of the T_b residuals at different T_a treatments between acclimation regimes ($B_{C,11}$=19.5, P=0.053). In contrast, *S. ooldea* was thermolabile across acclimation regimes, where average T_b for *S. ooldea* showed significant variation with T_a (two-way ANOVA, T_a, $F_{3,72}$=8.16, $P=9.77\times10^{-5}$) and acclimation regime (two-way ANOVA, acclimation, $F_{2,79}$=3.44, P=0.0377). The variance of T_b residuals differed significantly between acute T_a treatments and the acclimation regimes ($B_{C,11}$=64.9, $P=1.12\times10^{-9}$).

At all acclimation regimes, *S. macroura* showed decreasing SMR from T_a=10°C to BMR. Under the 12–22°C and 18–28°C acclimation regimes, BMR occurred at T_a=30°C, which is presumed to be within the TNZ because metabolism was higher at T_a=35°C (Table 2). Under the 25–35°C acclimation regime, however, SMR was lowest (i.e. BMR) at T_a=35°C. The mass-corrected ($M^{-0.75}$) BMR was not statistically different between the three acclimation regimes in respect to $\dot{V}_{O_2}$ ($F_{2,15}$=2.14, P=0.153) or $\dot{V}_{CO_2}$ ($F_{2,15}$=1.57, P=0.240). Despite the shift in TNZ under the warmest acclimation regime, the metabolic profile below the TNZ was not statistically

Table 1. Summary of metabolic physiology of *S. macroura* and *S. ooldea* at the three different acclimation regimes tested

Physiological variable	Acclimation regime		
	12–22°C	18–28°C	25–35°C
S. macroura			
Body mass (g)	16.1±0.4 (6, 24)	16.0±0.5 (6, 24)	19.1±0.3 (6, 24)*
T_b (°C)	33.7±0.4 (6, 21)	33.8±0.4 (6, 23)	34.1±0.4 (6, 23)
BMR (ml O_2 g^{-1} h^{-1})	1.43±0.29 (6)	1.57±0.34 (6)	0.80±0.14 (6)
BMR (ml CO_2 g^{-1} h^{-1})	1.25±0.34 (6)	0.88±0.18 (6)	0.58±0.14 (6)
MR regression slope	–0.28±0.04 (6, 18)	–0.27±0.03 (8, 18)	–0.16±0.04 (6, 18)
MR regression intercept	9.35±1.11 (6, 18)	8.61±0.83 (8, 18)	6.45±1.02 (6, 18)
PRWE (°C)	–9.0 (6, 22)	–8.7 (6, 23)	–4.8 (6, 24)
RWE regression slope	–0.02±0.003 (6, 22)	–0.02±0.003 (6, 23)	–0.02±0.002 (6, 24)
S. ooldea			
Body mass (g)	11.6±0.1 (8, 32)	10.6±0.3 (8, 32)	11.0±0.2 (8, 32)*
T_b (°C)	33.9±0.3 (8, 30)	34.6±0.4 (8, 21)	33.9±0.4 (8, 25)
BMR (ml O_2 g^{-1} h^{-1})	1.39±0.29 (8)	1.72±0.22 (8)	1.67±0.23 (8)
BMR (ml CO_2 g^{-1} h^{-1})	0.77±0.11 (8)	1.14±0.10 (8)	1.24±0.13 (8)
MR regression slope	–0.28±0.08 (8, 22)	–0.17±0.05 (8, 18)	–0.25±0.03 (8, 22)
MR regression intercept	9.47±0.72 (8, 22)	6.91±1.17 (8, 18)	9.08±0.72 (8, 22)
PRWE (°C)	9.0 (8, 31)	–15.0 (8, 28)	1.7 (8, 30)*
RWE regression slope	–0.03±0.004 (8, 31)	–0.02±0.003 (8, 28)	–0.03±0.003 (8, 30)*

Data are presented as means ± s.e.m.; sample sizes are presented in parentheses (*n*, *N*), where *n* is the number of individuals and *N* is the total sample size. Asterisks denote significant differences between acclimation regimes. See the List of symbols and abbreviations for definitions of variables and abbreviations.

different between the three acclimation regimes (slope, $F_{2,46}$=2.54, P=0.0890; intercept, $F_{2,48}$=0.691, P=0.506), with a common regression of $\dot{V}_{O_2}$=8.089–0.207T_a.

Sminthopsis ooldea maintained a pattern of decreasing SMR from T_a=10 to 30°C between all acclimation regimes. For the 12–22°C acclimation regime, metabolic rate increased between T_a=30°C (TNZ beginning between T_a=25 and 30°C and ending between T_a=30 and 35°C) and 35°C ($\dot{V}_{O_2}$, $t_{6.64}$=2.38, P=0.049; $\dot{V}_{CO_2}$, $t_{11.0}$=2.57, P=0.026; Table 2). For the 18–28°C acclimation regime there was no difference in metabolic rate between T_a=30 and 35°C ($\dot{V}_{O_2}$, t_{10}=0.831, P=0.425; $\dot{V}_{CO_2}$, $t_{5.04}$=0.811, P=0.454; Table 2), which is assumed to represent BMR in the TNZ (beginning between T_a=25 and 30°C and ending above T_a=35°C). For the 25–35°C acclimation regime, BMR at T_a=30°C was not different from that at T_a=35°C ($\dot{V}_{O_2}$, t_{11}=0.327, P=0.750; $\dot{V}_{CO_2}$, t_{11}=0.181, P=0.860; Table 2), again implying that the TNZ began between T_a=25 and 30°C and ended above T_a=35°C. The mass-corrected BMR (T_a=30°C) was not statistically different between the three acclimation regimes in respect to $\dot{V}_{O_2}$ ($F_{2,21}$=0.253, P=0.779) or $\dot{V}_{CO_2}$ ($F_{2,21}$=0.947, P=0.404). The metabolic response to T_a was not statistically different between the three acclimation regimes (slope, $F_{2,55}$=2.252, P=0.115; intercept, $F_{2,57}$=0.286, P=0.752), with a common regression of $\dot{V}_{O_2}$=10.602–0.288T_a.

The normothermic respiratory exchange ratio (RER) of *S. macroura* did not change significantly between ambient temperatures within the 12–22°C ($F_{3,18}$=0.26, P=0.850), 18–28°C ($F_{3,20}$=1.07, P=0.385) or 25–35°C regimes ($F_{3,20}$=0.46, P=0.708; see Tables 1, 2 for values). The RER, pooled by acclimation regime, was not significantly different between acclimation regimes (two-way ANOVA, acclimation, $F_{2,67}$=0.25, P=0.780) and there was no interaction with T_a (acclimation × T_a, $F_{6,63}$=0.64, P=0.697), averaging 0.79±0.04 (N=12). *Sminthopsis ooldea* also showed no significant difference in normothermic RER between ambient temperatures within the 12–22°C ($F_{3,25}$=0.865, P=0.472), 18–28°C ($F_{3,22}$=0.441, P=0.726) or 25–35°C regimes ($F_{3,24}$=0.277, P=0.841; see Tables 1, 2). RER pooled by acclimation regime showed no significant difference between acclimations (two-way ANOVA, acclimation, $F_{2,80}$=2.61, P=0.081), and no significant interaction with T_a (acclimation × T_a, $F_{6,76}$=0.440, P=0.850), averaging 0.77±0.02 (N=12).

Evaporative water loss, thermal conductance and water economy

There were significant differences in EWL of *S. macroura* across the experimental T_a range for the 12–22°C acclimation regime ($F_{3,18}$=3.76, P=0.029; Table 1) and the 18–28°C regime ($F_{3,20}$=4.69, P=0.0120), but not the 25–35°C regime ($F_{3,20}$=0.263, P=0.851). EWL increased at both T_a=10 and 35°C compared with the TNZ in the cool acclimation regimes, but not under the 25–35°C regime (Table 1). A pattern of stable C_{wet} and C_{dry} between T_a=10 and 30°C, with an increase at T_a=35°C, was consistent between the acclimation regimes. There was no significant effect of acclimation regime on C_{wet} (two-way ANOVA, acclimation, $F_{2,58}$=1.21, P=0.308; T_a, $F_{3,57}$=20.2, P=1.31×10^{-8}; acclimation × T_a, $F_{6,54}$=1.59, P=0.169) or C_{dry} (two-way ANOVA; acclimation, $F_{2,58}$=1.31, P=0.278; T_a, $F_{3,57}$=6.89, P=0.001; acclimation × T_a, $F_{6,54}$=1.37, P=0.244), but there were significant increases in both at T_a=35°C during all acclimation regimes. RWE increased with decreasing T_a under all three acclimation regimes (Tables 1, 2). The RWE profile was not statistically different for the three acclimation regimes (slope $F_{2,64}$=0.542, P=0.584; intercept $F_{2,66}$=0.375, P=0.688), with a common regression of RWE=0.85–0.02T_a. The extrapolated PRWE was similar between all three acclimation regimes and averaged –6.8±1.2°C (Table 1).

A constant EWL between T_a=10 and 35°C was evident for *S. ooldea* across all three acclimation regimes (12–22°C, $F_{3,26}$=1.759, P=0.180; 18–28°C, $F_{3,24}$=0.548, P=0.654; 25–35°C, $F_{3,25}$=1.296, P=0.298; Table 1). The pattern of stable C_{wet} and C_{dry} between T_a=10 and 30°C, with an increase at T_a=35°C, was also consistent between the acclimation regimes. There was no significant effect of acclimation regime on C_{wet} (two-way ANOVA, acclimation, $F_{2,71}$=0.342, P=0.711; T_a, $F_{3,70}$=12.9, P=1.13×10^{-6}; acclimation × T_a, $F_{6,67}$=0.277, P=0.946) or C_{dry} (two-way ANOVA; acclimation, $F_{2,71}$=0.368, P=0.694; T_a, $F_{3,70}$=4.443, P=0.007; acclimation × T_a,

Table 2. Thermoregulatory variables for *Sminthopsis macroura* and *S. ooldea* at the three different acclimation regimes tested

Thermoregulatory variable	Acclimation regime	10°C	25°C	30°C	35°C	*P*
S. macroura						
T_b (°C)	12–22°C	32.67±1.20	31.50±0.43	34.33±0.49	35.67±0.21	0.00006
	18–28°C	31.87±2.62	32.92±0.58	33.38±0.55	35.05±0.87	0.181
	25–35°C	33.40±1.36	32.33±0.21	35.00±0.63	35.50±0.34	0.016
$\dot{V}_{O_2}$ (ml g^{-1} h^{-1})	12–22°C	7.07±1.01	2.41±0.51	1.43±0.29	2.06±0.32	7.81×10^{-6}
	18–28°C	6.24±0.53	2.35±0.27	1.45±0.26	1.47±0.27	6.96×10^{-7}
	25–35°C	5.75±0.85	2.98±0.60	1.60±0.26	0.80±0.14	0.0003
$\dot{V}_{CO_2}$ (ml g^{-1} h^{-1})	12–22°C	6.49±0.88	2.12±0.62	1.25±0.34	1.48±0.35	2.58×10^{-5}
	18–28°C	5.58±0.55	1.88±0.51	1.05±0.28	0.95±0.17	1.01×10^{-6}
	25–35°C	4.63±0.70	2.35±0.64	1.43±0.40	0.58±0.14	0.002
RER	12–22°C	0.92±0.01	0.83±0.13	0.84±0.13	0.75±0.15	0.85
	18–28°C	0.89±0.04	0.75±0.10	0.71±0.08	0.65±0.05	0.385
	25–35°C	0.81±0.05	0.74±0.07	0.83±0.14	0.70±0.07	0.708
EWL (mg g^{-1} h^{-1})	12–22°C	8.30±1.71	3.72±0.89	5.68±1.52	8.70±0.82	0.029
	18–28°C	6.47±0.75	3.59±1.08	3.58±0.46	6.35±0.71	0.018
	25–35°C	5.68±0.77	5.37±1.46	6.02±1.63	6.89±1.07	0.851
C_{wet} (J g^{-1} h^{-1} °C^{-1})	12–22°C	7.20±1.24	7.61±1.70	6.76±1.43	35.14±7.18	0.000152
	18–28°C	5.69±0.47	6.31±1.09	11.76±4.37	20.39±4.92	0.032
	25–35°C	1.42±3.35	8.24±1.60	6.62±1.39	18.86±3.83	1.92×10^{-5}
C_{dry} (J g^{-1} h^{-1} °C^{-1})	12–22°C	6.15±0.98	5.83±1.43	3.86±1.23	16.87±4.48	0.011
	18–28°C	5.04±0.44	5.09±0.72	8.23±3.11	12.92±5.31	0.089
	25–35°C	4.12±0.62	6.45±1.20	3.61±0.56	6.26±3.28	0.049
RWE	12–22°C	0.57±0.06	0.34± 0.06	0.17± 0.02	0.13±0.01	2.31×10^{-6}
	18–28°C	0.58±0.07	0.44±0.06	0.20±0.02	0.13±0.02	1.21×10^{-5}
	25–35°C	0.61±0.03	0.37±0.04	0.18±0.01	0.08±0.02	8.72×10^{-12}
N	12–22°C	4	6	6	6	
	18–28°C	5	6	6	6	
	25–35°C	6	6	6	6	
S. ooldea						
T_b (°C)	12–22°C	33.32±0.70	33.60±0.53	32.68±0.60	35.93±0.31	0.001
	18–28°C	33.93±0.99	31.70±1.30	33.83±0.48	36.49±0.30	0.0003
	25–35°C	32.13±0.92	35.00±0.48	32.61±0.44	35.54±0.45	0.0002
$\dot{V}_{O_2}$ (ml g^{-1} h^{-1})	12–22°C	6.96±0.91	3.55±0.43	1.63±0.23	1.91±0.10	2.48×10^{-7}
	18–28°C	5.12±1.39	3.36±0.67	1.78±0.33	1.75±0.19	0.011
	25–35°C	7.64±0.59	4.01±0.48	1.99±0.51	1.78±0.23	1.00×10^{-7}
$\dot{V}_{CO_2}$ (ml g^{-1} h^{-1})	12–22°C	5.62±0.66	2.57±0.39	0.81±0.07	1.20±0.15	1.23×10^{-8}
	18–28°C	4.02±1.02	2.69±0.58	1.20±0.19	1.05±0.12	0.003
	25–35°C	6.28±0.53	3.32±0.40	1.08±0.26	1.18±0.13	1.80×10^{-7}
RER	12–22°C	0.82±0.02	0.72±0.07	0.58±0.10	0.66±0.09	0.472
	18–28°C	0.81±0.06	0.78±0.10	0.69±0.06	0.66±0.11	0.726
	25–35°C	0.82±0.04	0.84±0.05	0.69±0.13	0.73±0.09	0.841
EWL (mg g^{-1} h^{-1})	12–22°C	5.55±1.38	6.19±1.65	4.22±0.33	7.77±0.50	0.180
	18–28°C	5.87±1.75	5.89±1.84	4.80±1.52	7.46±1.30	0.298
	25–35°C	6.05±0.77	6.23±0.73	4.96±0.93	7.24±0.87	0.660
C_{wet} (J g^{-1} h^{-1} °C^{-1})	12–22°C	5.98±0.86	8.98±1.45	16.85±4.12	39.34±7.67	0.0001
	18–28°C	3.86±0.74	5.58±0.27	10.85±1.16	35.22±10.15	0.036
	25–35°C	6.83±0.46	7.94±1.36	18.00±7.79	29.49±5.95	0.092
C_{dry} (J g^{-1} h^{-1} °C^{-1})	12–22°C	5.41±0.79	7.14±1.23	11.97±3.31	21.09±4.97	0.010
	18–28°C	3.27±0.60	4.88±0.26	7.13±0.77	17.73±7.08	0.262
	25–35°C	6.16±0.34	6.57±1.36	12.67±6.42	15.44±5.69	0.602
RWE	12–22°C	0.94±0.14	0.45±0.08	0.20±0.02	0.13±0.01	6.46×10^{-9}
	18–28°C	0.54±0.07	0.41±0.06	0.28±0.05	0.14±0.02	3.93×10^{-6}
	25–35°C	0.76±0.07	0.44±0.07	0.23±0.04	0.14±0.02	2.84×10^{-9}
N	12–22°C	7	8	7	7	
	18–28°C	6	7	6	7	
	25–35°C	7	8	8	8	

Data are means ± s.e.m. Reduced *N*-values at low T_a are the result of individuals entering torpor early in a trial and not arousing for the 8 h duration. *P* is the probability of significant differences occurring between acute T_a treatments within each acclimation regime (significant differences occurred where $P<0.05$). See the List of symbols and abbreviations for definitions of variables and abbreviations.

$F_{6,67}$=0.180, P=0.981). RWE of *S. ooldea* showed the same pattern of increasing economy with decreasing T_a for all three acclimation regimes. The RWE profile was statistically different between the three acclimation regimes (slope $F_{2,83}$=7.221, P=0.001; Tables 1, 2), and each acclimation regime had a statistically significant RWE profile: 12–22°C ($F_{1,29}$=65.4, $P=6.46\times10^{-9}$), RWE=1.32±0.12–0.03±0.004T_a; 18–28°C ($F_{1,26}$=33.9, $P=3.93\times10^{-6}$), RWE=0.75±0.08–0.02±0.003T_a; and 25–35°C ($F_{1,28}$=72.8, $P=2.84\times10^{-9}$), RWE=1.04±0.08–0.03±0.003T_a.

The extrapolated PRWE was different between all three acclimation regimes, ranging from –15.0°C under the 18–28°C regime to 9.0°C under the 12–22°C regime (Table 1).

Maximum metabolic rate

There was a general decrease in MMR of *S. macroura* with an increase in the acclimation temperature, from 17.01±3.90 ml O_2 g^{-1} h^{-1} and 9.50±1.85 ml CO_2 g^{-1} h^{-1} at the 12–22°C acclimation regime to 9.53±1.21 ml O_2 g^{-1} h^{-1} and 8.07±0.36 ml CO_2 g^{-1} h^{-1} at the 25–35°C acclimation regime. After MMR was log-transformed to normalise unequal variances, maximum $\dot{V}_{O_2}$ ($\dot{V}_{O_2,max}$) showed a significant decrease from the coolest acclimation regime to the warmest ($F_{1,16}$=4.77, P=0.044; Table 3). There was no difference in the maximum $\dot{V}_{CO_2}$ ($\dot{V}_{CO_2,max}$) of *S. macroura* across the acclimation regimes ($F_{1,16}$=0.240, P=0.631). There was no change in MMR ($\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$) across the acclimation regimes for *S. ooldea* (O_2, $F_{2,18}$=0.796, P=0.466; CO_2, $F_{2,18}$=0.905, P=0.422; Table 3). The RER for *S. macroura* was 0.78±0.07, with no effect of acclimation regime ($F_{2,15}$=0.557, P=0.584), but RER was 0.69±0.07 for *S. ooldea*, with a significant increase in the 25–35°C acclimation regime ($F_{2,18}$=4.21, P=0.032).

Body temperature of *S. macroura* in helox at T_a=10°C decreased slightly below normothermic T_b in air at T_a=10°C for all of the acclimation regimes (12–22°C, T_b=27.5±1.15°C, ΔT_b=4.7±1.45°C, t_7=2.78, P=0.027; 18–28°C, T_b=27.5±1.02°C, ΔT_b=6.8±0.86°C, t_9=4.80, P=0.001; 25–35°C, T_b=26.8±0.75°C, ΔT_b=6.4±1.50°C, t_9=4.43, P=0.002). In contrast, the T_b of *S. ooldea* in helox at T_a=10°C was similar to normothermic T_b values in air at T_a=10°C for all of the acclimation regimes (12–22°C, T_b=30.6±2.08°C, ΔT_b=0.1±1.28°C, $t_{8.51}$=1.22, P=0.252; 18–28°C, T_b=30.3±1.26°C, ΔT_b=2.2±1.09°C, t_7=1.86, P=0.105; 25–35°C, T_b=28.7±2.67°C, ΔT_b=3.0±1.51°C, $t_{8.51}$=2.24, P=0.052).

Factorial thermogenic scope (i.e. MMR divided by BMR) of *S. macroura* (Table 3) did not differ between acclimation regimes (O_2, $F_{2,15}$=0.631, P=0.546; CO_2, $F_{2,15}$=0.314, P=0.735), averaging 12.7 (range 10.2–14.3) for $\dot{V}_{O_2}$ and 15.2 (range 13.4–18.2) for $\dot{V}_{CO_2}$. The thermogenic scope of *S. ooldea* was variable (Table 3), but was not significantly different between acclimation regimes (O_2, $F_{2,14}$=0.240, P=0.789; CO_2, $F_{2,16}$=0.381, P=0.690), averaging 7.23 (range 6.36–7.31) for $\dot{V}_{O_2}$ and 10.08 (range 8.58–10.12) for $\dot{V}_{CO_2}$. The thermogenic scope of *S. macroura* (15.21; range 13.41–18.16) in helox at T_a=10°C was 50% higher than that of *S. ooldea* (10.08; range 8.58–10.12), perhaps reflecting a difference in their propensities to thermoregulate in the face of thermal challenge.

Torpor

Torpor (with endogenous arousal) was observed for *S. macroura* only at T_a=10°C, in two trials (33%) in the 12–22°C acclimation regime and in three trials (50%) in the 18–28°C acclimation regime. In the 25–35°C acclimation regime, however, no torpor was recorded but there was one instance of hypothermia (a slow decline of metabolism compared with the rapid entry into torpor). *Sminthopsis ooldea* had a much greater propensity for torpor at both T_a=10 and 25°C. The proportion of *S. ooldea* entering torpor remained fairly constant for all acclimation regimes with four individuals entering torpor (50% of trials) at both T_a=10 and 25°C for the 12–22°C acclimation regime, and with five individuals entering torpor (62.5% of trials) at both T_a=10 and 25°C for the 18–28°C and 25–35°C acclimation regimes. The pattern of torpor for these dunnarts was to enter torpor and arouse again before the completion of a metabolic trial; as such, sample sizes for *S. macroura* are low and thus not amenable to statistical analysis of the effects of acclimation upon torpor. Significant differences in torpid metabolic rate (TMR) were found for *S. ooldea*, where there was no effect of T_a ($F_{1,25}$=0.093, P=0.763), but warm acclimation significantly reduced TMR (averaged between the two acute T_a treatments) from 1.78±0.27 ml O_2 g^{-1} h^{-1} at the 12–22°C acclimation regime to 0.77±0.20 ml O_2 g^{-1} h^{-1} at the 25–35°C acclimation regime (acclimation, $F_{2,24}$=6.64, P=0.006).

DISCUSSION

The broad context of this study was to examine whether *S. macroura*, a strongly thermoregulating dunnart with a broad geographic distribution, had a more flexible physiological response to chronic T_a acclimation regimes than a congeneric species, *S. ooldea*, a more thermolabile species with a narrow geographic distribution. The rationale was that a species with a narrow distribution should experience less selection for physiological flexibility in response to climatic variability than a species with a broad distribution, which is likely to have evolved greater phenotypic flexibility to different climatic regimes. It has already been shown that *S. macroura* is a stronger thermoregulator than *S. ooldea* across a broad T_a range within a single temperature acclimation regime (18–28°C) (Tomlinson, 2012). We show here that *S. macroura* (from a distribution spanning continental Australia), as well as

Table 3. Maximum metabolic rates and thermogenic scope for *S. macroura* and *S. ooldea* in helox and air atmospheres

		Acclimation regime		
		12–22°C	18–28°C	25–35°C
S. macroura				
$\dot{V}_{O_2,max}$ (ml g^{-1} h^{-1})		17.01±3.90 (6)	13.63±1.82 (6)	9.53±1.21 (6)
Scope	Helox	13.59±2.67 (6)	10.17±1.79 (6)	14.31±3.60 (6)
	Air	6.27±1.81 (4)	5.21±1.26 (4)	8.43±2.07 (4)
$\dot{V}_{CO_2,max}$ (ml g^{-1} h^{-1})		9.50±1.85 (6)	8.86±0.90 (6)	8.07±0.36 (6)
Scope	Helox	13.41±5.47 (6)	14.07±4.25 (6)	18.16±3.91 (6)
	Air	7.25±2.74 (4)	8.44±1.99 (4)	10.80±3.45 (4)
RER		0.71±0.16 (6)	0.74±0.14 (6)	0.89±0.07 (6)
S. ooldea				
$\dot{V}_{O_2,max}$ (ml g^{-1} h^{-1})		10.09±1.06 (8)	11.35±1.04 (6)	9.58±0.76 (7)
Scope	Helox	7.31±1.43 (8)	8.03±2.12 (4)	6.36±1.42 (7)
	Air	5.69±1.43 (8)	3.80±1.40 (4)	5.37±1.44 (7)
$\dot{V}_{CO_2,max}$ (ml g^{-1} h^{-1})		6.33±0.75 (8)	7.56±0.86 (6)	7.77±0.95 (7)
Scope	Helox	8.58±1.63 (8)	11.53±3.53 (4)	10.12±2.29 (7)
	Air	8.42±1.11 (8)	6.84±3.70 (4)	9.07±2.37 (7)
RER		0.61±0.04 (8)	0.66±0.03 (6)	0.81±0.07 (7)

Thermogenic scope (scope) is the maximum metabolic rate divided by BMR. RER is the amount of CO_2 produced per unit of O_2 consumed. Values are means ± s.e.m. (*n*). See the List of symbols and abbreviations for definitions of variables and abbreviations.

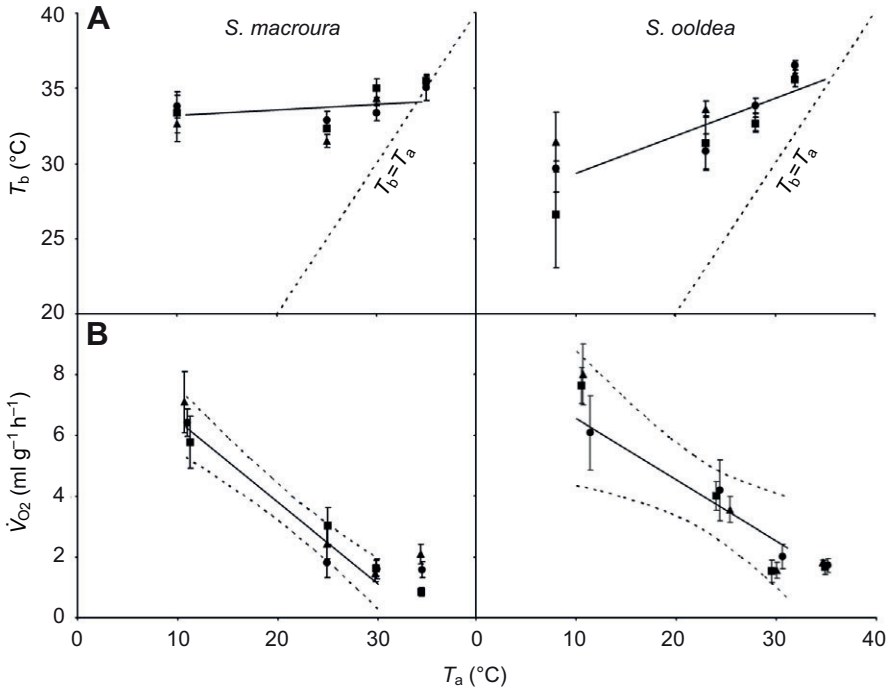

Fig. 1. Energetic variables of *Sminthopsis macroura* and *S. ooldea*, showing the ambient temperature (T_a) regression for the 18–28°C acclimation regime (circles), for comparison with the 12–22°C (triangles) and 25–35°C (squares) acclimation regimes. (A) Body temperature (T_b), showing significant effects of T_a for *S. ooldea* (P=0.0002) but not *S. macroura* (P=0.425) at all acclimation regimes. (B) Standard metabolic rate (SMR), showing no effects chronic acclimation on the SMR pattern of *S. ooldea,* but a change in the thermoneutral zone (TNZ) of *S. macroura* from T_a=30 to 35°C. Data are means ± s.e.m.

thermoregulating well in response to a broad range of acute T_a conditions, was flexible in its thermoregulatory strategies following acclimation to a broad range of chronic temperature regimes. Overall, the propensity to thermoregulate may impart resilience to climatic variability, which is expanded by flexibility in water loss and energetics, while thermolability provides a general reduction of energetic requirement, but potentially at the expense of water economy.

Metabolic acclimation

The basic metabolic responses to acute T_a exposures reported here are similar to previous reports for both species (Geiser and Baudinette, 1987; Hinds et al., 1993; Cooper et al., 2005; Tomlinson, 2012), but *S. macroura* was more flexible in response to chronic thermal acclimation. The similarity in T_b of *S. macroura* across acute T_a exposures and between chronic T_a acclimation regimes, and the similar T_b variances over all experimental treatments (Fig. 1), indicates robust thermoregulatory control. The variable T_b of *S. ooldea* and the different variances between experimental T_a values (Fig. 1) indicate substantial thermolability (see Tomlinson, 2012). At high T_a acclimation, although the BMR of *S. macroura* had not changed, the TNZ extended to a higher T_a (Fig. 1). *Sminthopsis ooldea*, however, showed no flexibility in its metabolic responses to chronic T_a regimes (Fig. 1). Although we define the TNZ here based upon metabolic and thermal parameters (as opposed to an increase in EWL), similar patterns can be seen by consideration of EWL, but these were less clear and less robust to statistical analysis. The results presented here suggest that *S. macroura* can modify their metabolic physiology in response to acclimation to low T_a regimes, but that *S. ooldea* are tightly constrained by a constancy of physiological responses to chronic T_a conditions.

The metabolic response to thermal acclimation differs between various groups of mammals and birds (Hart, 1971; Heldmaier et al., 1986; Dawson and Olson, 1988; Koteja, 1996; Rose et al., 1999; Russell and Chappell, 2007). Heteromyine rodents, for example, enhance metabolism in response to cold acclimation (Hill, 1983; Russell and Chappell, 2007), as do most bird species (McKechnie, 2008). The results presented here for *S. macroura* concur with this expectation [and those that have previously found effects of thermal

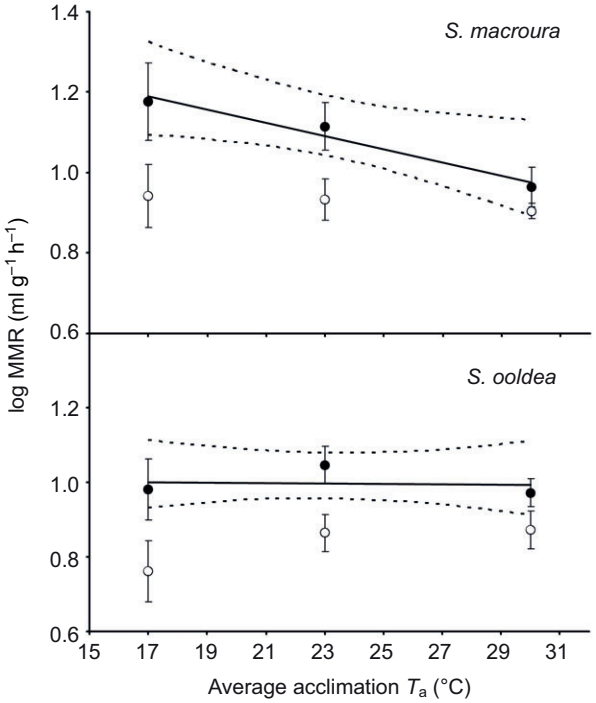

Fig. 2. Maximum metabolism in $\dot{V}_{O_2}$ (filled circles) and $\dot{V}_{CO_2}$ (open circles) at each acclimation regime, showing a significant decline in maximum $\dot{V}_{O_2}$, but no change in $\dot{V}_{CO_2}$ for *S. macroura*, with increasing acclimation T_a and no changes in maximum metabolic rate (MMR) $\dot{V}_{CO_2}$ or $\dot{V}_{O_2}$ for *S. ooldea* across acclimation regimes. The *x*-axis represents the average daily T_a of each acclimation regime from maximum to minimum. Data are means ± s.e.m.

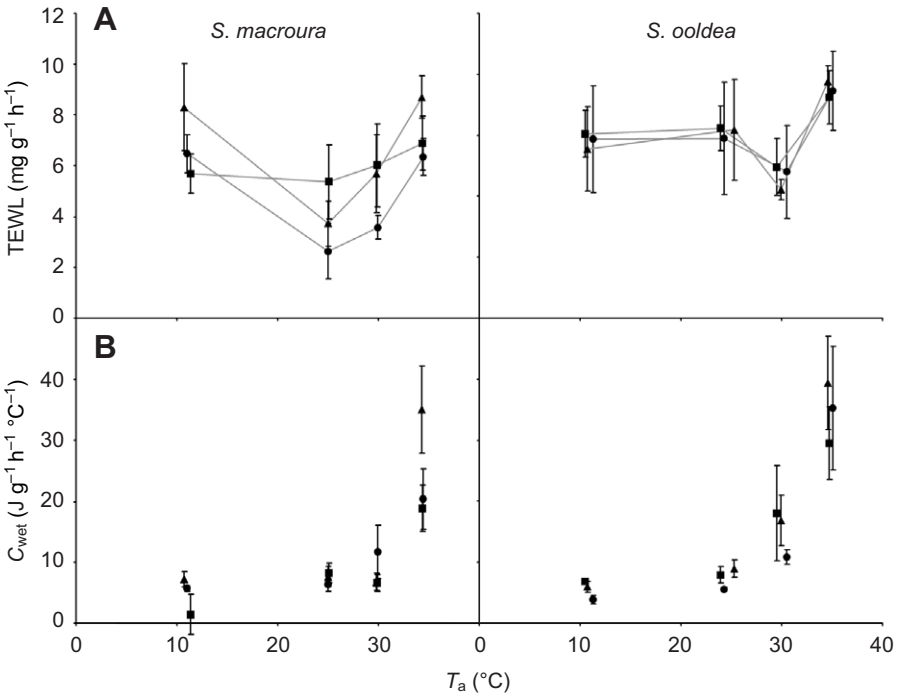

Fig. 3. Effects of T_a on water budget variables of *S. macroura* and *S. ooldea* for the 12–22°C (triangles), 18–28°C (circles) and 25–35°C (squares) acclimation regimes. (A) Total evaporative water loss (TEWL), showing a consistent pattern of increased EWL for *S. macroura* at high and low T_a for the 12–22°C and 18–28°C acclimation regimes, but not the 25–35°C acclimation regime, and relatively consistent values across the experimental range in all acclimation regimes for *S. ooldea*. (B) Wet thermal conductance (C_{wet}), showing relatively consistent values between T_a=10 and 30°C, but increases and higher variance at T_a=30°C at all acclimation regimes for both species. Data are means ± s.e.m.

acclimation in small marsupials (e.g. Geiser et al., 2003)], although studies of small marsupials have generally reported a lack of phenotypic change to thermal acclimation (Smith and Dawson, 1984; Dawson and Olson, 1988; Withers and Hulbert, 1988), as reported here for *S. ooldea*.

The most obvious criticism of the conclusion that *S. macroura* are more physiologically flexible than *S. ooldea* (and that *S. ooldea* are not flexible) relates to the limitations of the acclimation period and intensity. The choice of a 14-day acclimation was based on results from several other studies, suggesting that this is a suitable duration for physiological acclimation (Nespolo et al., 2001; Soobramoney et al., 2003). Other studies, particularly of similar small marsupials, used periods of up to 6 weeks (Reynolds and Hulbert, 1982; Smith and Dawson, 1984; Smith and Dawson, 1985; Dawson and Olson, 1988; Withers and Hulbert, 1988). For species such as the dunnarts studied here from arid environments, seasonal changes occur over approximately 2–4 months (Australian Bureau of Meteorology, unpublished data), so an acclimation period of 2 weeks may not be long enough to elicit a full seasonal acclimatisation. Further, the breadth of the experimental acclimations described here may not be wide enough (from cool acclimation at 12–22°C to warm acclimation at 25–35°C) to trigger the fullest extent of acclimation. This seems unlikely, however, given that the range of acclimations encompasses the full extent of climatic conditions within their natural distributions. Further, at a colder acclimation regime (5–15°C) neither species was able to maintain body mass with food restriction.

There is some ambiguity in the acclimation of MMR in the dunnarts studied here. Although both dunnart species had similar MMR at the 25–35°C acclimation regime, the MMR of *S. ooldea* was not affected by thermal acclimation, whereas *S. macroura* showed evidence of acclimation in maximal $\dot{V}_{O_2}$ (Fig. 2). The thermogenic scopes calculated here for both species are quite similar to the scopes of 8–10 in air reported by Smith and Dawson for kowari (Smith and Dawson, 1985), but much greater than the 2.6 in air and the 5.1 in helox previously reported for *S. macroura* (Geiser et al., 1996). The difference almost certainly reflects the T_a exposure during the trials, where we used helox at T_a=10°C, but Geiser et al. (Geiser et al., 1996) used helox at T_a=18°C. *Sminthopsis macroura* nearly doubled its thermogenic scope in response to cold acclimation, but *S. ooldea* showed no change in maximal metabolism, and we conclude that *S. macroura* adjusted MMR as an acclimatory response, but that *S. ooldea* did not. The different acclimation patterns of MMR between species concur with the patterns of acclimation for SMR, where *S. macroura* showed phenotypic flexibility but *S. ooldea* did not. A lack of acclimation capacity of MMR for *S. ooldea* is in disagreement with many other findings that peak metabolism is decreased in magnitude (Dawson and Olson, 1988; Withers and Hulbert, 1988; Chappell and Hammond, 2004; Rezende et al., 2004; Russell and Chappell, 2007) or duration (Smith and Dawson, 1985) for warm-acclimated mammals. This may reflect the reliance of *S. ooldea* on thermolability rather than metabolic heat production in response to cold. We conclude that *S. macroura* seems to be more reliant on plasticity of MMR as an element of its thermoregulation in the face of chronic exposure to different thermal environments. The thermolability of *S. ooldea* may contribute to the lower thermogenic scopes and conservative MMRs measured here, and it would be of interest to assess the thermoregulation of the kowari studied by Smith and Dawson (Smith and Dawson, 1985).

Acclimation of EWL and RWE

The EWL measured here for *S. macroura* within the TNZ was not significantly different from that predicted allometrically by Withers et al. (Withers et al., 2006) under the 12–22°C regime (t_5=1.256, P=0.264), the 18–28°C regime (t_5=0.636, P=0.553) or the 25–35°C regime (t_5=1.523, P=0.188). Similarly, the EWL of *S. ooldea* within the TNZ was not different from that predicted by Withers et al. (Withers et al., 2006) during any of the acclimation regimes (12–22°C, t_7=0.052, P=0.960; 18–28°C, t_7=0.339, P=0.745; 25–35°C, t_7=0.782, P=0.460).

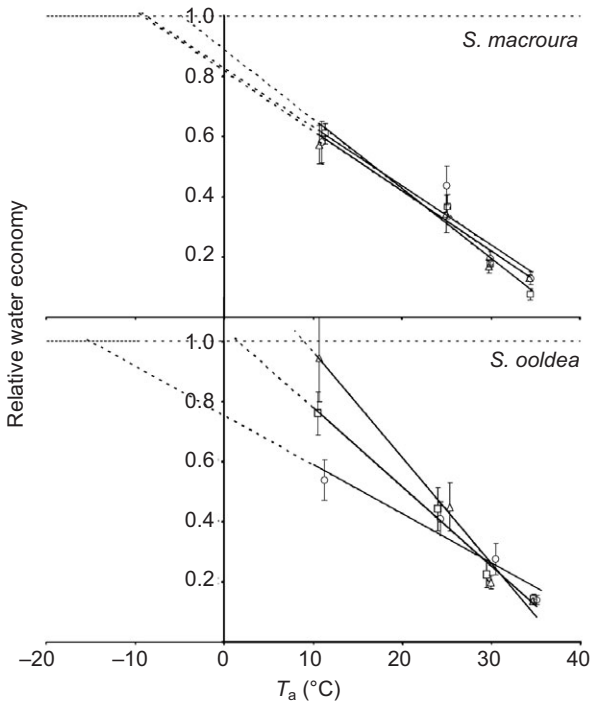

Fig. 4. Relative water economy profiles of *S. macroura* and *S. ooldea* under different acclimation regimes, showing a consistent pattern for *S. macroura* between T_a=10 and 30°C for the 12–22°C (triangles), 18–28°C (circles) and 25–35°C (squares) acclimation regimes, but significantly variable responses for *S. ooldea*. Data are means ± s.e.m.

The EWL of *S. macroura* at both ends of the T_a range (10 and 35°C) decreased with warm acclimation (Fig. 3). Although this should theoretically result in increased RWE, this was not the case (Fig. 4), but the reason for the failure of this mechanism remains unclear. *Sminthopsis ooldea*, in contrast, showed no change in EWL in response to acute T_a exposures or acclimation (Fig. 3). Although this inflexibility, coupled with the inflexibility of SMR (implying inflexible MWP), should result in consistent RWE between acclimation regimes, there were significant changes of RWE for *S. ooldea*. The pattern of these differences does not concur with any expectations of thermal acclimation, in that the warmest and the coolest acclimations had similar RWE patterns and both acclimations had generally higher RWE than the intermediate acclimation regime (Fig. 4). Furthermore, there was a strong correlation of $\dot{V}_{O_2}$ with EWL for both *S. macroura* (r=0.73) and *S. ooldea* (r=0.71) at low T_a, implying that the increased EWL was due to the ventilatory demands of increased metabolic activity. The high thermal conductance of *S. ooldea* at T_a=10°C suggests that, at all acclimation regimes other than 18–28°C, the dunnarts did not rest properly, and this elevated metabolism and EWL could reasonably account for the increased RWE. Finally, the order of increase in conductance and RWE was not consistent with the increasing magnitude of chronic acclimation T_a, but in the temporal order of the experimental process, suggesting that there may be uncontrolled responses of *S. ooldea* to captivity that interact with the physiological lability of the species to produce this unexpected result (see Geiser and Ferguson, 2001). The difficulty in partitioning avenues of water loss, however, limits our current interpretation of similar levels of EWL flexibility. For example, it is likely for *S. macroura* that its similar levels of EWL (and hence EHL) at T_a=35 and 10°C represent different thermoregulatory and evaporative challenges: elevated EHL to dissipate heat T_a=35°C, and increased EWL at T_a=10°C resulting largely from increased ventilatory requirements for increased thermoregulatory SMR. Thus, the lack of change in EWL of *S. ooldea* at low T_a is presumed also to reflect the more broadly thermolabile response of this species to a T_a gradient.

Torpor and acclimation

The propensity towards torpor decreased with increasing acclimation temperatures for *S. macroura*. Acclimation to higher T_a regimes may shift the torpor regulation point upwards (the T_a where TMR increase to regulate torpid T_b); as such, the dunnarts would no longer have a lower critical point of T_a>10°C [established by Geiser and Baudinette (Geiser and Baudinette, 1985) and Tomlinson (Tomlinson, 2012)]. If T_a=10°C fell below the acclimated lower critical point, then entry into torpor may have become prohibitively dangerous (analogous to lower T_a thresholds in cooler acclimation regimes; see S.T., unpublished data) and the dunnarts may not attempt torpor as often under these conditions. *Sminthopsis ooldea*, however, seemed to have no differences in their patterns of torpor use between acclimation regimes. They did, however, have a higher TMR for the 12–22°C regime, which may be analogous to the higher BMRs that were expected (but not observed) under the same acclimation regime. Given that their normothermic SMR and BMR did not change with chronic thermal challenge, and their reliance on torpor and heterothermy at T_a that nominally approach the TNZ of most small mammals [i.e. T_a=30°C; Tomlinson et al. (Tomlinson et al., 2007) and references therein], it seems that broad thermolability and torpor are the thermoregulatory responses of *S. ooldea* to cold challenge, not phenotypic flexibility with chronic acclimation.

Ecophysiological and evolutionary interpretations

The ecophysiological implications of our acclimation experiments can only be generalised to possible acclimatisation because of the distinction between laboratory ‘acclimation’ and field ‘acclimatisation’ discussed by McKechnie (McKechnie, 2008) and Piersma and Drent (Piersma and Drent, 2003). With due consideration of these semantic distinctions, however, species that exhibit phenotypic flexibility in response to chronic acclimation could be expected to inhabit broad, variable geographical distributions. This is the pattern for the dunnarts studied here: *S. macroura* showed flexibility in BMR, MMR and thermoregulatory EWL and has a broad continental distribution, whereas *S. ooldea* showed less flexibility, exhibiting the same poor thermoregulatory response at all acclimation regimes, and has a comparatively small distribution that falls within a fairly predictable and restricted climatic envelope.

Given that the Sminthopsini are variable in their morphology on the basis of habitat and climate (where *S. ooldea* exhibits the most derived morphological characters associated with an arid environment) (Archer, 1981), finding different responses of these two species during acclimation to chronic temperature regimes accords with expectations. Aside from *S. macroura* and *S. crassicaudata* (Godfrey, 1968; Morton, 1978; Geiser and Baudinette, 1985; Nagy et al., 1988; Frey, 1991; Holloway and Geiser, 1995; Song and Geiser, 1997; Song et al., 1998; Zosky, 2002; Zosky and O’Shea, 2003; Cooper et al., 2005), very few other sminthopsines have been studied in physiological terms. *Sminthopsis macroura* and *S. ooldea* also represent different lineages of the Sminthopsini (Blacket et al., 1999). If responses to the realised ecological niche of these taxa differ along phylogenetic lines, then other members of the *S. psammophila* species group (which includes *S. ooldea*)

may be similarly thermally labile and as dependent upon torpor as *S. ooldea*, compared with members of the *S. macroura* species group (e.g. *S. crassicaudata* and *S. macroura*) that are not so dependent upon torpor or thermolability. Tight homeothermy is presumably ancestral, not derived, in the Sminthopsini. This allowed *S. macroura* and *S. crassicaudata* to expand into broad distributions, as it was selection for thermolability that allowed *S. ooldea* to adapt specifically to a hyper-arid distribution. Following the conclusions of Felsenstein (Felsenstein, 1985) and Garland and Adolph (Garland and Adolph, 1994) that two-species comparisons suggest associations that can only be 'proved' by multi-specific phylogenetic replication, broader interest in the physiology of a wider range of the Sminthopsini may provide great insight into the costs and benefits of maintaining homeothermy in ecosystems of varying productivity.

LIST OF ABBREVIATIONS

B_C	Bartlett's comparison of variance
BMR	basal metabolic rate
C_{dry}	dry thermal conductance
C_{wet}	wet thermal conductance
EHL	evaporative heat loss
EWL	evaporative water loss
MHP	metabolic heat production
MMR	maximum metabolic rate
MWP	metabolic water production
PRWE	point of relative water economy
RER	respiratory exchange ratio
RWE	relative water economy
SMR	standard metabolic rate
SNK	Student–Newman–Keuls *post hoc* test
T_a	ambient temperature
T_b	body temperature
TEWL	total evaporative water loss
TMR	torpid metabolic rate
TNZ	thermoneutral zone
$\dot{V}_{CO_2}$	rate of carbon dioxide uptake
$\dot{V}_{O_2}$	rate of oxygen uptake

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