TECHNICAL COMMENT

Experimental Sources of Variation in Avian Energetics: Estimated Basal Metabolic Rate Decreases with Successive Measurements

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ABSTRACT

Basal metabolic rate (BMR) is one of the most widely used metabolic variables in endotherm ecological and evolutionary physiology. Surprisingly few studies have investigated how BMR is influenced by experimental and analytical variables over and above the standardized conditions required for minimum normothermic resting metabolism. We tested whether avian BMR is affected by habituation to the conditions experienced during laboratory gas exchange measurements by measuring BMR five times in succession in budgerigars (*Melopsittacus undulatus*) housed under constant temperature and photoperiod. Both the magnitude and the variability of BMR decreased significantly with repeated measurements, from 0.410 ± 0.092 W ($n = 9$) during the first measurement to 0.285 ± 0.042 W ($n = 9$) during the fifth measurement. Thus, estimated BMR decreased by ~30% within individuals solely on account of the number of times they had previously experienced the experimental conditions. The most likely explanation for these results is an attenuation with repeated exposure of the acute stress response induced by birds being handled and placed in respirometry chambers. Our data suggest that habituation to experimental conditions is potentially an important determinant of observed BMR, and this source of variation needs to be taken into account in future studies of metabolic variation among individuals, populations, and species.

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Introduction

One of the most widely used metabolic variables in endotherm ecological and evolutionary physiology is basal metabolic rate (BMR), the lower limit of normothermic energy expenditure measured in resting, nonreproductive, and postabsorptive individuals during the rest phase of their circadian cycle at a thermoneutral environmental temperature (Dawson and Whittow 2000). In terms of practical considerations, BMR is often far easier to measure than total energy requirements (i.e., field metabolic rate) or other standardized metabolic rates, such as summit metabolism (i.e., maximum resting heat production). As a result, BMR has been measured for relatively large numbers of species (in excess of 500 birds and 600 mammals; McNab 2008, 2009) in comparison with the other metabolic variables mentioned above. Comparative analyses of the evolutionary and ecological correlates of endotherms' energy requirements have relied heavily on the identification of patterns of intra- and interspecific variation in BMR (e.g., Reynolds and Lee 1996; Ricklefs et al. 1996; Lovegrove 2000; McKechnie and Wolf 2004; White and Seymour 2004), as have investigations of phenotypic flexibility in metabolic machinery (e.g., Tieleman et al. 2003; Smit and McKechnie 2010; Maldonado et al. 2012).

Considering the importance of analyses of BMR for understanding sources of metabolic diversity among endotherms, surprisingly few studies have investigated the effects of variables associated with laboratory respirometry systems and experimental protocols (Hayes et al. 1992; Cooper and Withers 2009; Page et al. 2011). Many studies involve an implicit assumption that, if BMR is measured under standard conditions, it is directly comparable among species, populations, and/or individuals. However, the importance of examining the influence of variables related to experimental protocols on estimates of avian BMR was recently highlighted by Page et al. (2011), who investigated the effects of experimental variables on BMR in budgerigars (*Melopsittacus undulatus*). These authors found that measurement duration and the time at which measurements started significantly influenced the observed minimal metabolic rates, and they argued that measurements of BMR in small birds should involve durations of at least 9 h in order to avoid elevations in metabolic rate elicited by handling.

Stress has been considered as a factor potentially affecting BMR measurements by several authors (Weathers et al. 1983; Buttemer et al. 1991; Hinsley 1992; Weimerskirch et al. 2002), and interspecific variation in endocrine stress responses has been invoked to explain observed differences in the effect of handling on measured metabolic rates (Hayes et al. 1992;

Speakman et al. 1993). The possibility of stress responses being attenuated through repeated exposure to an experimental setup, however, has seldom been considered. Habituation to repeated stressors has been observed in several vertebrate taxa, with subsequent repeated stress events associated with reduced secretion of glucocorticoid stress hormones (Lynn et al. 2010). It seems reasonable, therefore, to expect that animals will respond differently to being handled and placed in metabolic chambers if they have experienced the procedure several times previously compared with if they are naive. Here we investigate the possibility that habituation to experimental procedures and handling influences observed BMR and/or the time taken for minimum metabolic rate to be reached after the start of measurements.

Another variable that potentially influences measured BMR is the sampling interval (i.e., the period over which O_2 consumption and/or CO_2 production is averaged), since the metabolic rate of endotherms does not remain precisely constant during measurements. A cursory examination of the literature reveals sampling periods ranging from 1 to 60 min (Chappell et al. 1999; Buttemer et al. 2008; Smit et al. 2008; Cory Toussaint and McKechnie 2012). Shorter sampling intervals are more likely to exclude periods of activity but are also more likely to be influenced by the transient reductions in metabolic rate that characterize most gas exchange traces (Hayes et al. 1992; Bech et al. 1999; Withers 2001; Cooper and Withers 2010; Page et al. 2011). Hence, sampling interval is potentially an important variable in measurements of BMR (Hayes et al. 1992; Bech et al. 1999), and we explore this issue further here.

Material and Methods

Study Animals and Housing

We opted to use captive-bred budgerigars (*Melopsittacus undulatus*) for this study, for two reasons. First, this is the same species used by Page et al. (2011) in their examination of the effects of experimental start time and duration on BMR measurement. Second, by using birds that had spent their entire lives in captivity, we avoided any metabolic consequences of bringing wild birds into captivity, such as the initial decrease in the BMR of laughing doves (*Streptopelia senegalensis*) noted by McKechnie et al. (2007) or increases in metabolic rate associated with captivity stress (Swanson and King 2013). We obtained 11 budgerigars from a local breeder and housed the birds individually in cages (38 cm long × 38 cm high × 24 cm wide) placed in a constant environment room at the Department of Zoology and Entomology, University of Pretoria, with mixed seed, fresh greens, and drinking water provided ad lib. The air temperature in the room was maintained at ~23°C with a photoperiod of 12 h, conditions that remained constant throughout the study. Budgerigars were maintained under these conditions for 25 d before the start of metabolic measurements to ensure full acclimation and steady-state physiological conditions before the commencement of data collection. Body mass was measured (± 0.01 g) using a calibrated electronic scale (Scout Pro SPU402, Ohaus, Pine Brook, NJ).

Measurements of Body Temperature and Gas Exchange

Body temperatures were monitored using temperature-sensitive passive integrated transponder (PIT) tags (Bio-Thermo 12-mm Microchip, Destron Fearing, St. Paul, MN) injected intraperitoneally using a sterile syringe at least 1 wk before measurement. A topical antiseptic (Betadine) was applied to the site with each injection. During measurements, PIT tags were typically scanned every 15 s using a PIT tag reader (model FS2001F-ISO, Biomark, Boise, ID).

We estimated BMR from oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) at an air temperature (T_a) of 30°C, which is within the thermoneutral zone of this species (Page et al. 2011). Budgerigars were placed individually in 4-L plastic chambers in a darkened temperature-controlled cabinet (model KMF 720, Binder, Tuttlingen, Germany). A layer of mineral oil (1 cm) was placed at the bottom of each chamber to prevent evaporation from feces and urine from influencing water vapor pressure measurements, with the birds perching on a piece of plastic mesh positioned ~10 cm above the mineral oil. Air temperatures in the chambers were continuously measured using thermistor probes (Sable Systems, Las Vegas, NV). Atmospheric air supplied by a compressor was dried and scrubbed of CO_2 using an adsorption drier (model K-NT3, Parker-Zander, Essen, Germany) before being supplied to chambers by mass flow controllers (model FMA 5400/5500, Omega Engineering, Bridgeport, NJ), regularly calibrated using a soap bubble flow meter (Baker and Pouchot 1983). Flow rates of approximately 1 L min^{-1} were used, which maintained a difference in $[O_2]$ of less than 0.5% between incurrent and excurrent air and dewpoints below −12°C in the chamber. The 99% equilibrium time for this system, calculated following Lasiewski et al. (1966), was 18.4 min.

Two parallel systems were set up in order to measure gas exchange in two birds simultaneously without the need to alternate between the two chambers. In each system, excurrent air from the chamber was subsampled at ~150 mL min^{-1}, using a Sable Systems RM-8 respirometry multiplexor (Sable Systems), before being pulled through either (1) an LI-840 H_2O/CO_2 analyzer (Li-Cor, Lincoln, NE) followed by an FC-10A oxygen analyzer (Sable Systems); or (2) an RH-300 water vapor analyzer (Sable Systems), a CA-10A CO_2 analyzer (Sable Systems), and an FC-10B oxygen analyzer (Sable Systems). The oxygen analyzers were regularly spanned using atmospheric air scrubbed of CO_2 and water vapor using soda lime and magnesium perchlorate (Merck, Modderfontein, South Africa), respectively. The CO_2 and water vapor analyzers were regularly zeroed using pure nitrogen (Afrox, Johannesburg, South Africa) and spanned using a certified calibration gas containing 2,000 ppm CO_2 (Afrox) for CO_2 and the oxygen dilution method of Lighton (2008) for water vapor. An SS-3 subsampling pump (Sable Systems) pulled subsampled air through system 2, whereas a custom-built pump and rotometer (FL-2012, Omega Engineering) was used for system 1. Voltage outputs from all sensors and instruments were acquired and recorded every 5 s

using a UI-2 analog-digital convertor and Expedata software (Sable Systems).

Experimental Protocol

Metabolic rates were measured five times in each budgerigar, with 4–8 d between successive measurements. Sample sizes varied slightly between 9 and 11 individuals. Measurements took place between April 29 and June 14, 2013, with the date of each individual's first measurement ranging from April 29 to May 29. All measurements conformed to the standard criteria for BMR, such that individuals were nonreproductive adults and postabsorptive (6–10 h since removal of food) before start of measurements. Birds were caught by hand inside their cages, immediately placed in cloth bags, and taken to the lab containing the respirometry systems. Birds were weighed before and after measurements, with the average of these two body mass values used for calculations. The time birds spent being handled or kept in cloth bags before the start of measurements was kept approximately constant. Gas exchange measurements commenced at 18:00 to coincide with the start of the scotophase in the room where the birds were housed (Page et al. 2011). Baseline $[O_2]$, $[CO_2]$, and water vapor pressure were initially recorded for 30 min, whereafter excurrent values were recorded continuously for 12 h. Following these measurements—that is, at 06:30 the following morning—a second 30-min baseline measurement was obtained. All data were corrected for lag and drift, using the appropriate algorithms in Expedata.

Data Analyses

Values of $\dot{V}O_2$, $\dot{V}CO_2$, and evaporative water loss were calculated using the appropriate equations of Lighton (2008). The respiratory exchange ratio was determined as $\dot{V}CO_2/\dot{V}O_2$, and gas exchange was converted to metabolic rate (W) using the thermal equivalence data in table 4.2 of Withers (1992). Mean ± SD respiratory exchange ratio during the study was 0.766 ± 0.109, indicating predominantly lipid metabolism.

In order to investigate the influence of sampling interval (i.e., the period over which gas exchange was averaged to identify the minimum metabolic rate) on estimated BMR, we identified the lowest running average values of metabolic rate over 1-, 5-, 10-, 20-, 30-, 40-, 50-, and 60-min intervals. We then fitted regression models (linear, quadratic, and exponential decay) to BMR as a function of sampling interval and identified the model that provided the best fit, using the approach of Song and Geiser (1997). On the basis of this analysis (see "Results"), we used a 30-min interval for estimating BMR for all further analyses. Times to minimum values were taken as the midpoint times of the 30-min average minima identified within each 12-h trace. In order to quantify changes in metabolic rate during the course of measurements, we calculated mean metabolic rate during the 30-min period immediately following the initial baseline measurement (i.e., 30–60 min after the bird was placed in the chamber) as well as the lowest 30-min average for each 2-h period during measurements.

Assumptions of normality and homoscedasticity were verified using Shapiro-Wilk and Levene's tests, respectively. The effects of successive measurements on BMR and related variables were examined, using general linear mixed models (GLMMs) implemented in R 3.0.2 (R Development Core Team 2011) using the *nlme* package (Pinheiro et al. 2009), with either observed BMR, mass-specific BMR, time to reach BMR, T_b or evaporative water loss as a response variable. Predictor variables were body mass, measurement sequence (fixed, 1–5), bird identity (random), and the Julian date of each individual's first measurement (random). The latter variable was included to account for any temporal changes in BMR that may have occurred during the course of the study, despite the birds being housed under conditions of constant temperature and photoperiod. Post hoc tests (Tukey's HSD) were conducted using the R packages *multcomp* (Hothorn et al. 2008) and *mvtnorm* (Genz et al. 2011). We calculated the repeatability (r) of BMR from the variance components of the GLMM following Lessells and Boag (1987) and the 95% confidence intervals (CIs) following Becker (1984). Repeatability was considered significant when the 95% CI excluded 0 (Swanson and King 2013). Additional statistics, such as regression analyses, were conducted using SigmaPlot 12.5 (Systat Software). Values are presented as means ± SD.

Results

Metabolic rate and T_b gradually declined after measurements commenced until a steady state was reached. Minimum metabolic rate (i.e., BMR) was typically reached between 4.5 and 7.5 h after the start of measurements. Estimated BMR varied with the interval over which metabolic rate was averaged (fig.

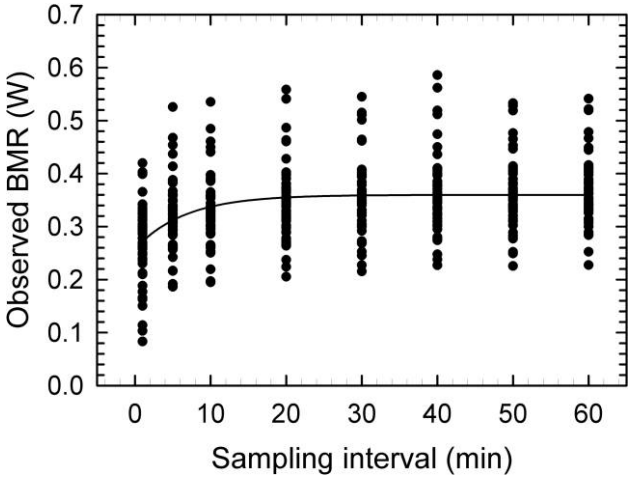

Figure 1. Observed basal metabolic rate (BMR) in budgerigars (*Melopsittacus undulatus*) as a function of the sampling interval over which metabolic rate was averaged to estimate BMR. BMR was measured in 9–11 birds over five successive sets of measurements (total = 51 measurements). The solid line shows an exponential decay regression model ($y = 0.360 - 0.103e^{-0.152x}$, $r^2 = 0.156$, $P < 0.001$), which provided a better fit than either a linear or quadratic model.

1). Linear, quadratic, and exponential decay regression models all yielded significant fits to the BMR versus sampling interval data. The exponential decay model ($F_{2, 393} = 36.320$, $P < 0.001$) provided the best fit ($r^2 = 0.156$), indicating that observed BMR reached an asymptote within the range of sampling intervals examined here. Mean BMR estimated from the lowest 1-, 5-, and 10-min intervals was equivalent to 73.91%, 88.06%, and 92.35%, respectively, of that averaged over 60 min. On the basis of the latter observation, we used a 30-min interval for estimating BMR for all further analyses (fig. 1).

Body mass varied among individuals from 29.12 ± 0.52 to 39.93 ± 1.17 g (mean per individual over course of entire study), with an overall mean for all birds of 33.74 ± 3.57 g. Body mass did not vary significantly during the course of experiments ($F_{1, 43} = 2.010$, $P = 0.164$). Body mass was not significantly related to BMR ($F_{1, 41} = 1{,}844$, $P = 0.182$) nor time to reach BMR ($F_{1, 41} = 1.458$, $P = 0.234$) but emerged as a significant predictor of mass-specific BMR ($F_{1, 41} = 9.358$, $P = 0.004$). The mean T_b associated with BMR was 38.67° ± 0.53°C. Body temperature was not significantly correlated with BMR (Pearson product moment $r = 0.041$, $P = 0.800$) and was not significantly influenced by measurement sequence ($F_{1, 32} = 0.350$, $P = 0.561$). Similarly, evaporative water loss was not significantly related to M_b ($F_{1, 40} = 0.507$, $P = 0.481$) or significantly influenced by measurement sequence ($F_{1, 40} = 0.308$, $P = 0.586$).

Measurement sequence had a significant effect on both BMR ($F_{1, 41} = 19.738$, $P < 0.001$) and mass-specific BMR ($F_{1, 41} = 17.051$, $P < 0.001$). Observed BMR declined significantly with successive measurements, and the mean BMR during the fifth set of measurements (0.285 ± 0.042 W; $n = 9$) was equivalent to 69.51% ± 0.46% of BMR during the first set of measurements (0.410 ± 0.092 W; $n = 9$; fig. 2). Moreover, the standard deviation of BMR was significantly related to measurement sequence, decreasing linearly with repeated measurements (fig. 3). BMR was highly and significantly repeatable ($r = 0.842$; 95% CI = 0.549–1.134). The relationship between time to reach BMR and measurement sequence was not significant ($F_{1, 42} = 3.879$, $P = 0.056$), with the time taken to reach BMR during the fifth set of measurements being 4.53 ± 2.13 h ($n = 9$) compared with 7.09 ± 3.61 h ($n = 9$) during the first set of measurements.

Mean metabolic rate between 30 and 60 min after the birds were placed in the chambers decreased significantly with successive measurements ($F_{1, 41} = 14.178$, $P < 0.001$; fig. 4). Moreover, relative to these initial metabolic rates shortly after being placed in the chambers, the extent to which measured BMR decreased during the course of the night varied with measurement number, with the magnitude of this reduction decreasing from ~40% during the first measurement to 20%–30% by the third and fifth measurements (fig. 4).

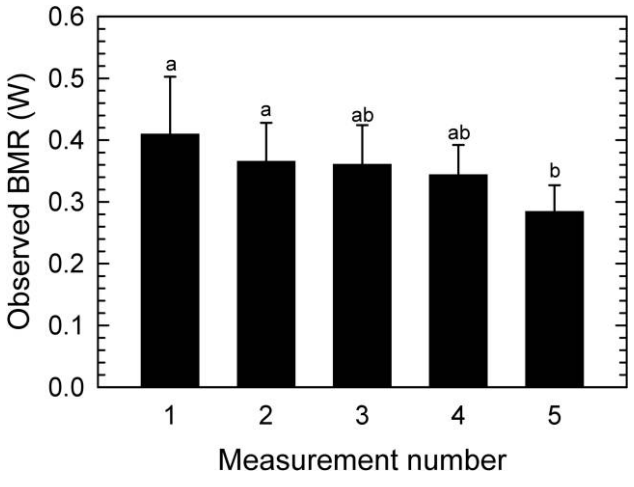

Figure 2. Mean ± SD observed basal metabolic rate (BMR) in budgerigars (*Melopsittacus undulatus*) decreased significantly within individuals with successive measurements. BMR was measured in the same 9–11 birds over five successive sets of measurements. Different letters indicate significant differences ($P < 0.05$) identified in post hoc tests.

Discussion

Our data highlight several ways in which experimental and analytical variables other than the standardized criteria for basal metabolism can influence observed BMR in birds. Most notably, the magnitude and variability of BMR decreased significantly with repeated exposure to experimental conditions, indicating that individual habituation is an important determinant of BMR in birds. Moreover, there was significant variation in estimated BMR associated with an analytical variable, namely the sampling interval over which minimum metabolic rate was averaged.

The large differences in observed BMR associated with various sampling intervals reiterate the need for careful consideration of the latter variable (Hayes et al. 1992; Speakman et al. 1993). The relationship between estimated BMR and sampling interval was best described by an exponential model that reached an asymptote when sampling period was 20–30 min (fig. 1). Shorter sampling intervals were associated with rapid decreases in estimated BMR. Our results are quantitatively similar to those of Bech et al. (1999), who estimated BMR in kittiwakes (*Rissa tridactyla*) using sampling intervals of 2–45 min and concluded that a 25-min interval was optimal. These authors noted that shorter intervals resulted in very low values on account of transient decreases in metabolic rate, whereas longer intervals were more likely to include periods of restlessness that elevate metabolic rate above basal levels. Thus, the limited available data suggest that for measurements of avian BMR, sampling intervals of 20–30 min should be used.

The fractional magnitude of the within-individual decreases in budgerigar BMR associated with successive measurements (~30%) is quantitatively similar to the decreases in resting metabolism associated with habituation to experimental conditions in a single Amazonian manatee and harp seal (Gallivan and Best 1980; Gallivan 1992). In both these animals, which were housed in tanks set up for gas exchange measurements

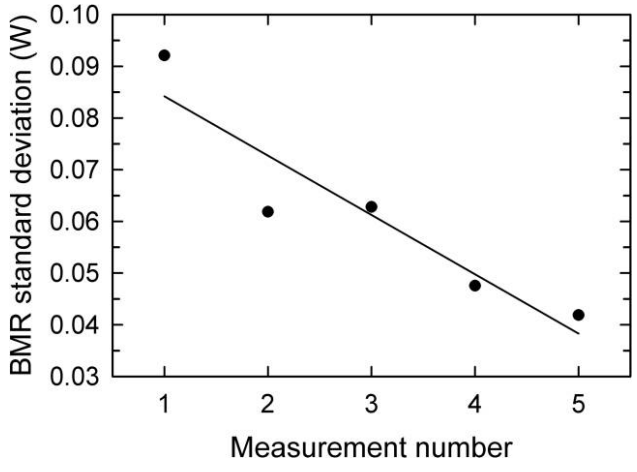

Figure 3. Standard deviation of observed basal metabolic rate (BMR) in budgerigars (*Melopsittacus undulatus*) decreased significantly within individuals with successive measurements. BMR was measured in the same 9–11 birds over five successive sets of measurements. Solid line shows a linear regression model ($y = 0.096 - 0.115x$, $r^2 = 0.868$, $P = 0.021$).

through a mask above the water surface, resting metabolic rate declined by 33%–36% over the course of the first 3–4 d of measurements (Gallivan and Best 1980; Gallivan 1992). However, whereas resting metabolic rate in the manatee and seal leveled off at approximately constant levels after the initial habituation period, the BMR of budgerigars did not show any indication of reaching an asymptote under the conditions of our study. This observation suggests that, had we continued with additional measurements, the observed BMR of the budgerigars may have declined yet further.

The repeatability for BMR in this study (0.842) is near the upper end of the range reported for avian BMR (Versteegh et al. 2008). Typically, repeatability is estimated for metabolic rates measured preceding and following acclimation or acclimatization (e.g., Bech et al. 1999; Tieleman et al. 2003; Vézina et al. 2006; McKechnie et al. 2007), and the high r for BMR in budgerigars probably partly reflects the steady-state conditions that the birds experienced preceding and during the study. However, it also indicates that changes in BMR associated with increasing habituation are highly consistent among individuals.

Our data also reiterate the point made by Page et al. (2011) that birds need to spend relatively long periods in respirometry chambers to ensure that minimal metabolic rates are attained. During our study, BMR was reached in <9 h in 43 of 51 cases, supporting the suggestion of Page et al. (2011) that 9 h should be viewed as the minimum period budgerigars need to spend in chambers. The marginal nonsignificance ($P = 0.056$) of the relationship between time required to reach BMR and measurement sequence suggests that this variable may be worth investigating further. Our data do indicate, however, that the magnitude of the effect of measurement duration on estimated BMR may vary with successive measurements, with smaller reductions in metabolic rate relative to initial values in birds that had experienced experimental conditions several times (fig. 4). Hayes et al. (1992) found that BMR estimated over 30 min at the start of measurements was 65% higher in wood mice and 13% higher in field moles compared with BMR estimated over a duration of 6 h. These authors suggested that the differences between these species could be attributed to the relative docility of voles compared to wood mice. Downs and Brown (2012) noted that from a total of 294 studies, only 22% performed BMR measurements over 9 h or more, 28.6% estimated BMR in 2 h or less, and 21.2% did not report a measurement duration. During our measurements, in only one case was BMR reached in less than 2 h.

The most obvious explanation for the decreases in the magnitude and variability of BMR with increasing familiarity with the experimental setup is an attenuation of the acute stress response associated with the birds being handled and placed in the metabolic chambers. It is striking that mean metabolic rate soon (30–60 min) after birds were placed in chambers decreased with successive measurements. The latter effect could well arise from lower activity levels immediately after being placed in the chamber, but we did not collect behavioral data to explore this possibility. Stress responses in birds and other animals can vary widely among and within species (Cockrem 2007, 2013), and many birds are known to reduce the magnitude of glucocorticoid-mediated acute stress responses with repeated exposure to a stressor (Love et al. 2003; Lynn et al. 2010). Such variation in acute stress responses can translate into large differences in observed metabolic rates among species, populations, and individuals and potentially explains much of the metabolic variation observed among similarly sized animals (Careau et al. 2008). Moreover, seasonal modulation of avian adrenocortical responses to stress (e.g., Wingfield et

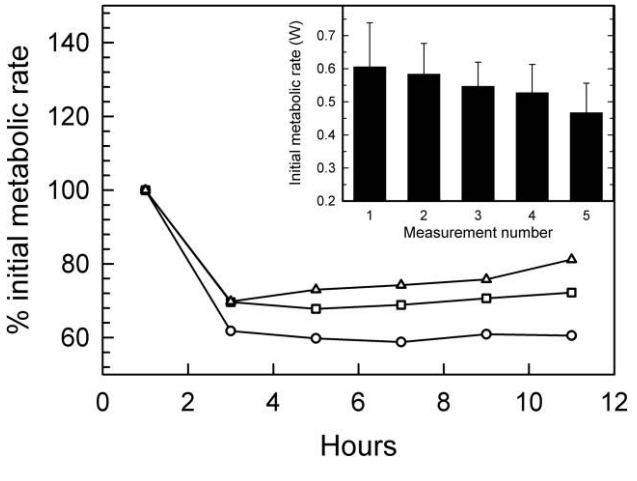

Figure 4. Lowest 30-min average metabolic rate of budgerigars (*Melopsittacus undulatus*) in each 2-h period during overnight measurements expressed as a percentage of initial metabolic rate 30–60 min after birds were placed in the chambers for the first (circles), third (squares), and fifth (triangles) measurements. Inset shows the relationship between mean ± SD initial metabolic rate and measurement number.

al. 1992; Wingfield et al. 1994; Romero and Remage-Healey 2000) raise the possibility that observed seasonal variation in BMR may partly reflect annual cycles in the magnitude of stress responses.

Decreases in BMR with progressive habituation to experimental conditions have a host of potential implications for comparative analyses. It is striking, for instance, that the range of mean BMR values in this study (0.410 and 0.285 W during the first and fifth sets of measurements, respectively) exceeds the range of BMR for this species reported in other studies and includes values substantially lower than those previously reported (0.37–0.43 W; Weathers and Schoenbaechler 1976; Hinds et al. 1993; Page et al. 2011). If our results for budgerigars are typical of other species, variation in experimental protocol among studies may add a significant amount of noise to comparative BMR data sets (Bennett and Harvey 1987; White et al. 2007; Jetz et al. 2008; McNab 2009). The magnitude of the changes in BMR (~30%) associated with habituation in our study exceeds that of some patterns of adaptive variation identified in comparative analyses (e.g., 17% lower BMR on average in desert birds compared with nondesert birds; Tieleman and Williams 2000).

The effect of habituation on BMR we report here poses many more questions and challenges than it resolves. One obvious implication is that experimental designs for future studies of intra- and interspecific metabolic variation will need to place greater emphasis on standardizing subjects' habituation to the experimental conditions than they have in the past. Most studies do not specify the number of times individuals experience the experimental conditions before measurement of BMR; we encourage authors to report this information in future. We also encourage workers to re-examine their published data sets and test for possible habituation effects on measured BMR. For instance, is BMR measured in individuals previously used for measurements of resting metabolic rate in order to determine the lower critical limit of thermoneutrality lower than BMR measured in conspecifics with no previous experience of the respirometry chambers? We suspect that there are many existing data sets potentially suitable for testing for correlations between BMR and individuals' prior experience with experimental conditions.

Evaluating the magnitude of the noise introduced into comparative BMR data sets by the effect of habituation to experimental conditions and understanding the implications thereof for comparative analyses will require studies on the relationships between habituation and metabolic variables. Not only should these involve taxa that differ in the magnitude of their acute stress responses and endocrine studies quantifying changes in glucocorticoid-mediated stress responses associated with successive metabolic measurements, but also they will need to examine the effects of variation in endocrine stress responses associated with factors such as season, sex, age, and body mass. Another key question is whether habituation effects are more pronounced in wild birds than captive populations. The latter is particularly pertinent in light of differences in the scaling of BMR between wild-caught and captive birds (McKechnie et al. 2006).

Finally, we need more studies comparing BMR measured under laboratory conditions with that estimated via heart rate telemetry in free-ranging individuals, in order to gauge the overall effect of laboratory conditions on BMR. In wandering albatrosses (*Diomedea exulans*), the BMR estimated during gas exchange measurements under laboratory conditions was 163% of that estimated from heart rates in free-ranging individuals on their nests (Weimerskirch et al. 2002), and free-ranging spotted antbirds (*Hylophylax naevioides*) also appeared to reduce their metabolic rates to levels below those seen at comparable air temperatures in the laboratory (Steiger et al. 2009). Comparisons of BMR measured under artificial laboratory conditions and those that occur in free-ranging individuals under natural conditions are a prerequisite for improving our understanding of the ecological and evolutionary relevance of this metabolic variable.

Comparative analyses of BMR and other standardized metabolic variables remain one of the most widespread approaches to testing hypotheses regarding metabolic adaptation in endotherms. However, the data we have presented here add to a growing body of evidence that simply meeting the standard criteria of postabsorptive individuals experiencing thermoneutral conditions during the rest phase of the circadian cycle is not always sufficient to ensure comparability among individuals, populations, and species.

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Literature Cited

Baker W.C. and J.F. Pouchot. 1983. The measurement of gas flow. Pt. II. J Air Pollut Control Assoc 33:156–162.

Bech C., I. Langseth, and G.W. Gabrielsen. 1999. Repeatability of basal metabolism in breeding female kittiwakes *Rissa tridactyla*. Proc R Soc B 266:2161–2167.

Becker W.A. 1984. A manual of quantitative genetics. Academic Enterprises, Pullman, WA.

Bennett P.M. and P.H. Harvey. 1987. Active and resting metabolism in birds: allometry, phylogeny and ecology. J Zool (Lond) 213:327–363.

Buttemer W.A., L.B. Astheimer, and J.C. Wingfield. 1991. The effects of corticosterone on standard metabolic rates of small passerine birds. J Comp Physiol B 161:427–432.

Buttemer W.A., S. Warne, C. Bech, and L.B. Astheimer. 2008. Testosterone effects on avian basal metabolic rate and aerobic

performance: facts and artefacts. Comp Biochem Physiol A 150:204–201.

Careau V., D. Thomas, M.M. Humphries, and D. Réale. 2008. Energy metabolism and animal personality. Oikos 117:641–653.

Chappell M.A., C. Bech, and W.A. Buttemer. 1999. The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. J Exp Biol 202: 2269–2279.

Cockrem J.F. 2007. Stress, corticosterone responses and avian personalities. J Ornithol 148:169–178.

———. 2013. Individual variation in glucocorticoid stress responses in animals. Gen Comp Endocrinol 181:45–58.

Cooper C.E. and P.C. Withers. 2009. Effects of measurement duration on the determination of basal metabolic rate and evaporative water loss of small marsupials: how long is long enough? Physiol Biochem Zool 82:438–446.

———. 2010. Effect of sampling regime on estimation of basal metabolic rate and standard evaporative water loss using flow-through respirometry. Physiol Biochem Zool 83:385–393.

Cory Toussaint D. and A.E. McKechnie. 2012. Interspecific variation in thermoregulation among three sympatric bats inhabiting a hot, semi-arid environment. J Comp Physiol B 182:1129–1140.

Dawson W.R. and G.C. Whittow. 2000. Regulation of body temperature. Pp. 343–390 in G.C. Whittow, ed. Sturkie's avian physiology. Academic Press, New York.

Downs C.T. and M. Brown. 2012. Is respirometry a standardized technique? a review of measurement of avian resting metabolic rates. J Therm Biol 37:531–536.

Gallivan G.J. 1992. What are the metabolic rates of cetaceans? Physiol Zool 65:1285–1297.

Gallivan G.J. and R.C. Best. 1980. Metabolism and respiration of the Amazonian manatee (*Trichechus inunguis*). Physiol Zool 53:245–253.

Genz A., F. Bretz, T. Miwa, X. Mi, F. Leisch, F. Scheipl, and T. Hothorn. 2011. *mvtnorm*: multivariate normal and t distributions. R package version 0.9-9991.

Hayes J.P., J.R. Speakman, and P.A. Racey. 1992. Sampling bias in respirometry. Physiol Zool 65:604–619.

Hinds D.S., R.V. Baudinette, R.E. MacMillen, and E.A. Halpern. 1993. Maximum metabolism and the aerobic factorial scope of endotherms. J Exp Biol 182:41–56.

Hinsley S.A. 1992. Bioenergetics of a desert specialist, the double-banded sandgrouse, and the problems of stress induced by experimental conditions. Comp Biochem Physiol 102A: 433–439.

Hothorn T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical J 50:346–363.

Jetz W., R.P. Freckleton, and A.E. McKechnie. 2008. Environment, migratory tendency, phylogeny and basal metabolic rate in birds. PLoS ONE 3:e3261.

Lasiewski R.C., A.L. Acosta, and M.H. Bernstein. 1966. Evaporative water loss in birds. I. Characteristics of the open flow method of determination, and their relation to estimates of thermoregulatory ability. Comp Biochem Physiol 19:445–457.

Lessells C.M. and P.T. Boag. 1987. Unrepeatable repeatabilities: a common mistake. Auk 104:116–121.

Lighton J.R.B. 2008. Measuring metabolic rates: a manual for scientists. Oxford University Press, Oxford.

Love O.P., L.J. Shutt, J.S. Silfies, and D.M. Bird. 2003. Repeated restraint and sampling results in reduced corticosterone levels in developing and adult captive American kestrels (*Falco sparverius*). Physiol Biochem Zool 76:753–761.

Lovegrove B.G. 2000. The zoogeography of mammalian basal metabolic rate. Am Nat 156:201–219.

Lynn S.E., L.E. Prince, and M.M. Phillips. 2010. A single exposure to an acute stressor has lasting consequences for the hypothalamo-pituitary-adrenal response to stress in free-living birds. Gen Comp Endocrinol 165:337–344.

Maldonado K., F. Bozinovic, G. Cavieres, C.A. Fuentes, A. Cortés, and P. Sabat. 2012. Phenotypic flexibility in basal metabolic rate is associated with rainfall variability among populations of rufous-collared sparrow. Zoology 115:128–133.

McKechnie A.E., K. Chetty, and B.G. Lovegrove. 2007. Phenotypic flexibility in basal metabolic rate in laughing doves: responses to short-term thermal acclimation. J Exp Biol 210: 97–106.

McKechnie A.E., R.P. Freckleton, and W. Jetz. 2006. Phenotypic plasticity in the scaling of avian basal metabolic rate. Proc R Soc B 273:931–937.

McKechnie A.E. and B.O. Wolf. 2004. The allometry of avian basal metabolic rate: good predictions need good data. Physiol Biochem Zool 77:502–521.

McNab B.K. 2008. An analysis of the factors that influence the level and scaling of mammalian BMR. Comp Biochem Physiol A 151:5–28.

———. 2009. Ecological factors affect the level and scaling of avian BMR. Comp Biochem Physiol A 152:22–45.

Page A.J., C.E. Cooper, and P.C. Withers. 2011. Effects of experiment start time and duration on measurement of standard physiological variables. J Comp Physiol B 181:657–665.

Pinheiro J., D. Bates, S. DebRoy, D. Sarkar, and R Development Core Team. 2009. *nlme*: linear and nonlinear mixed effects models. R package version 3.1-94.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Reynolds P.S. and R.M. Lee. 1996. Phylogenetic analysis of avian energetics: passerines and nonpasserines do not differ. Am Nat 147:735–759.

Ricklefs R.E., M. Konarzewski, and S. Daan. 1996. The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. Am Nat 147:1047–1071.

Romero L.M. and L. Remage-Healey. 2000. Daily and seasonal variation in response to stress in captive starlings (*Sturnus vulgaris*): corticosterone. Gen Comp Endocrinol 118:52–59.

Smit B., M. Brown, and C.T. Downs. 2008. Thermoregulatory

responses in seasonally acclimatized captive southern white-faced scops-owls. J Therm Biol 33:76–86.

Smit B. and A.E. McKechnie. 2010. Avian seasonal metabolic variation in a subtropical desert: basal metabolic rates are lower in winter than in summer. Funct Ecol 24:330–339.

Song X. and F. Geiser. 1997. Daily torpor and energy expenditure in *Sminthopsis macroura*: interactions between food and water availability and temperature. Physiol Zool 70:331–337.

Speakman J.R., R.M. McDevitt, and K.C. Cole. 1993. Measurement of basal metabolic rates: don't lose sight of reality in the quest for comparability. Physiol Zool 66:1045–1049.

Steiger S.S., J.P. Kelley, W.W. Cochran, and M. Wikelski. 2009. Low metabolism and inactive lifestyle of a tropical rain forest bird investigated via heart-rate telemetry. Physiol Biochem Zool 82:580–589.

Swanson D.L. and M.O. King. 2013. Short-term captivity influences maximal cold-induced metabolic rates and their repeatability in summer-acclimatized American goldfinches *Spinus tristis*. Curr Zool 59:439–448.

Tieleman B.I. and J.B. Williams. 2000. The adjustment of avian metabolic rates and water fluxes to desert environments. Physiol Biochem Zool 73:461–479.

Tieleman B.I., J.B. Williams, M.E. Buschur, and C.R. Brown. 2003. Phenotypic variation of larks along an aridity gradient: are desert birds more flexible? Ecology 84:1800–1815.

Versteegh M.A., B. Helm, N.J. Dingemanse, and B.I. Tieleman. 2008. Repeatability and individual correlates of basal metabolic rate and total evaporative water loss in birds: a case study in European stonechats. Comp Biochem Physiol A 150: 452–457.

Vézina F., K. Jalvingh, A. Dekinga, and T. Piersma. 2006. Acclimation to different thermal conditions in a northerly wintering shorebird is driven by body mass-related changes in organ size. J Exp Biol 209:3141–3154.

Weathers W.W. and D.C. Schoenbaechler. 1976. Regulation of body temperature in the budgerygah, *Melopsittacus undulatus*. Aust J Zool 24:39–47.

Weathers W.W., D.L. Weathers, and C. van Riper. 1983. Basal metabolism of the apapane: comparison of freshly caught birds with long-term captives. Auk 100:977–978.

Weimerskirch H., S.A. Shaffer, G. Mabille, J. Martin, O. Boutard, and J.L. Rouanet. 2002. Heart rate and energy expenditure of incubating wandering albatrosses: basal levels, natural variation, and the effects of human disturbance. J Exp Biol 205:475–483.

White C.R., T.M. Blackburn, G.R. Martin, and P.J. Butler. 2007. The basal metabolic rate of birds is associated with habitat temperature and precipitation, not productivity. Proc R Soc B 274:287–293.

White C.R. and R.S. Seymour. 2004. Does basal metabolic rate contain a useful signal? mammalian BMR allometry and correlations with a selection of physiological, ecological, and life-history variables. Physiol Biochem Zool 77:929–941.

Wingfield J.C., P. Deviche, S. Sharbaugh, L.B. Astheimer, R. Holberton, R. Suydam, and K. Hunt. 1994. Seasonal changes of the adrenocortical responses to stress in redpolls, *Acanthis flammea*, in Alaska. J Exp Zool 270:372–380.

Wingfield J.C., C.M. Vleck, and M.C. Moore. 1992. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran desert. J Exp Zool 264:419–428.

Withers P.C. 1992. Comparative animal physiology. Saunders College , Fort Worth, TX.

———. 2001. Design, calibration and calculation for flow-through respirometry systems. Aust J Zool 49:445–461.