TECHNICAL COMMENT

Using A Priori Contrasts for Multivariate Repeated-Measures ANOVA to Analyze Thermoregulatory Responses of the Dibbler (*Parantechinus apicalis*; Marsupialia, Dasyuridae)

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ABSTRACT

Physiological studies often involve the repeated measurement of individuals over a range of ordered categorical conditions, for example, varying ambient temperature. We illustrate here the use of a priori contrasts for multivariate repeated-measures ANOVA by analyzing the thermal responses of various physiological variables for a small marsupial, the dibbler (*Parantechinus apicalis*). Our analyses showed that dibblers conform closely to the Scholander-Irving model of endothermy. Body temperature was constant at low air temperatures, was $36.3 \pm 0.24°C$ at thermoneutrality (30°C), and increased at 35°C. Metabolic rate decreased with increasing ambient temperature to a basal rate of 0.619 ± 0.036 mL O_2 g^{-1} h^{-1} at 30°C; it extrapolated closely to thermoneutral body temperature. Increased oxygen demand at lower ambient temperature was met by increased respiratory minute volume, achieved by increased respiratory frequency and tidal volume; oxygen extraction was constant at about 19%. Evaporative water loss and wet and dry thermal conductance increased markedly at high ambient temperatures but not sufficiently to maintain constant body temperature. Relative water economy was similar to that of other small marsupials, increasing linearly at lower air temperatures with a point of relative water economy of 20.3°C. We conclude that a priori contrasts provide a statistically appropriate and powerful analysis that can be used routinely to statistically describe the pattern of response of

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physiological variables to a categorical factor and are especially useful for repeated-measures ANOVA designs common to many physiological studies.

Introduction

A priori contrasts provide a powerful tool to evaluate expected patterns with a categorical factor for ANOVA (Cohen 2008), univariate repeated-measures ANOVA (Potvin et al. 1990; Park et al. 2009), and multivariate repeated-measures ANOVA (Rencher 2002), because they examine specific hypotheses using an appropriate contrast matrix (e.g., Bock 1975; Hand and Taylor 1987). For example, a simple contrast tests whether a control category is different from each other category. For ordered categorical data, a Helmert contrast tests whether a category is different from all subsequent categories pooled together; a reverse-Helmert contrast tests whether a category is different from all previous categories pooled. For ordered numeric categories (e.g., ambient temperature T_a measured in °C), a polynomial contrast examines whether there is a linear, quadratic, or other polynomial relationship. A priori contrasts can be more powerful than the overall ANOVA hypothesis (that all means are equal) because they address more specific hypotheses than general post hoc tests and because they address fewer specific uncorrelated hypotheses (e.g., experimental groups differ from a control group, or there is a linear relationship for ordered categories). However, contrast tests have not been widely embraced by comparative physiologists despite their usefulness for examining specific patterns in physiological data sets and especially their particular value in overcoming the issue of an absence of post hoc tests for repeated-measures ANOVA.

Many physiological studies involve the repeated measurement of individuals subjected to different categorical treatments on an ordered scale, for example, measurement of a physiological variable at different ambient temperatures. Multivariate repeated-measures analysis of variance (MANOVAR) is an appropriate statistical analysis for such data, with the repeated measure (e.g., T_a) being the multivariate factor (Potvin et al. 1990; Rencher 2002; Park et al. 2009). However, a major limitation of MANOVAR is that it does not have formal comparison tests equivalent to ANOVA post hoc tests (e.g. Tukey, Dunnett, Student-Newman-Keuls, etc). Consequently, it is difficult to statistically discern the pattern of the effect of a sig-

nificant repeated factor (e.g., the pattern of a T_a effect for a physiological variable) within the MANOVAR model. The recent physiological literature includes various approaches to this problem. Some repeated-measures studies simply do not evaluate the pattern of the repeated measure (e.g., Beaupre and Zaidan 2001; Gomez et al. 2006), and some use a Bonferroni-type adjustment for multiple comparison tests (e.g., Rezende et al. 2004; Berg and Biewener 2008; Park et al. 2009; Wu et al. 2009; Dunlap et al. 2010) or post hoc ANOVA tests that do not account for repeated measurements (e.g., McLean and Speakman 2000; Gilmour et al. 2001; Zerm et al. 2004; Zhao and Cao 2009; Dupont-Prinet et al. 2010).

We illustrate here the use of a priori contrasts, by examining the thermoregulatory responses of the dibbler (*Parantechinus apicalis*), a 40–100-g endangered dasyurid marsupial characterized by a white eye ring and freckled fur (Woolley 2008). The dibbler's current distribution is restricted to three small islands near Jurien, Western Australia, and a few scattered mainland sites on the south coast of Western Australia, where it inhabits dense heath on sandy soils (Menkhorst and Knight 2004; Cronin 2008; Woolley 2008). We characterize body temperature, metabolic rate, thermal conductance, respiratory ventilation, evaporative water loss, and relative water economy over a range of ambient temperatures and then examine the various patterns of T_a effects below and above thermoneutrality using a priori contrasts and MANOVAR (Rencher 2002), based on the typical responses of endotherms (e.g., McNab 1980; Withers 1992) and more specifically of other dasyurid marsupials (e.g., Dawson and Wolfers 1978; Morton and Lee 1978; Geiser 1986; Geiser and Baudinette 1988; Hallam and Dawson 1993; Larcombe 2002; Schmidt et al. 2009; Cooper and Withers 2010; Warnecke et al. 2010). We use polynomial contrasts to examine linear changes with T_a, as might be expected for metabolic rate (MR), ventilatory variables including respiratory rate (f_R), tidal volume (V_T) and minute volume (V_I), and possibly evaporative water loss (EWL). We use simple contrasts to compare thermoneutrality with the other T_a treatments and Helmert and reverse-Helmert contrasts to identify a pattern of relative constancy but an increase at low or high T_a, as might be expected for body temperature (T_b), wet (C_{wet}) and dry (C_{dry}) thermal conductance, and possibly EWL.

Material and Methods

Animals

Eight dibblers (four male, four female) were studied at Perth Zoo, Western Australia (31°58′S, 115°51′E), during August/September 2009. One individual was wild caught at Fitzgerald River National Park (34°4′S, 119°25′E), and the others were captive-born descendants of other individuals from this location. Dibblers were housed indoors in individual cages with a natural photoperiod and were fed a diet of minced meat, ground cat biscuits, boiled eggs, and Wombaroo small-carnivore food, along with baby rats, crickets, and mealworms, with water ad lib.

Respirometry

Metabolic rate (oxygen consumption, Vo_2; carbon dioxide production, Vco_2) and EWL were measured by flow-through respirometry for individuals that had been fasted for 20 h before the commencement of experiments. Dibblers were measured for 5–9 h at each T_a during their inactive phase (day) until Vo_2, Vco_2, and EWL were stable and minimal; individuals were measured at only one T_a per day and not more frequently than every 3 days. Observations of dibblers in the metabolic chamber by video indicated when dibblers were resting and asleep; these periods were easily identified in the continuous traces of physiological data and used for analysis, so we are confident that our values are for resting, inactive dibblers. T_b was measured when dibblers were removed from the chamber at the end of the experiment, using a plastic-tipped thermocouple (connected to a Radio Spares 611–234 thermocouple meter) inserted ∼1.5 cm into the cloaca.

Mass flow controllers (Aalborg GFC17 and Cole-Palmer 32708-26) regulated the flow of ambient air at a constant rate of 500–700 mL min^{-1} through cylindrical glass chambers (500 mL), located in a temperature-controlled cabinet. Individuals were measured at each ambient temperature (T_a: 9.4°, 15.3°, 19.9°, 25.3°, 30.8°, and 35.3°C) in random order for independence of data from the order of measurement. Excurrent air passed over a thin-film-capacitance relative humidity (RH) and T_a probe (Vaisala HMP45A), with a subsample passing through a column of Drierite (W. A. Hammond Drierite) and then an oxygen analyzer (Sable Systems FoxBox or Servomex 574) and a carbon dioxide analyzer (Sable Systems FoxBox or CA-2A). The data were recorded by a PC every 20 s throughout the experimental period using custom-written Visual Basic (v6) software, via the serial port of the Sable Systems FoxBox or a Pico Technology ADC11 A/D converter. A custom-written Visual Basic program was used for metabolic and hygric calculations (after Withers 2001).

Ventilatory data were measured by whole-body plethysmography (Malan 1973; Withers 1977; Szewczak and Powell 2003), whereby pressure changes in the metabolic chamber due to respiration were detected with a custom-made pressure transducer (Motorola MPX2010 sensor). The voltage output from the pressure transducer was monitored using a Pico Technology ADC11 A/D converter and was recorded on a PC every 15 ms for approximately 30 s using PicoScope. Between two and six sets of ventilatory data were obtained for an individual dibbler at each T_a, and a single mean was calculated for each ventilatory variable for each individual at each T_a. Ventilatory measurements were made toward the end of each experiment, when a low stable metabolic rate and video observation confirmed that the dibbler was quiet and resting. A custom-written Visual Basic program was used for ventilatory calculations (after Malan 1973; Szewczak and Powell 2003). Ventilatory variables are presented at body temperature and pressure saturated (BTPS) conditions. Oxygen extraction (Eo_2) was calculated using the standard temperature and pressure dry (STPD) values for Vo_2 and V_I at the time of ventilatory measurements, using

a fractional inspired oxygen content calculated as the mean of 0.2095 and the measured fractional excurrent oxygen at the time of ventilatory measurements.

The FoxBox oxygen analyzer was two-point calibrated by its electronic zero function and dry ambient air (20.95%) and the Servomex with compressed N_2 and dry ambient air. Carbon dioxide analyzers were calibrated with compressed N_2 and a certified gas mix (0.53% CO_2). The calibration of the relative humidity probes was confirmed using 1% RH air (dried with Drierite) and 100% RH air (saturated; by breathing on the RH probe). Flowmeters were calibrated using a bubble flowmeter (Bubble-O-Meter). The plethysmography system was calibrated by injecting known air volumes (0.3–0.5 mL) into the chamber and then accounting for the pressure decay characteristics (i.e., mathematically converting the open-system measurement to a closed system; see Szewczak and Powell 2003).

Respiratory exchange ratio (RER) was calculated as V_{CO_2}/V_{O_2}, and C_{wet} (J g^{-1} h^{-1} $°C^{-1}$) was calculated from metabolic heat production (MHP; converted from MR using the measured RER, by interpolation from Table 4.2 in Withers 1992) as MHP/$(T_b - T_a)$. C_{dry} (J g^{-1} h^{-1} $°C^{-1}$) was calculated as (MHP $-$ EHL)/$(T_b - T_a)$, where evaporative heat loss (EHL) waw converted from EWL assuming 2.4 J mg^{-1} H_2O (McNab 2002). Metabolic water production (MWP; mg g^{-1} h^{-1}) was also calculated from MR using the measured RER after Withers (1992). Relative water economy (RWE) was calculated as MWP/EWL and the point of relative water economy (PRWE) as the T_a at which RWE interpolated to 1.

Statistics

Values are presented as mean $\pm$ standard error, with $N = 8$ individuals. Basic statistical analyses were conducted with statistiXL (v1.8). Effects of T_a on physiological variables were examined using MANOVAR, with the eight dibblers as replicates and T_a as the repeated measure (Rencher 2002). We examined various a priori contrasts, depending on the expected T_a pattern, including linear, quadratic, and cubic polynomial contrasts, simple contrasts for comparing a specified group with each other group, and Helmert and reverse-Helmert contrasts for sequential comparisons of categories with all previous or subsequent T_a categories.

The t statistic for an a priori contrast was calculated for each row of the contrast matrix (C) as

$$t_i = \frac{n^{0.5}\mathbf{c}_i^T\bar{\mathbf{y}}}{(\mathbf{c}_i^T\mathbf{S}\,\mathbf{c}_i)^{0.5}},$$

where n is the number of subjects, $\mathbf{c}_i$ is a row and $\mathbf{c}_i^T$ is the transpose of a row of the contrast matrix, $\bar{\mathbf{y}}$ is a vector consisting of the means for each repeated category, and $\mathbf{S}$ is the covariance matrix (which can be obtained from various statistical procedures in many statistics packages, e.g., from principal component analysis in statistiXL); the test statistic has $n - 1$ degrees of freedom (Rencher 2002). Contrast tests were accomplished in an Excel spreadsheet using a custom-written a priori contrast

macro, based on Rencher (2002); this spreadsheet is available in an Excel file in the online edition of *Physiological and Biochemical Zoology*. Simple, Helmert and reverse-Helmert contrast matrices were calculated after Bock (1975) and Hand and Taylor (1987). Polynomial contrast matrices were calculated for unequally spaced repeated measures (Robson 1959). The contrast matrices for our data set with $T_a = 9.4°$, 15.3°, 19.9°, 25.3°, 30.8°, and 35.3°C are shown in Table 1.

Results

Mean body mass of the dibblers was 78.3 ± 5.1 g ($N = 8$). There were mass differences between individuals (range 63.5–99.3 g; $F_{7,40} = 204$, $P < 0.001$), but there was no change in mass of individuals over the five measurements ($F_{5,3} = 6.25$, $P = 0.081$).

Observations of dibblers in the metabolic chamber indicated that they rested and appeared to be asleep for most of the time, except for occasional short periods of activity (e.g., grooming) that corresponded to increased V_{O_2}, V_{CO_2} and EWL. The dibblers were curled up with erect fur at low T_a, and their fur was more depressed and posture more stretched out as T_a increased. None of the dibblers entered torpor, defined as $V_{O_2} < 75\%$ of basal metabolic rate (BMR) at any T_a (Geiser and Baudinette 1988).

Dibblers' T_b ranged from $36.3° \pm 0.2°C$ at $T_a = 9°C$ to $37.7° \pm 0.2°C$ at $T_a = 35°C$ (Fig. 1). Individuals did not differ with respect to T_b ($F_{7,40} = 0.84$, $P = 0.558$), but there was a significant effect of T_a ($F_{5,3} = 13.1$, $P = 0.030$). Excluding the $T_a = 35°C$ data removed the T_a effect ($F_{4,4} = 0.316$, $P = 0.855$), suggesting a pattern of higher T_b at $T_a = 35°C$ compared with all other T_a's. A priori contrasts supported this pattern. Linear ($P = 0.002$), quadratic ($P = 0.046$), and cubic contrasts ($P = 0.015$) were significant when $T_a = 35°C$ data were included, but not when $T_a = 35°C$ data were excluded ($P \geq 0.379$), indicating that the $T_a = 35°C$ data differed from T_b at the other T_a's. Similarly, Helmert contrasts were significant only for comparison of $T_a = 31°C$ with $T_a = 35°C$ ($P = 0.004$), and the only significant reverse Helmert contrast was for comparison of $T_a = 35°C$ with all of the lower T_a's ($P < 0.001$). The thermolability of T_b ($\Delta T_b/\Delta T_a$) was $-0.00042°C$ $°C^{-1}$ from $T_a = 9°$ to 31°C (MANOVAR linear contrast); it was $+0.283° \pm 0.067°C$ $°C^{-1}$ from $T_a = 31°$ to 35°C.

The V_{O_2} of dibblers decreased from 2.65 ± 0.12 mL O_2 g^{-1} h^{-1} at $T_a = 9°C$ to a minimum of 0.619 ± 0.036 mL O_2 g^{-1} h^{-1} ($n = 8$) at $T_a = 31°C$ (Fig. 1); we consider this minimal value at 31°C to be BMR. There were no differences in V_{O_2} between individuals ($F_{7,40} = 0.131$, $P = 0.995$), but there was a significant effect of T_a ($F_{5,3} = 58.2$, $P = 0.004$). The pattern of V_{O_2} with T_a (Fig. 1) suggests that V_{O_2} decreased linearly with increasing T_a but increased at $T_a = 35°C$. Polynomial a priori contrasts confirmed this pattern. Linear ($P < 0.001$), quadratic ($P = 0.001$), and cubic ($P = 0.029$) polynomials were significant when $T_a = 35°C$ data were included, but there was only a significant linear pattern ($P < 0.001$) when $T_a = 35°C$ data were omitted ($P \geq 0.475$ for quadratic and cubic contrasts),

Table 1: Contrast matrices used for a priori comparison tests of dibbler physiological variables at ambient temperatures (T_a's) of 9.4°, 15.3°, 19.9°, 25.3°, 30.8°, and 35.3°C

Contrast	T_a (°C)					
	9.4	15.3	19.9	25.3	30.8	35.3
Simple	1	0	0	0	−1	0
	0	1	0	0	−1	0
	0	0	1	0	−1	0
	0	0	0	1	−1	0
	0	0	0	0	−1	1
Helmert	1	−1/5	−1/5	−1/5	−1/5	−1/5
	0	1	−1/4	−1/4	−1/4	−1/4
	0	0	1	−1/3	−1/3	−1/3
	0	0	0	1	−1/2	−1/2
	0	0	0	0	1	−1
Reverse Helmert	−1	1	0	0	0	0
	−1/2	−1/2	1	0	0	0
	−1/3	−1/3	−1/3	1	0	0
	−1/4	−1/4	−1/4	−1/4	1	0
	−1/5	−1/5	−1/5	−1/5	−1/5	1
Polynomial:						
Linear	−13.3	−7.4	−2.8	2.6	8.1	12.6
Quadratic	92.4	−27.0	−71.9	−70.5	−9.1	86.1
Cubic	−379.9	594.5	288.6	−403.0	−547.6	447.4

Note.   Contrasts: simple compares a specified category with all other categories (specified group is 30.8°C), Helmert tests whether a category is different from all subsequent categories pooled together, reverse Helmert tests whether a category is different from all previous categories pooled, and polynomial test is for an ordered pattern such as a linear or quadratic relationship for a variable with categories on a numeric scale.

indicating that $\dot{V}o_2$ decreased linearly with T_a to 31°C; it did not increase significantly at $T_a = 35°C$ (simple contrast $P = 0.128$). The linear relationship (excluding $T_a = 35°C$) was $\dot{V}o_2 = 3.44 − 0.095T_a$, which extrapolates to a predicted T_b of 36.2°C. The pattern in $\dot{V}co_2$ was similar to that of $\dot{V}o_2$ and is therefore not analyzed or presented separately. The RER was independent of individual ($F_{7,40} = 1.34$, $P = 0.258$) and T_a ($F_{5,3} = 5.88$, $P = 0.088$), at 0.72 ± 0.015 (mean for all 6 T_a's).

Dibblers' C_{wet} varied little from $T_a = 9°C$ (1.93 ± 0.083 J g^{-1} h^{-1} °C^{-1}) to 25°C (1.77 ± 0.085 J g^{-1} h^{-1} °C^{-1}), then increased slightly at 31°C (2.27 ± 0.15 J g^{-1} h^{-1} °C^{-1}) and substantially at 35°C to 6.51 ± 0.70 J g^{-1} h^{-1} °C^{-1} (Fig. 1). There was no difference in C_{wet} between individuals ($F_{7,40} = 0.099$, $P = 0.998$), but there was a significant effect of T_a ($F_{5,3} = 130$, $P = 0.001$). There were highly significant linear, quadratic, and cubic patterns ($P < 0.001$) for all T_a's but no linear ($P = 0.200$), quadratic ($P = 0.060$), or cubic effects ($P = 0.140$) for $T_a \leq 31°C$. This pattern was supported by reverse Helmert contrasts being significant for C_{wet} only at 35°C ($P < 0.001$) and 31°C ($P = 0.037$) compared to lower T_a's, and significant Helmert contrasts for all T_a's ($P < 0.0012$). C_{dry} was constant from $T_a = 9°C$ (1.84 ± 0.081 J g^{-1} h^{-1} °C^{-1}) to 31°C (1.89 ± 0.135 J g^{-1} h^{-1} °C^{-1}) and then increased substantially at $T_a = 35°C$ to 4.74 ± 0.56 J g^{-1} h^{-1} °C^{-1} (Fig. 1). There was no difference in C_{dry} between individuals ($F_{7,40} = 0.148$, $P =$

0.993), but there was a significant effect of T_a ($F_{5,3} = 154$, $P < 0.001$). As for C_{wet}, there were highly significant linear, quadratic, and cubic effects ($P < 0.003$) for all T_a's but no linear, quadratic, or cubic effects ($P > 0.186$) for $T_a \leq 31°C$. This pattern was supported by the reverse Helmert contrast being significant only for C_{dry} at 35°C compared to lower T_a ($P = 0.002$) and significance for all Helmert contrasts of C_{dry} for all T_a's ($P < 0.003$) at 35°C compared to lower T_a's ($P < 0.003$).

Individual dibblers did not differ with respect to EWL ($F_{7,40} = 1.63$, $P = 0.155$) and there was no overall T_a effect by MANOVAR ($F_{5,3} = 4.07$, $P = 0.139$). However, examination of the pattern of EWL with T_a suggests that EWL decreased from $T_a = 9°$ to 31°C, then increased to the highest value of 1.65 ± 0.12 mg H$_2$O g^{-1} h^{-1} at 35°C (Fig. 2). A priori contrasts indicated a significant linear ($P = 0.043$), quadratic ($P = 0.001$) and cubic ($P = 0.003$) effect over all T_a's but only a significant linear effect ($P = 0.016$) if $T_a = 35°C$ was excluded. This suggests a negative linear effect of T_a on EWL from 9°C (1.10 ± 0.10 mg g^{-1} h^{-1}) to 31°C (0.86 ± 0.065 mg g^{-1} h^{-1}) and a marked increase in EWL at $T_a = 35°C$. The linear relationship (excluding $T_a = 35°C$) was EWL = 1.31 − 0.017T_a. EWL was also significantly higher at $T_a = 35°C$ than all lower T_a's (simple and reverse Helmert contrasts, $P < 0.001$) and EWL at 20°, 25° and 31°C was lower than at 35°C by Helmert contrasts ($P < 0.037$).
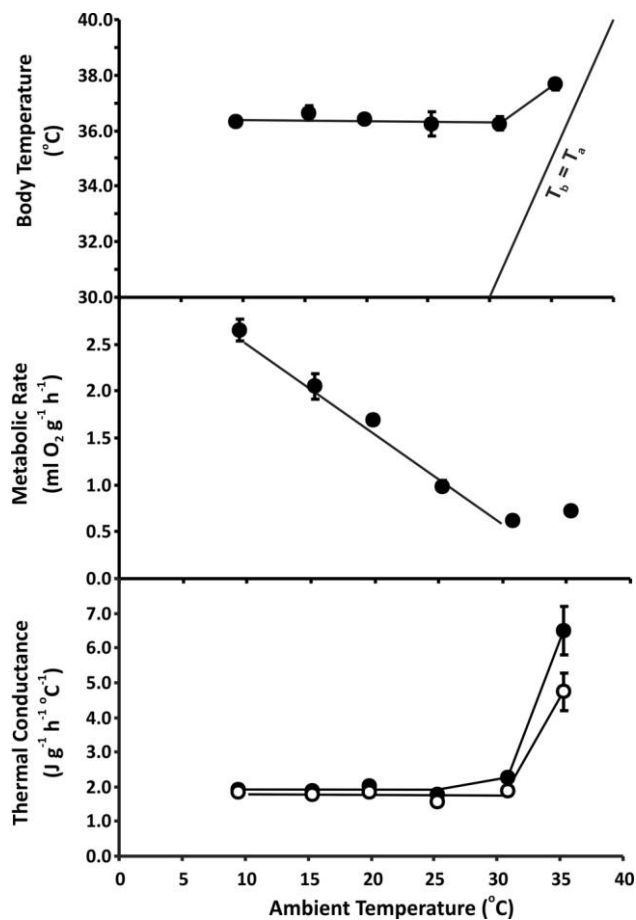
Figure 1. Body temperature, metabolic rate, and wet (black symbols) and dry (white symbols) thermal conductance of dibblers (*Parantechinus apicalis*) at a range of ambient temperatures. Solid lines indicate patterns supported by a priori contrast analysis (see text). Values are mean ± standard error; $N = 8$.

RWE (Fig. 2) decreased significantly with T_a ($F_{5,3} = 34.2$, $P = 0.008$), from 1.45 ± 0.13 at $T_a = 9°C$ to 0.26 ± 0.04 at 35°C but did not differ between individuals ($F_{7,40} = 0.501$, $P = 0.828$). The pattern was highly linear for all T_a's ($P < 0.001$) but not quadratic or cubic ($P > 0.085$); RWE $= 1.92 - 0.052\ T_a$, and PRWE $= 20.3°C$.

There was a significant effect of T_a on f_R, V_T, and V_I (Fig. 3; $F_{5,3} \geq 10.5$, $P \leq 0.041$), but not on Eo_2 ($F_{5,3} = 0.552$, $P = 0.738$), with no differences between individual dibblers for any respiratory variable ($F_{7,40} \leq 1.70$, $P \geq 0.138$). Contrasts indicated linear ($P < 0.001$) and quadratic ($P = 0.023$) components for the effect of all T_a's on f_R but only a linear component when $T_a = 35°C$ was excluded ($f_R = 105 - 2.59\ T_a$; $P < 0.001$); f_R increased significantly from 31° to 35°C (simple contrast $P = 0.018$). V_T decreased linearly from $T_a = 9.4°C$ (2.10 ± 0.259 mL) to $T_a = 35°C$ (1.01 ± 0.55 mL); $V_T = 2.46 - 0.044\ T_a$ ($P = 0.003$) for all T_a's, with no significant quadratic effect ($P = 0.790$). For V_I there was a significant ($P < 0.001$) linear decline from 9°C (166.4 ± 25.5 mL min^{-1}) to 31°C ($27.7 \pm$

2.09 mL min^{-1}) for both $T_a \leq 31°C$ and $T_a \leq 35°C$ but no significant quadratic or cubic contrasts ($P > 0.071$). For $T_a \leq 31°C$, $V_I = 196 - 5.49\ T_a$. V_I was not significantly different between 31° and 35°C (simple contrast $P = 0.227$). There were no significant polynomial contrasts for Eo_2 ($P > 0.350$) and no decrease in Eo_2 at $T_a = 35°C$ compared to the lower T_a's (reverse Helmert $P = 0.440$); mean Eo_2 was $19.3\% \pm 0.5\%$ over all T_a's.

Discussion

This study of the thermal, metabolic, hygric, and ventilatory physiology of the dibbler follows a repeated-measures experimental design that is common in physiological (and many other) studies, that is, the measurement of a parameter (e.g., metabolic rate) repeated for the same individuals over a range of ordered categorical experimental conditions (e.g., T_a). We demonstrate here the power of a priori contrasts in a multivariate repeated-measures analysis in the absence of post hoc tests, by examining specific hypotheses for thermal patterns in the first physiological data available for dibblers. This approach enables us to describe the presence as well as statistically confirming the pattern of physiological responses with T_a for this endangered dasyurid marsupial.

MANOVAR consistently found significant effects for those physiological variables that we anticipated would be influenced by T_a, that is, T_b, Vo_2, C_{wet}, C_{dry}, RWE, f_R, V_T, and V_I. An overall T_a effect was insignificant for EWL and Eo_2, but a T_a effect is
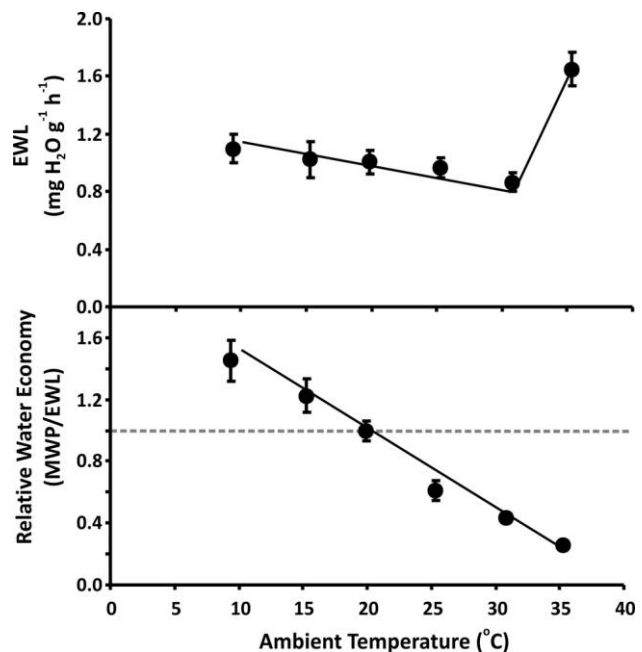

Figure 2. Evaporative water loss and relative water economy (metabolic water production/evaporative water loss) of dibblers (*Parantechinus apicalis*) at a range of ambient temperatures. Solid lines indicate patterns supported by a priori contrast analysis (see text). The dashed line indicates relative water economy = 1. Values are mean ± standard error; $N = 8$.
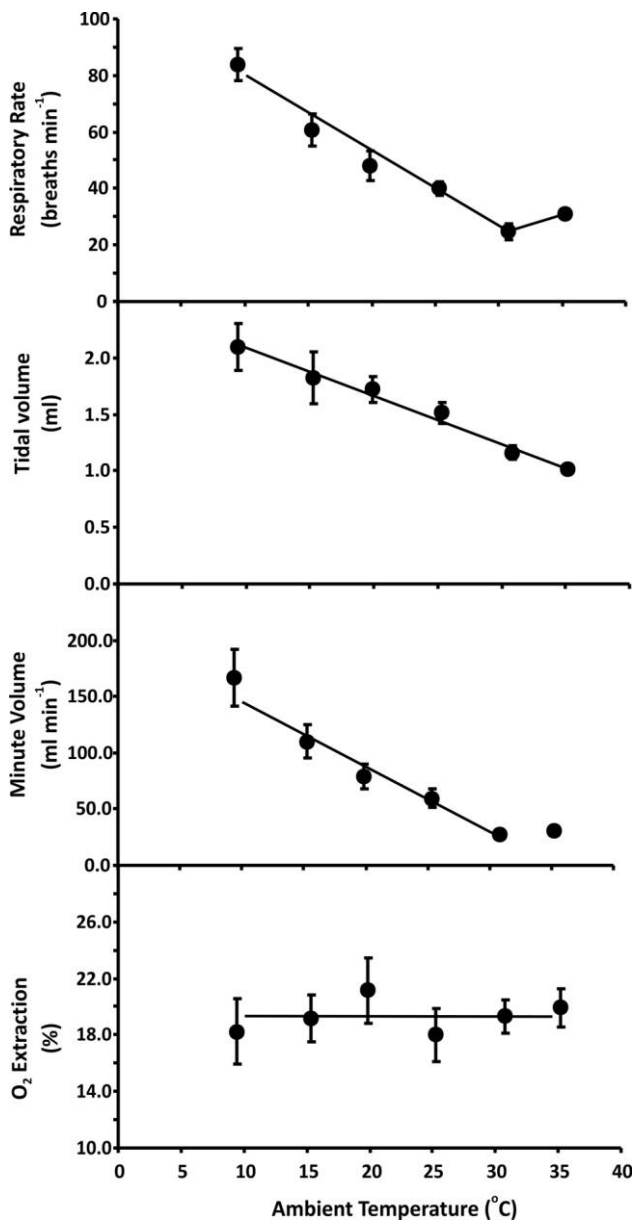
even if the overall MANOVAR test is not significant. They are also useful for examining specific hypotheses in non-repeated-measures ANOVA and univariate repeated-measures ANOVA models.

For T_b, the overall significant MANOVAR was supported by various significant a priori contrasts (polynomial, Helmert, reverse Helmert) that reflected a pattern of constant T_b from $T_a = 10°$ to $31°C$ and then an increase in T_b above thermoneutrality ($T_a = 35°C$). This pattern of T_b consistency at lower T_a and then hyperthermia at high T_a is expected for a euthermic mammal (e.g., McNab 1980; Withers 1992) and is consistent with that observed for other species of dasyurid marsupial (e.g., Robinson and Morrison 1957; Nicol and Maskrey 1980; Dawson and Dawson 1982; Geiser 1986; Schmidt et al. 2009; Cooper and Withers 2010). Many smaller dasyurid species are relatively thermolabile at low T_a (Morton and Lee 1978; Dawson and Wolfers 1978; Withers and Cooper 2009a, 2009b; Warnecke et al. 2010), but dibblers maintained a constant T_b below thermoneutrality and showed no tendency for torpor during experiments.

For Vo_2, the overall significant MANOVAR resulted from a slightly different pattern to T_b, of a linear decrease in Vo_2 with increasing T_a and then an increase at $T_a = 35°C$; there were significant differences between the thermoneutral T_a and all other T_a's, and there was a significant quadratic relationship for Vo_2 at all T_a's but only a linear relationship for $T_a < 35°C$. This is the expected pattern from the Scholander-Irving model for an endotherm (e.g., McNab 1980; Withers 1992), reflecting the increased metabolic heat production required for thermoregulation of a constant T_b at low T_a, and it is typical of normothermic dasyurid marsupials (e.g., Nicol and Maskrey 1980; Geiser 1986; MacMillen and Dawson 1986; Schmidt et al. 2009; Withers and Cooper 2009a, 2009b; Cooper and Withers 2010; Warnecke et al. 2010). The predicted T_b from the relationship of Vo_2 with T_a below thermoneutrality of $36.2°C$ was very similar to the measured T_b ($36.3°C$), confirming the near conformity of dibblers to the Scholander-Irving model.

Similar use of a priori contrasts demonstrated the constancy of C_{wet} and C_{dry} at low T_a but elevated values at high T_a. This pattern is typical for endotherms in general (e.g., McNab 1980; Withers 1992) and for other dasyurid marsupials in particular (Schmidt et al. 2009; Withers and Cooper 2009a, 2009b; Cooper and Withers 2010; Warnecke et al. 2010).

Ventilatory parameters of dibblers accommodated changing O_2 demand with T_a. The pattern of change in V_I with T_a mirrored that of Vo_2 and was mediated by a decrease in f_R with increasing T_a and then an increase at $T_a = 35°C$ (like Vo_2) and a linear decrease in V_T. Such f_R and V_T changes are typical of similar-sized marsupials. Smaller species generally increase only f_R and larger species only V_T to increase V_I at low T_a (Hallam and Dawson 1993; Chappell and Dawson 1994; Schmidt et al. 2009; Withers and Cooper 2009b; Cooper and Withers 2010; Warnecke et al. 2010).

Even for EWL and Eo_2, for which the overall MANOVAR was insignificant, it was useful to examine specific a priori patterns. EWL of dibblers would be expected to increase at T_a

Figure 3. Ventilatory variables; respiratory frequency, tidal volume, minute volume, and oxygen extraction for dibblers (*Parantechinus apicalis*) at various ambient temperatures. Solid lines indicate patterns supported by a priori contrast analysis (see text). Values are mean ± standard error; $N = 8$.

not necessarily anticipated for these variables (e.g., Hinds and MacMillen 1986; Cooper and Withers 2002, 2004; Larcombe 2002; Withers and Cooper 2009a, 2009b). For any T_a effects, it was of interest to establish what that pattern was and/or for which categories of the repeated factor there were significant differences. Although an absence of post hoc tests makes this difficult for MANOVAR, a priori contrasts provided a mechanism by which we could statistically describe the observed patterns of response to T_a. A priori contrasts can also be valuable in examining specific hypotheses (e.g., a linear effect)

above thermoneutrality as a thermoregulatory response when heat challenged (e.g., MacMillen and Dawson 1986; Schmidt et al. 2009; Withers and Cooper 2009a, 2009b; Cooper and Withers 2010). We confirmed this response using polynomial contrasts including and excluding $T_a = 35°C$ and simple and reverse Helmert contrasts. The effect of lower T_a on EWL varies amongst dasyurids, from none to negative to positive (Hinds and MacMillen 1986; MacMillen and Dawson 1986; Cooper et al. 2005; Schmidt et al. 2009; Withers and Cooper 2009a, 2009b; Cooper and Withers 2010; Warnecke et al. 2010). Differences in patterns of change in the respiratory component of total EWL at low T_a presumably contribute to this variation between species. We found a significant negative effect of $T_a \leq 31°C$ for dibblers by linear contrast, and Helmert contrasts reinforced this interpretation. The negative EWL-T_a relationship for dibblers suggests that they have a relatively high respiratory EWL, presumably reflecting a comparatively poor nasal countercurrent exchange system. Contrasts demonstrated the expected linear decrease in RWE with increasing T_a, and the PRWE of 20.3°C was consistent with that for other dasyurids (Schmidt et al. 2009; Withers and Cooper 2009a, 2009b; Cooper and Withers 2010; Warnecke et al. 2010).

No a priori contrasts were significant for Eo_2, although we expected a decrease in Eo_2 to accompany increased f_R at high T_a (e.g., Cooper and Withers 2010). However, f_R only increased slightly (but V_I did not) for dibblers at $T_a = 35°C$, suggesting that 35°C was not sufficiently above the thermoneutral zone to initiate panting and decrease Eo_2, consistent with results for some other dasyurids at a similar T_a (Chappell and Dawson 1994; Schmidt et al. 2009; Withers and Cooper 2009b).

We conclude that a priori contrasts, especially in conjunction with MANOVAR since post hoc tests are unavailable, provide a powerful statistical tool to examine expected patterns for repeated-measures physiological data. Judicious use of various a priori contrast tests for specific expected hypotheses provided considerably more information and power than just the overall MANOVAR hypothesis (that all means are equal), allowing us to statistically support the physiological patterns in response to changing T_a expected for the dibbler. It was found to be a typical endotherm, that closely conformed to the Scholander-Irving model, but was less thermolabile than many other small dasyurid marsupials.

Acknowledgments

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