The Journal of Experimental Biology 210, 2723-2729
Published by The Company of Biologists 2007
doi:10.1242/jeb.005009

Ventilation patterns in red kangaroos (*Macropus rufus* Desmarest): juveniles work harder than adults at thermal extremes, but extract more oxygen per breath at thermoneutrality

Adam J. Munn[1,*,†], Terence J. Dawson[1] and Shane K. Maloney[1,2]

[1]*School of Biological Science, University of New South Wales, Sydney, NSW, 2052, Australia and* [2]*Physiology: School of Biomedical, Biomolecular and Chemical Science, University of Western Australia, Perth, Western Australia*

*Author for correspondence (e-mail: a.munn@unswalumni.com)

†Present address: School of Biological Sciences, The University of Sydney, Sydney, NSW, 2006, Australia

Accepted 16 May 2007

Summary

Juvenile mortalities in large mammals are usually associated with environmental extremes, but the basis for this vulnerability is often unclear. Because of their high surface area to volume ratio, juveniles are expected to suffer greater thermal stresses relative to adults. Coping with thermal stress requires the ventilatory system to accommodate increases in oxygen demand and respiratory water loss at thermal extremes. Because juveniles are smaller than adults, these demands may set up different constraints on their ventilatory system. Using red kangaroos (*Macropus rufus* Desmarest), an arid zone species, we compared the ventilatory capabilities of juveniles and adults at thermoneutral (25°C) and extreme (–5°C and 45°C) ambient temperatures. We used an allometry to compare juvenile to adult ventilation, using predicted body mass scaling exponents for oxygen consumption (0.75), respiration rate (–0.25), tidal volume (1.0), ventilation rate (0.75) and oxygen extraction (0.0). At ambient 25°C, the juveniles' resting metabolic rate was 1.6 times that of the mature females (ml min^{-1} kg$^{-0.75}$), accommodated by significantly higher levels of oxygen extraction of 21.4±1.8% *versus* 16.6±1.9% (P<0.05). At thermal extremes, juveniles showed typical mammalian responses in their ventilation, mirrored by that of adults, including higher metabolic and ventilation rates at ambient –5°C and shallow panting at 45°C. However, at thermal extremes the juvenile kangaroos needed to work harder than adults to maintain their body temperature, with higher rates of ventilation at ambient –5°C and 45°C, accomplished *via* larger breaths at –5°C and higher respiratory rates at 45°C.

Key words: allometry, kangaroo, marsupials, juveniles, thermoregulation, ventilation.

Introduction

Red kangaroos (*Macropus rufus* Desmarest) are one of the largest extant marsupials and inhabit much of arid and semi-arid inland Australia. Their unpredictable environment is typified by stochastic rainfall and extremes of ambient temperature, ranging from below freezing at night to over 50°C diurnally (Dawson, 1995). The survival of juvenile red kangaroos in this environment is problematic and significant population recruitment occurs mainly after periods of high or extended rainfall. Such events provide juvenile kangaroos with access to the high-quality forage needed for their long-term growth and survival (Munn et al., 2006; Munn and Dawson, 2006). However, in addition to their requirement for high-quality forage, which occurs over a relatively long physiological time-scale (i.e. weeks to months), juvenile red kangaroos must be able to cope with the more immediate challenges of their environment. These challenges include high radiant heat loads, daily thermal extremes and scarce free water (Dawson, 1995).

The red kangaroos' reproductive strategy affords their offspring some protection from environmental extremes in the form of maternal care while the young develop inside the pouch, but only during the early stages of development. By 190 days old (d) and weighing around 2 kg, the young is fully furred and will venture out of the pouch for short periods. At this 'in–out' stage, the young kangaroo is still unable to maintain deep body temperature (T_b) for long periods, regularly returning to the pouch for warmth and safety (Frith and Sharman, 1964; Dawson, 1995). By 230–250 d, thermoregulation is well developed (Munn and Dawson, 2001) and the young kangaroo permanently leaves the pouch, becoming a young-at-foot (YAF; mass 4–5 kg). YAF red kangaroos forage in association with their mothers but also continue to take milk by putting their head into the pouch to access the same teat used during pouch-life. It is not for another 100 days or so that the YAF is fully weaned, at around 360 d and weighing 10–11 kg (Frith and Sharman, 1964; Dawson, 1995). Once young kangaroos have permanently left the mother's pouch they face the same environmental challenges as adults, but their smaller body size and high-energy requirements for growth (Munn and Dawson, 2001; Munn and Dawson, 2003) potentially impact on their ability to meet such thermal challenges.

Munn and Dawson showed that the energy and water requirements of YAF red kangaroos differ from those of adults (Munn and Dawson, 2001; Munn and Dawson, 2003). For example, at thermoneutral ambient temperatures (T_a) around 25°C, YAF red kangaroos had absolute rates of oxygen consumption (ml min^{-1}) that were 58% that of mature, non-lactating females, despite being only 30% of maternal body mass. To maintain heat balance at cold T_as, at –5°C, the absolute oxygen consumption (ml min^{-1}) of YAFs increased to 68% that of mature females (Munn and Dawson, 2001). In addition, YAF also had high water requirements at high T_a (~45°C), where juvenile kangaroos evaporated 2.5-times more water per unit body mass than mature females. These high evaporative water losses were achieved largely through elevated rates of respiratory evaporative cooling (i.e. panting) (Munn and Dawson, 2001). There is clearly a requirement for a larger relative capacity of the ventilatory system of the juvenile kangaroo compared to the adult. Therefore, we here explore the ventilatory physiology of juvenile red kangaroos by asking whether their ventilatory characteristics are similar to those of adults.

To evaluate the capabilities of the juvenile kangaroo ventilatory system, relative to adults, we used an allometric approach (Schmidt-Nielsen, 1984). That is, we compared the juvenile and adult red kangaroos' ventilation/respiratory patterns (including oxygen consumption) using the relevant body-mass exponents predicted from quarter-power scaling (West et al., 2000). The body mass (m) exponents that we used for oxygen consumption (ml min^{-1}; $m^{0.75}$), respiration rate (breaths min^{-1}; $m^{-0.25}$), tidal volume (ml; $m^{1.0}$), ventilation rate (ml min^{-1}; $m^{0.75}$) and oxygen extraction (%; $m^{0.0}$) are supported empirically from studies on both eutherians (Stahl, 1967) and marsupials (Dawson and Needham, 1981; Frappell and Baudinette, 1995; Withers et al., 2006). Because there is some controversy about exponent values for various parameters (Dodds et al., 2001; Kozlowski and Konarzewski, 2004; West and Brown, 2005; White and Seymor, 2005), we also present results using an alternative exponent of 0.67 for oxygen consumption and ventilation. We therefore compared allometrically adjusted data for the ventilation/respiration characteristics of juvenile and adult kangaroos at thermoneutral (25°C) and extreme T_as of –5°C and 45°C.

Materials and methods

Metabolism and ventilation

Experimental animals

Seven juvenile red kangaroos (five females and two males) were hand-reared. Four weeks prior to experimentation the animals were transferred to our laboratory at the University of New South Wales. During this time the kangaroos were familiarised with the experimental procedure and implanted intraperitoneally with temperature-sensitive radio transmitters (±0.1°C) (Sirtrack, Havelock North, Hawkes Bay, New Zealand), which had been calibrated (±0.1°C) against a certified mercury in glass thermometer (National Testing Authorities, Sydney, NSW, Australia). The kangaroos were maintained in pens (1.65×0.9×1.2 m) under a 12 h:12 h light:dark regime. Feed [Gordons rabbit pellets (Gordons Specialty Stock Feeds, Yanderra, NSW, Australia), Kangaroo cubes (Doust and Rabbidge, Forbes, NSW, Australia) and Lucerne/Bran mix (Kensington Stock Feeds, Sydney, NSW, Australia)] and water were available *ad libitum*. The diet of YAF kangaroos was supplemented with a ration of low lactose milk (Digestelact, Sharpe Laboratories, Sydney, Australia), the quantity of which was reduced over time until it was eliminated at normal weaning age. Juvenile red kangaroos on this diet regimen show growth and development patterns that are comparable to those raised on natural diets (Frith and Sharman, 1964; Sharman et al., 1964; Munn and Dawson, 2003). Food was withheld for 24 h prior to experimentation. During the major part of this study the juvenile red kangaroos were at an average age of 313±5 d, with a mass of 8.6±0.3 kg (mean ± s.e.m.).

Experimental procedure

For metabolic and ventilatory measurements, kangaroos were weighed to the nearest 0.1 kg (Salter Scales, Sydney, Australia) and placed in an open-circuit metabolism chamber (69.5×45×58 cm) within a temperature-controlled room. T_a was regulated to ±0.5°C of a set point and each animal was tested at T_as of –5°, 25° and 45°C. T_a was measured (±0.1°C) using a thermocouple placed in the excurrent port of the chamber. The metabolism chamber had a mesh floor above a bath of vegetable oil to trap excreta. The walls of the metabolism chamber were painted flat black to reduce radiation reflection (Porter, 1969; Maloney and Dawson, 1994). Experiments were performed between 08.00 and 16.00 h, corresponding to the resting phase of adult red kangaroo circadian rhythm (Watson and Dawson, 1993). Animals were monitored throughout each experiment *via* a low-light CCD camera (Oatley Electronics, Sydney, Australia) mounted inside the chamber.

After an animal was sealed into the chamber, at least 3 h was allowed for equilibration at any given temperature; data collection then commenced when the animal's body temperature had stabilised. Flow rate (FR) of dry air into the metabolism chamber was measured upstream using a Hastings Mass flowmeter (model HFM-201; John Morris Scientific, Sydney, Australia). Flow rate was adjusted to prevent water vapour pressure inside the chamber from exceeding 15 mmHg (1 mmHg=133.3 Pa). A sub-sample of air (125 ml min^{-1}) was drawn from the excurrent port using a flow controller (Ametek Applied Electrochemistry R2 flow controller; Pittsburgh, PA, USA) and passed through a capacitance type relative humidity (%RH) sensor (±0.1%) (CHK-Engineering, Sydney, Australia), which was calibrated regularly using saturated solutions of lithium chloride, sodium chloride and magnesium chloride (Winston and Bates, 1960). After leaving the humidity sensor, the excurrent air was dried with drierite, scrubbed of CO_2 with ascarite and re-dried before passing through an oxygen analyser (Ametek Applied Electrochemistry S3A-III).

Sensor outputs (FR, %RH, $\%O_2$) were logged on a personal computer at 5 s intervals using Warthog Labhelper software (Warthog Systems, University of California, Riverside, CA, USA) running a 12-bit analog/digital (A/D) converter (National Instruments Lab-NB card, North Ryde, NSW, Australia). This system averaged approximately 120 readings for each recorded value and gave a maximum resolution of 0.006% for $\%O_2$. The whole system was calibrated regularly using the iron-burn method (Young et al., 1984). Oxygen consumption

($\dot{V}_{O_2}$) (ml min^{-1}) was calculated as described in detail elsewhere (Munn and Dawson, 2001). At least 20 min of continuous minimal $\dot{V}_{O_2}$ during an exposure was used to determine the mean metabolic rate at each T_a.

The metabolism chamber acted as a whole-body plethysmograph, which allowed measurement of respiratory frequency (f_R; breaths min^{-1}) and tidal volume (V_T; ml) (Malan, 1973; Maloney and Dawson, 1994). Described in detail by Maloney and Dawson (Maloney and Dawson, 1994), plethysmography measures changes in the chamber air pressure caused by the humidification and warming/cooling of chamber air during inspiration. Chamber pressure was measured with a pressure transducer (PT-100; Sable Systems International, Las Vegas, NV, USA) and logged on a personal computer *via* an analog/digital converter. Data were logged at 0.05 s intervals at –5 and 25°C and at 0.03 s intervals at 45°C. The system was calibrated by injecting 115 ml of air into the chamber at the end of each experiment. Calibrations were repeated until the injection deflections were stable and matched the deflection kinetics exhibited by the respiring animal (Maloney and Dawson, 1994).

Data analysis

Tidal volume was estimated using equation 6 from Malan (Malan, 1973), assuming lung temperature was equal to T_b:

$$V_T = V_K \times \frac{\Delta P_T}{\Delta P_K} \times \left[\frac{P - P_{a,H_2O}}{(P - P_{a,H_2O}) - \left(\frac{T_a}{T_b}\right)} \times \frac{1}{P - P_{T_b}} \right], \quad (1)$$

where V_K=volume of calibration injection (115 ml); ΔP_T=change in chamber pressure with animal inspiration (volts); ΔP_K=change in chamber pressure with calibration injection (volts); P=chamber pressure (mmHg); P_{a,H_2O}=chamber water vapour pressure (mmHg); P_{T_b}=lung vapour pressure at T_b; and T_a and T_b are in degrees Kelvin. Vapour pressures were calculated from steam tables (Weast and Astle, 1982) using chamber relative humidity and assuming air in the lungs was saturated at T_b. Chamber pressure was estimated from barometric pressure corrected for chamber pressure relative to ambient air measured with a manometer connected to the chamber.

For each animal, V_T was established as the mean of five sets of respiratory traces taken throughout the 20-min period for minimal $\dot{V}_{O_2}$ at each T_a. V_T and respiratory minute volume (ventilation rate, $\dot{V}_I = V_T \times f_R$) was measured at body temperature and pressure, saturated, but converted to standard temperature and pressure, dry, for analysis.

Oxygen extraction (E_{O_2}), or the percentage of inspired oxygen ultimately used by the animal, was calculated as:

$$E_{O_2} = \frac{\dot{V}_{O_2}}{F_{I_{O_2}} \times V_T \times f_R} \times 100, \quad (2)$$

where $F_{I_{O_2}}$ is the fractional concentration of oxygen entering the chamber.

Data for juveniles were compared directly to that for adult red kangaroos obtained previously (Dawson et al., 2000a) (N=7). Mean mass of the adults was 23.5±1.1 kg.

Statistical analysis

The effect of temperature on ventilation in the YAF red kangaroos was assessed by comparing responses at T_as of –5°C, 25°C and 45°C using one-way repeated-measures ANOVA. Assumptions for ANOVA were tested by Levene's test for variances and Kolmogorov–Smirnov test for normality. To account for heterogeneity of variances in YAF, f_R, $\dot{V}_I$ and E_{O_2}, these data sets were $\log_{10}$ transformed (Zar, 1999). Allometrically adjusted data for the YAF and adult red kangaroos were compared at –5°, 25° and 45°C using a two-way repeated measures ANOVA. Assumptions for ANOVA were tested by Levene's test for variances and Kolmogorov–Smirnov test for normality. To account for heterogeneity of variances in the YAF *versus* adult f_R, $\dot{V}_I$ (for data using body-mass exponents of 0.67 or 0.75) and E_{O_2}, these data sets were $\log_{10}$ transformed (Zar, 1999). When ANOVA yielded significant differences, a Student–Newman–Keuls (SNK) test was performed to compare individual means. All statistical analyses were performed using Statistica 4.1 for Macintosh (Statsoft, Tulsa) and results are presented as means ± s.e.m.

Results

YAF kangaroos

Under cold (–5°C) and thermoneutral (25°C) T_as, the YAF had similar, stable T_bs ranging from 36.9°C to 37.4°C. At T_a –5°C, the YAF kangaroos' $\dot{V}_{O_2}$ was 2.6 times higher than it was under thermoneutral conditions at T_a 25°C (P<0.05, N=7; Table 1). The YAF accommodated their higher $\dot{V}_{O_2}$ at T_a –5°C by taking significantly deeper breaths (i.e. higher V_T) and more of them (i.e. higher f_R), leading to a 2.1 times higher $\dot{V}_I$ than at T_a 25°C (P<0.05, N=7; Table 1). The high $\dot{V}_{O_2}$ was also accommodated by a slight, though non-significant, rise in E_{O_2} of around 4.3% units (P=0.2; Table 1).

At 45°C, the YAF kangaroos were unable to maintain their T_b at the levels exhibited under thermoneutrality. Although stable, the YAF T_b at T_a 45°C was significantly higher than that at T_a 25°C, being 38.3±0.2°C (P<0.05, N=7; Table 1). At T_a 45°C, the YAF panted heavily, with respiratory rates 30 times higher than at T_a 25°C (Table 1). This was accompanied by significant reductions in V_T. There was also a significant reduction in E_{O_2} to around 1.5% at T_a 45°C, compared with 21% at T_a 25°C (P<0.05; Table 1). Notably, $\dot{V}_I$ in the YAF at T_a 45°C was 20 times that seen at thermoneutral T_as of 25°C (P<0.05; Table 1).

YAF versus adult red kangaroos

Using the exponents predicted from quarter-power scaling (West et al., 2000), we compared the YAF's ventilatory characteristics directly with those obtained from adult red kangaroos (Dawson et al., 2000a). The YAF red kangaroos showed respiratory responses to T_as of –5°, 25° and 45°C that were similar to those of mature females (Fig. 1) but at all T_as the YAF $\dot{V}_{O_2}$ (ml min^{-1} kg$^{-0.75}$) was around 1.6 times that of the mature females (P<0.05; Fig. 1A). At a thermoneutral T_a of 25°C, we found no significant difference between the YAF and adult red kangaroos' allometrically adjusted f_R (breaths min^{-1} kg$^{0.25}$; Fig. 1B), V_T (ml kg^{-1}; Fig. 1C) or $\dot{V}_I$ (ml min^{-1} kg$^{-0.75}$; Fig. 1D), although differences were apparent

Table 1. *Ventilatory characteristics for young-at-foot (YAF) red kangaroos at a range of ambient temperatures*

	Ambient temperature (°C)		
	–5.5±0.3	25.7±0.3	45.5±0.1
Mass (kg)	8.7±0.6[a]	7.2±0.4[a]	9.0±0.9[a]
T_b (°C)	37.4±0.2[a]	36.9±0.2[a]	38.3±0.2[b]
$\dot{V}_{O_2}$ (ml min^{-1})	128.9±5.8[a]	50.5±4.5[b]	68.2±6.8[c]
f_R (breaths min^{-1})	14.94±1.79[a]	11.14±0.89[b]	323.16±17.9[c]
V_T (ml)	168.6±10.9[a]	106.1±9.6[b]	73.4±8.8[c]
$\dot{V}_I$ (ml min^{-1})	2495±249[a]	1171±105[b]	23698±3064[c]
E_{O_2} (%)	25.7±1.90[a]	21.4±1.81[a]	1.47±0.15[b]

Values are means ± s.e.m.; values within rows and with different superscripts are significantly different (Student–Newman–Keuls test; P<0.05, N=7); see text for explanations of abbreviations.

at thermal extremes. The higher oxygen use by juveniles at 25°C compared to adults was accommodated by higher E_{O_2} (%), being 21.4±1.8% compared to 16.6±1.9% in the adults (P<0.05; Fig. 1E).

At cold T_as of –5°C, both the YAF and mature female red kangaroos significantly increased $\dot{V}_{O_2}$ (Fig. 1A) and $\dot{V}_I$ (Fig. 1D) by around 1.5 and 2.2 times, respectively, from thermoneutral levels. The YAF $\dot{V}_I$ at T_a –5°C was significantly higher than that of the mature females by a factor of 1.5 (Fig. 1D). This was associated with the YAF's V_T being 35% greater at T_a –5°C than it was at T_a 25°C (P<0.01), and it was also 40% higher than that of the mature females (P<0.05; Fig. 1C). Furthermore, the mature females did not significantly increase V_T at T_a –5°C compared with that at T_a 25°C (Fig. 1; P>0.05). Notably, the YAF and adult red kangaroos showed distinctly higher f_Rs at T_a –5°C compared with those seen at T_a 25°C, by between 7.4 and 4.5 breaths min^{-1} kg$^{0.25}$, respectively, and these differences showed a strong tendency for significance (P=0.06) in each case (see Fig. 1B).

At high T_as of around 45°C, well above the T_b of juvenile and adult red kangaroos, the $\dot{V}_I$ of YAF was over 20 times higher than at thermoneutral conditions and was over 1.9 times that of the mature females on an allometrically adjusted basis (Fig. 1D). There was no significant difference between the V_T of the YAF and the mature female kangaroos at 45°C (Fig. 1C). Therefore, the higher rate of $\dot{V}_I$ exhibited by the YAF at T_a 45°C compared with mature females (Fig. 1D) was due solely to the YAF's higher f_R (Fig. 1B), which exceeded 320 breaths min^{-1} on average (Table 1), compared with 150 breaths min^{-1} for the mature females.

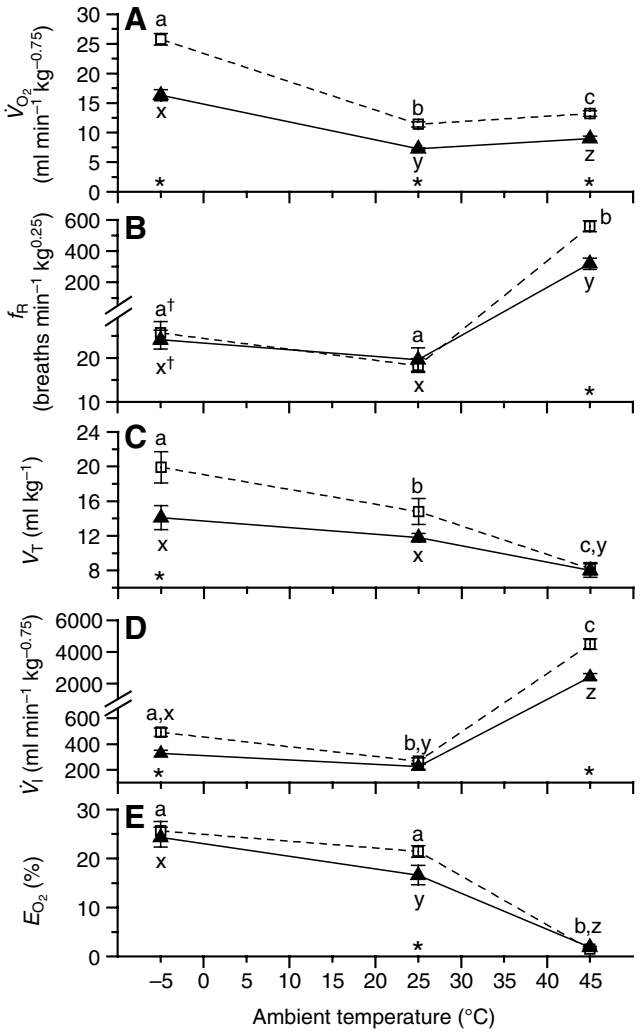

Fig. 1. (A) Oxygen consumption ($\dot{V}_{O_2}$), (B) respiratory rate (f_R), (C) tidal volume (V_T), (D) ventilation rate ($\dot{V}_I$) and (E) oxygen extraction (E_{O_2}) for young-at-foot (YAF; open squares; N=7) and mature female (filled triangles; N=7) red kangaroos at three different ambient temperatures. Letters denote significant differences (SNK; P<0.05) within each age class; a, b, c for the YAF, and x, y, z for the adult animals. Asterisks associated with the x-axis indicate significant differences between the YAF and adult red kangaroos at that ambient temperature (SNK, P<0.05). †Note: f_R within the YAF and adult data was not significantly different between ambient temperatures –5°C and 25°C, but they showed a strong tendency to be higher (P=0.06) within each age group (see text).

Discussion

Are juvenile red kangaroos just small adults?

Munn and Dawson (Munn and Dawson, 2001) found that the resting metabolic rate (ml O_2 min^{-1} kg$^{-0.75}$) of YAF red kangaroos was around 1.5 times that of fully mature, non-reproductive females, indicating that juvenile red kangaroos have intrinsically higher rates of energy turnover. We have re-iterated that finding here, placing our study of their ventilation patterns within the appropriate context. Juvenile mammals generally have higher basal metabolic rates than adults, due mainly to the extra energy turnover associated with growth (Robbins, 1993; Munn and Dawson, 2003). Here, we have shown that the YAF's higher $\dot{V}_{O_2}$ was not accommodated simply through higher f_R or even a higher overall $\dot{V}_I$ but was accommodated by a higher level of oxygen being extracted from each breath (i.e. a higher E_{O_2}; Fig. 1E). It is generally assumed that E_{O_2} is a fairly constant fraction of $\dot{V}_I$ across adult animals from different mammal groups, but we have shown that E_{O_2} can vary with animal age. This is reminiscent of the conclusion of Chappell et al. (Chappell et al., 2003) that E_{O_2} decreased with age in deer mice. Chappell's results (Chappell et al., 2003) were most likely due to senescence, but the higher E_{O_2}s reported here

for the very young animal deserves further investigation. Differences in E_{O_2} can arise from either changes in ventilation/perfusion matching or differences in the oxygen content of mixed venous blood because arterial blood is nearly 100% saturated under non-pathological conditions (Bartels, 1971; Withers, 1992). Thus, an increased E_{O_2} reflects either improved ventilation/perfusion matching or increased O_2 unloading from haemoglobin in tissues. Whichever the reason, it points to differences in the handling of oxygen with age in these large macropods.

Our red kangaroos showed typical mammalian responses to the higher O_2 demands imposed by cold T_as (Fig. 1A). Both the YAF and mature females increased $\dot{V}_{O_2}$ by around 2-fold at T_a –5°C compared with that at T_a 25°C. In both age groups, the higher $\dot{V}_{O_2}$ at T_a –5°C was met largely by increases in $\dot{V}_I$. In the adult animals, a significant increase in E_{O_2} also contributed (Fig. 1E), which is a typical mammalian response to cold exposure (Mortola and Frappell, 2000). That the YAF's E_{O_2} did not increase at T_a –5°C relative to that seen at T_a 25°C, as was the case for the adults, suggests that they may be operating near their maximum level of extraction even under standard conditions. Therefore, unlike adult kangaroos, the YAF would appear to have little reserve capacity in their E_{O_2} potential to ameliorate the extreme cold (Fig. 1E), and their higher $\dot{V}_{O_2}$ at T_a –5°C was met solely through increased $\dot{V}_I$ (Table 1). Notably, we were unable to push our juveniles (or adults) to the limits of their abilities, as indicated by the maintenance of constant T_bs at all T_as; further research is required to determine their maximum levels of E_{O_2} at even lower T_as or in response to exercise. Nonetheless, the higher $\dot{V}_I$ of the YAF at a T_a of –5°C, compared with that at T_a 25°C, was accomplished by an increase in V_T, while V_T of the adults remained unchanged between thermoneutral and cold T_as. Therefore, the juvenile kangaroos' ventilation system needed to work harder than that of adults at T_a –5°C in order to maintain T_b. Arid-zone kangaroos routinely face such cold conditions, particularly in winter when T_a regularly falls below freezing at night (http://www.bom.gov.au/climate/averages/ – date viewed 01/12/2006).

In addition to managing homeostasis during cold conditions, free-ranging red kangaroos often encounter particularly hot conditions. During summer months, for example, the average incident radiation across much of the red kangaroo's range exceeds 1000 W m^{-2} (Dawson, 1995), and they experience average daily T_as of 30 to >45°C (http://www.bom.gov.au/climate/averages/ – date viewed 01/12/2006). Munn and Dawson (Munn and Dawson, 2001) and Dawson et al. (Dawson et al., 2000a; Dawson et al., 2000b) found that both YAF and mature female red kangaroos, respectively, showed typical mammalian responses to high T_as of ~45°C. These principally included increases in evaporative cooling through both cutaneous (i.e. licking) and respiratory (i.e. panting) routes. Notably, panting was the major source of evaporative cooling under hot conditions for the adult red kangaroos, accounting for ~60% of total evaporative heat loss (Dawson et al., 2000b). Mature kangaroos do not sweat in response to thermal heat loads (although they do in response to exercise) (Dawson et al., 1974), and so the remaining portion of their evaporative heat loss at T_a 45°C can be attributed to insensible cutaneous evaporation and licking of the body surface, especially the forearms (Needham et al., 1974; Dawson et al., 2000b). Conversely, the YAF red kangaroos relied equally on cutaneous and respiratory evaporative cooling to maintain T_b at T_a 45°C (Munn and Dawson, 2001). We do not know if the YAF were sweating. Sweating in adult kangaroos occurs only during exercise, when T_b increases markedly. But if there is a threshold T_b above which sweating is initiated in resting kangaroos, then perhaps the YAF reached that T_b while the adults did not. Juvenile T_b reached 38.3°C while adults reached only 36.8°C at T_as around 45°C (see Munn and Dawson, 2001). By weaning age, when they had increased to a body mass of around 11–12 kg, the juveniles became more reliant on respiratory evaporative cooling at high T_as (Munn and Dawson, 2001), suggesting that the smaller YAF kangaroos' ventilatory system alone was not sufficient to support the higher rates evaporation required to maintain T_b, thereby necessitating the higher levels of cutaneous evaporation observed. The data presented here support this conclusion, and at a T_a of 45°C, well in excess of T_b, there were indications that the YAFs' ventilatory system was working harder than that of adults. In particular, at T_a 45°C, the YAF red kangaroos showed one of the highest f_Rs that we are aware of for any mammal, once body mass was taken into account. On an allometric basis, the fastest previously recorded f_Rs were 189 breaths min^{-1} kg$^{0.25}$ for an exercising 2 g Etruscan shrew (*Suncus etruscus*) (Jürgens et al., 1996) and 253 breaths min^{-1} kg$^{0.25}$ for the masked shrew (*Sorex cinereus*) (Morrison et al., 1959) (but see Jürgens et al., 1996). These values are less than half our measured f_R of 560 breaths min^{-1} kg$^{0.25}$ (i.e. 323 breaths min^{-1}; Table 1) for the heat-stressed YAF red kangaroos at an average body mass of 9 kg.

A note on allometry

In this study we have focussed on scaling exponents empirically derived for adult mammals (Stahl, 1967; Dawson and Needham, 1981; Frappell and Baudinette, 1995; Withers et al., 2006) and that are consistent with quarter-power scaling (West et al., 2000). However, we recognise that there is considerable controversy about the appropriate scaling exponent for metabolic rate in mammals (e.g. Dodds et al., 2001; Kozlowski and Konarzewski, 2004; West and Brown, 2005; White and Seymor, 2005). For example, a body-mass exponent of 0.67 has been suggested as more appropriate for both intra- and inter-specific comparisons (Heusner, 1991; White and Seymour, 2003). Choosing an appropriate exponent is further complicated when juvenile animals are considered (Wieser, 1984), but our objective was to examine whether juveniles were small adults in terms of ventilatory physiology (Munn and Dawson, 2001). We therefore chose an exponent of 0.75 for comparisons of $\dot{V}_{O_2}$ and $\dot{V}_I$, which is consistent with adult data (Stahl, 1967; Dawson and Needham, 1981; Frappell and Baudinette, 1995; Withers et al., 2006). Our results, however, were consistent both in pattern and implication when we compared $\dot{V}_{O_2}$s and $\dot{V}_I$s of the YAF and adult kangaroos using a body-mass exponent of 0.67 rather than 0.75 (Table 2). Specifically, using a scaling exponent for body mass of 0.67, we found that the YAFs' resting $\dot{V}_{O_2}$ (at T_a 25°C) was still 1.4 times that of adult animals (P<0.01; Table 2). We are unaware of alternative scaling exponents for V_T, f_R or E_{O_2}, but regardless of which body-mass exponent is used for $\dot{V}_{O_2}$ and $\dot{V}_I$, the

Table 2. *Oxygen consumption ($\dot{V}_{O_2}$) and ventilation ($\dot{V}_I$) by young-at-foot (YAF; N=7) and mature female (adult; N=7) red kangaroos at a range of ambient temperatures† and compared using a body-mass exponent of 0.67 (see Fig. 1 for comparisons with exponent 0.75)*

	Ambient temperature (°C)		
	–5.0	25.0	45.0
$\dot{V}_{O_2}$ (ml kg$^{-0.67}$ min^{-1})			
YAF	30.6±1.1[a,*]	13.4±0.5[b,*]	15.7±0.5[c,*]
Adult	21.0±1.3[a]	9.3±0.3[b]	11.6±0.6[b]
$\dot{V}_I$ (ml kg$^{-0.67}$ min^{-1})			
YAF	584±42[a,*]	312±29[b]	5364±430[c,*]
Adult	421±32[a]	290±31[b]	3130±259[c]

Values are means ± s.e.m.; values within rows and with different superscripts are significantly different (Student–Newman–Keuls test; $P<0.05$). Asterisks associated with YAF data denote significant differences between YAF and adults at that ambient temperature ($P<0.01$). †See Table 1 for measured ambient temperatures (T_a) for the YAF; respective adult data were collected at T_as –4.4±0.6, 25.2±0.7 and 44.8±0.9°C (Dawson et al., 2000a; Dawson et al., 2000b).

theoretical and measured body-mass exponent for E_{O_2} should remain constant at zero. In other words, regardless of the choice of exponent, our conclusion that the juvenile kangaroos meet higher resting oxygen consumptions by extracting more oxygen per breath is supported. Moreover, even with the more conservative body-mass exponent of 0.67, the YAF kangaroos had significantly higher $\dot{V}_{O_2}$s and $\dot{V}_I$s compared with those of adults at T_a –5°C and 45°C (Table 2).

Conclusions

There are two primary conclusions that can be drawn from this study. Firstly, in terms of their f_R, V_T and $\dot{V}_I$, the ventilatory system of juvenile red kangaroos at thermoneutral T_as was not significantly different from that expected for an adult marsupial of similar body mass. However, the YAF did have a higher E_{O_2} (%) at T_a 25°C, compared with adults, which explains how they meet their comparatively higher O_2 demands at this T_a. How this may be involved with active growth, which is usually cited as the main reason for juveniles having higher $\dot{V}_{O_2}$s, relative to adults (Robbins, 1993), is unknown. The second conclusion from this study is that, in the face of thermal extremes, the juvenile kangaroo ventilatory system must work considerably harder than that of adult animals to maintain heat balance. Overall, juvenile kangaroos appear more sensitive to extreme conditions, not only with respect to long-term stresses, such as food limitation (Munn and Dawson, 2006), but also to short-term extremes, such as severe cold or heat (present study) (Munn and Dawson, 2001). Short-term stress has been implicated in the mortalities of other large herbivores, such as caribou (*Rangifer tarandus*), where severe winter storms, rather than starvation, appeared to be the immediate cause of mortality in animals that were also exposed to long-term malnutrition (Dau, 2005). It is therefore important that these facets of juvenile mammal physiology be taken into account when considering the possible impacts of a changing climate. Currently, the bulk of models that evaluate species' extinction risks in relation to climate change often neglect the potential impacts on juvenile survival (e.g. Thomas et al., 2004; Humphries et al., 2004). Variation in juvenile survival, however, is particularly important for large mammalian herbivores from a range of climate and habitat types (Gaillard et al., 1998).

An Australian Research Council Postgraduate Award supported A.J.M. This research was in part supported by a grant from the Australian Research Council to T.J.D. Kangaroos were held under a licence from the New South Wales National Parks and Wildlife Service, and the University of New South Wales Animal Care and Ethics Committee gave approval for this project. Prof. Mark Chappell kindly provided Warthog Lab helper/analysist software for respirometry and plethysmography. Special thanks must go to the animal carers who participated in this project, especially Anne Williams.

References

Bartels, H. (1971). Blood oxygen dissociation curves: mammals. In *Respiration and Circulation* (ed. P. L. Altman and S. W. Dittmer), pp. 195-204, Bethesda, MD: Pergamon Press, Federation of the American Societies for Experimental Biology.

Chappell, M. A., Rezende, E. L. and Hammond, K. A. (2003). Age and aerobic performance in deer mice. *J. Exp. Biol.* **206**, 1221-1231.

Dau, J. (2005). Two caribou mortality events in Northwest Alaska: possible causes and management implications. *Rangifer* **16**, 37-50.

Dawson, T. J. (1995). *Kangaroos: Biology of the Largest Marsupials*. Sydney: University of New South Wales Press.

Dawson, T. J. and Needham, A. D. (1981). Cardiovascular characteristics of two resting marsupials: an insight into cardio-respiratory allometry in marsupials. *J. Comp. Physiol.* **145**, 95-100.

Dawson, T. J., Roberstshaw, D. and Taylor, C. R. (1974). Sweating in the kangaroo: a cooling mechanism during exercise, but not in the heat. *Am. J. Physiol.* **227**, 494-498.

Dawson, T. J., Munn, A. J., Blaney, C. E., Krockenberger, A. and Maloney, S. K. (2000a). 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*). *Physiol. Biochem. Zool.* **73**, 382-388.

Dawson, T. J., Blaney, C. E., Munn, A. J., Krockenberger, A. and Maloney, S. K. (2000b). 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*). *Physiol. Biochem. Zool.* **73**, 374-381.

Dodds, P. S., Rothman, D. H. and Weitz, J. S. (2001). Re-examination of the '3/4-law' of metabolism. *J. Theor. Biol.* **209**, 9-27.

Frappell, P. B. and Baudinette, R. V. (1995). Scaling of respiratory variables and the breathing pattern in adult marsupials. *Respir. Physiol.* **100**, 83-90.

Frith, H. J. and Sharman, G. B. (1964). Breeding in wild populations of the red kangaroo, *Megaleia rufa*. *Wildl. Res.* **9**, 86-114.

Gaillard, J., Festa-Bianchet, M. and Yoccoz, N. (1998). Population dynamics of large herbivores: variable recruitment with constant adult survival. *Trends Ecol. Evol.* **13**, 58-63.

Heusner, A. A. (1991). Size and power in mammals. *J. Exp. Biol.* **160**, 25-54.

Humphries, M. M., Umbanhowar, J. and McCann, K. S. (2004). Bioenergetic prediction of climate change impacts on northern mammals. *Integr. Comp. Biol.* **44**, 152-162.

Jürgens, K. D., Fons, R., Peters, T. and Sender, S. (1996). Heart and respiratory rates and their significance for convective oxygen transport rates in the smallest mammal, the Etruscan shrew *Suncus etruscus*. *J. Exp. Biol.* **199**, 2579-2584.

Kozlowski, J. and Konarzewski, M. (2004). Is West, Brown and Enquist's model of allometric scaling mathematically correct and biologically relevant? *Funct. Ecol.* **18**, 283-289.

Malan, A. (1973). Ventilation measured by body plethysmography in hibernating mammals and poikilotherms. *Respir. Physiol.* **17**, 32-44.

Maloney, S. K. and Dawson, T. J. (1994). 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. *J. Comp. Physiol. B* **164**, 473-481.

Morrison, P., Ryser, F. A. and Dawe, A. (1959). Studies on the physiology of the masked shrew *Sorex cinereus*. *Physiol. Zool*. **32**, 256-271.

Mortola, J. P. and Frappell, P. B. (2000). Ventilatory responses to changes in temperature in mammals and other vertebrates. *Annu. Rev. Physiol*. **62**, 847-874.

Munn, A. J. and Dawson, T. J. (2001). Thermoregulation in juvenile red kangaroo (*Macropus rufus*) after pouch exit: higher metabolism and evaporative water requirements. *Physiol. Biochem. Zool*. **74**, 917-927.

Munn, A. J. and Dawson, T. J. (2003). Energy requirements of the red kangaroos (*Macropus rufus*): impacts of age, growth and body size in a large desert-dwelling herbivore. *J. Comp. Physiol. B* **173**, 575-582.

Munn, A. J. and Dawson, T. J. (2006). Forage fibre digestion, rates of feed passage and gut fill in juvenile and adult red kangaroos (*Macropus rufus* Desmarest): why body size matters. *J. Exp. Biol.* **209**, 1535-1547.

Munn, A. J., Dawson, T. J. and Hume, I. D. (2006). Endogenous nitrogen excretion by red kangaroos (*Macropus rufus*): effects of animal age and forage quality. *Physiol. Biochem. Zool*. **79**, 424-436.

Needham, A. D., Dawson, T. J. and Hales, J. R. S. (1974). Forelimb blood flow and saliva spreading in the thermoregulation of the red kangaroo, *Megaleia rufa*. *Comp. Biochem. Physiol*. **49A**, 555-565.

Porter, W. P. (1969). Thermal radiation in metabolic chambers. *Science* **166**, 115-117.

Robbins, C. T. (1993). *Wildlife Feeding and Nutrition*. New York: Academic Press.

Schmidt-Nielsen, K. (1984). *Scaling: Why is Animal Size so Important?* Cambridge: Cambridge University Press.

Sharman, G. B., Frith, H. J. and Calaby, J. H. (1964). Growth of the pouch young, tooth eruption and age determination in the red kangaroo, *Megaleia rufa*. *Wildl. Res.* **9**, 20-49.

Stahl, W. R. (1967). Scaling of respiratory variables in mammals. *J. Appl. Physiol*. **22**, 453-460.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F. N., de Siqueira, M. F., Grainger, A., Hannah, L. et al. (2004). Extinction risk from climate change. *Nature* **427**, 145-148.

Watson, D. M. and Dawson, T. J. (1993). The effects of age, sex, reproductive status and temporal factors on the time-use of free-ranging red kangaroos (*Macropus rufus*) in western New South Wales. *Wildl. Res*. **20**, 785-801.

Weast, R. C. and Astle, M. J. (1982). *CRC Handbook of Chemistry and Physics*. Boca Raton, FL: CRC Press.

West, G. B. and Brown, J. (2005). The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. *J. Exp. Biol.* **208**, 1575-1592.

West, G. B., Brown, J. H. and Enquist, B. J. (2000). The origin of universal scaling laws in biology. In *Scaling in Biology* (ed. J. H. Brown and G. B. West), pp. 87-112. New York: Oxford University Press.

White, C. R. and Seymour, R. S. (2003). Mammalian basal metabolic rate is proportional to body mass 2/3. *Proc. Natl. Acad. Sci. USA* **100**, 4046-4049.

White, C. R. and Seymour, R. S. (2005). Allometric scaling of mammalian metabolism. *J. Exp. Biol.* **208**, 1611-1619.

Wieser, W. (1984). A distinction must be made between the ontogeny and the phylogeny of metabolims in order to understand the mass exponent of energy metabolism. *Respir. Physiol*. **55**, 1-9.

Winston, P. W. and Bates, D. H. (1960). Saturated solutions for the control of humidity in biological research. *Ecology* **41**, 232-237.

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

Withers, P. C., Cooper, C. E. and Larcombe, A. N. (2006). Environmental correlates of physiological variables in marsupials. *Physiol. Biochem. Zool*. **79**, 437-453.

Young, B. A., Fenton, T. W. and McLean, J. A. (1984). Calibration methods in respiratory calorimetry. *J. Appl. Physiol.* **56**, 1120-1125.

Zar, J. H. (1999). *Biostatistical Analysis* (4th edn). Sydney: Prentice-Hall.