Effects of Measurement Duration on the Determination of Basal Metabolic Rate and Evaporative Water Loss of Small Marsupials: How Long Is Long Enough?

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ABSTRACT

We examined the time course for measurement of basal metabolic rate (BMR; measured as O_2 consumption and CO_2 production) and standard evaporative water loss (EWL) for six species of small marsupial to determine the minimum time required to achieve basal/standard values. There was a highly significant effect of measurement duration on measured physiological variables with values for O_2 consumption, CO_2 production, and EWL decreasing with time for all species. The time required to attain values statistically indistinguishable from minimal differed significantly between species, but in general O_2 consumption rate reached basal values after 4.3 h, CO_2 production after 4.5 h, and evaporative water loss after 5.2 h. For 16 BMR measurements of small marsupial species in the literature, with experimental duration provided, 10 were for less than 4 h, suggesting that their BMR values might be overestimates. For EWL, three of the four published values for small marsupials may be overestimates. It is clear that appropriate experimental duration is an important component of the measurement protocol for both BMR and standardized water loss, which needs to be rigorously observed in future studies.

Introduction

Basal metabolic rate (BMR) is one of the variables most frequently measured by comparative physiologists. It is the lowest sustainable metabolic rate for a euthermic endotherm (Withers 1992) and is important because it is a metabolic standard that can be used for intra- and interspecific comparison (McNab 1997). To ensure that the measurement of BMR is indeed minimal and standardized, a stringent set of conditions are applied to its measurement. The conditions that satisfy BMR were first described for measurement of human metabolic rate (DuBois 1924, 1930) and were subsequently employed to standardize measurement of metabolic rate for a range of domestic animals (Kleiber 1932, 1961). Further modifications to account for a range of nondomesticated animals (e.g., heterotherms) and other factors that may influence BMR (e.g., circadian rhythm) have been added to the definition (Aschoff and Pohl 1970; McNab 1997) to ensure that factors that may increase metabolism above basal levels are eliminated. To qualify as a true measure of BMR, animals must be adult, nonreproductive, postabsorptive, endothermic individuals that are measured at rest within their thermoneutral zone (TNZ) during euthermy in the inactive phase of their circadian rhythms (McNab 1997).

Evaporative water loss (EWL) is another commonly measured physiological variable, although it is not measured as frequently as BMR. As such, a standard for EWL measurement is not as well defined as for metabolism, and a number of methodological inconsistencies have contributed to variability in EWL measurement (Lasiewski et al. 1966; Bernstein et al. 1977; Cooper et al. 2005). We use the term "standard EWL" here to refer to the comparative BMR equivalent for EWL rather than basal EWL, because EWL is not necessarily minimal under standard BMR conditions; EWL may decrease with decreasing ambient temperature (T_a) below the TNZ. Generally, standard EWL is considered to be EWL measured under the same conditions as BMR. It should be noted that the TNZ is defined as the T_a zone where temperature regulation is achieved without regulatory changes in metabolic heat production or evaporative heat loss, not just the broader range of T_a where metabolic rate is constant (Bligh and Johnson 1973; Anon. 1987, 2003). EWL tends to increase within the TNZ at lower T_a's above the lower critical temperature (T_{lc}) than metabolic rate does (e.g., Weathers and Caccamise 1975). Thus, measurement of standard EWL requires a more restricted range of T_a (TNZ) than measurement of BMR. Therefore, both BMR and standard EWL are best measured near the T_{lc}.

Most of the conditions necessary to measure BMR and EWL can be imposed on an animal, but this is not always possible for all criteria, and it has been suggested that true BMR can be achieved only in cooperative human subjects (Kleiber 1961; Blaxter 1989). For example, ruminants (e.g., bovids, camelids), pseudoruminants (e.g., macropods), and hindgut fermenters (e.g., phalangerids, sloths) may never be postabsorptive (Blaxter 1989; McNab 1997; White and Seymour 2005). A resting state

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is particularly difficult to attain for echolocating or continuously swimming species (e.g., bats, cetaceans; Speakman et al. 1993). It may also be difficult to achieve a complete resting state for animals if activity or anxiety as a result of human contact or unfamiliar surroundings increases metabolic rate and EWL. This may be particularly true for wild-caught individuals or animals that are not accustomed to being handled or to the experimental procedure. However, as there can be an effect of long-term captivity on physiological variables (Skadhauge and Bradshaw 1974; Geiser et al. 1990; Warkentin and West 1990; Geiser and Ferguson 2001), it is often desirable to measure "field-fresh" individuals. Presumably, the period of time that the animal is allowed to settle in the metabolic chamber before BMR or EWL are determined will influence the measured values of these variables, as the animals will take time to settle and attain a truly resting state. Gallivan (1992) noted that experiment duration and familiarity with the metabolic chamber influenced measurement of oxygen consumption for harp seals (*Phoca groenlandica*) and Amazonian manatees (*Trichechus inunguis*), and Hayes et al. (1992) found that measurement duration influenced the measurement of metabolic rate of wood mice (*Apodemus sylvaticus*) and to a lesser extent short-tailed field voles (*Microtus agrestis*). Measurement of oxygen consumption of various fish species is also influenced by experimental duration (Steffensen et al. 1994; Steffensen 2002). Here, we systematically examine the influence of experiment duration on the measurement of BMR and EWL for six species of small marsupial to determine the minimum experimental period necessary to obtain BMR and to provide the first data quantifying the effect of measurement duration on EWL.

Material and Methods

Metabolic rate was measured for six species of small marsupial: western pygmy possum (*Cercartetus concinnus*, $n = 6$), little red kaluta (*Dasykaluta rosamondae*, $n = 8$), gracile mouse opossum (*Gracilinanus agilis*, $n = 6$), mallee ningaui (*Ningaui yvonneae*, $n = 6$), fat-tailed dunnart (*Sminthopsis crassicaudata*, $n = 6$), and hairy-footed dunnart (*Sminthopsis hirtipes*, $n = 7$). All individuals were wild caught and were maintained in captivity on a diet of either cat food, mince, and mealworms (kalutas, ningauis, and dunnarts); fruit, cat food, and mince (mouse opossums); or high-protein baby food and honey (pygmy possums). All were provided with water ad lib. Animals were housed indoors at approximately 21°C with about a 12L : 12D cycle. Measurements were made in the laboratory within 3 wk of capture, and these measurements were the first time that the individuals had been measured in a metabolic system.

Measurements conformed to the criteria for measuring BMR and standard EWL; all individuals were adult, nonreproductive, postabsorptive (fasted for at least 24 h before the commencement of experiments because passage time for small marsupials is <24 h; Hume 1982) and were measured at thermoneutrality near the T_{lc} (30°C; C. E. Cooper and P. C. Withers, unpublished data) during their rest phase (daytime), with experiment durations of up to 9 h.

The details of the metabolic systems used varied slightly between species, but the general system consisted of a mass flow controller that regulated the flow of dry compressed air through a Perspex metabolic chamber (125–360 mL) at a rate of 50–500 mL min^{-1}. The metabolic chamber was located inside a controlled temperature cabinet or room. Excurrent air passed through a thin-film capacitance hygrometer, a column of Drierite, an oxygen analyzer, and a carbon dioxide analyzer. Custom-written data acquisition software (P. C. Withers; Visual Basic v6) was used to record the outputs of the humidity meter and gas analyzers and T_a every 10–20 s throughout the experimental period. This system relied on the dry incurrent air to dry any feces or urine produced by the animal during the experiment. The metabolic systems were calibrated after Withers (2001).

Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and EWL were calculated based on Withers (2001), using a custom-written data analysis program (P. C. Withers; Visual Basic v6). For each individual animal, the minimum 20-min (e.g., Cooper et al. 2005; Cooper et al. 2009) mean $\dot{V}O_2$, $\dot{V}CO_2$, and EWL were determined for each hour of the experiment (e.g., Fig. 1). These hourly values were converted to a percentage of the lowest hourly value. If the percentage was higher than an earlier percentage, then the earlier lower percentage was used. In this way, it was possible to determine the lowest metabolic rate or EWL that would have been measured if the experiment had run for 1 h, 2 h, 3 h, and so on. This hourly percent of minimum was then used for all subsequent statistical analyses.

We examined whether the decline in hourly minima over time reflected the mathematical inevitability that would have resulted as a consequence of random fluctuations in metabolic rate (or EWL) measurements of individual animals or whether there was a systematic temporal pattern of a decline in $\dot{V}O_2$, $\dot{V}CO_2$, or EWL resulting from the animals being more alert and active at the start of the measurement period. As experimental duration increases, there is a larger sample from which to choose the lowest 20-min metabolic rate for each animal up to that point in the experiment. Thus, any random fluctuation in measurement will lead to an hourly average decline using the methods outlined here until all individuals have been sampled for their minimum value. Therefore, for each species, we randomly reassorted the hourly minimal $\dot{V}O_2$ (or $\dot{V}CO_2$, EWL) values for each individual over the experimental period and then calculated the percentage of experimental minimum for each hour. We repeated this random reassortment and calculation of hourly minimum 10,000 times. We determined the number of times that the measured experimental mean hourly percentages were significantly higher than or equal to the randomly reassorted mean hourly percentages with the probability that the mean for the hour was greater than random, calculated as this number divided by 10,000. We interpret $P < 0.05$ as indicating significance of a nonrandom animal effect, that is, evidence of a higher $\dot{V}O_2$ (or $\dot{V}CO_2$, EWL) for that measurement period than expected based on random fluctuations in metabolic rate.

Statistical analyses were completed using statistiXL (ver. 1.6),

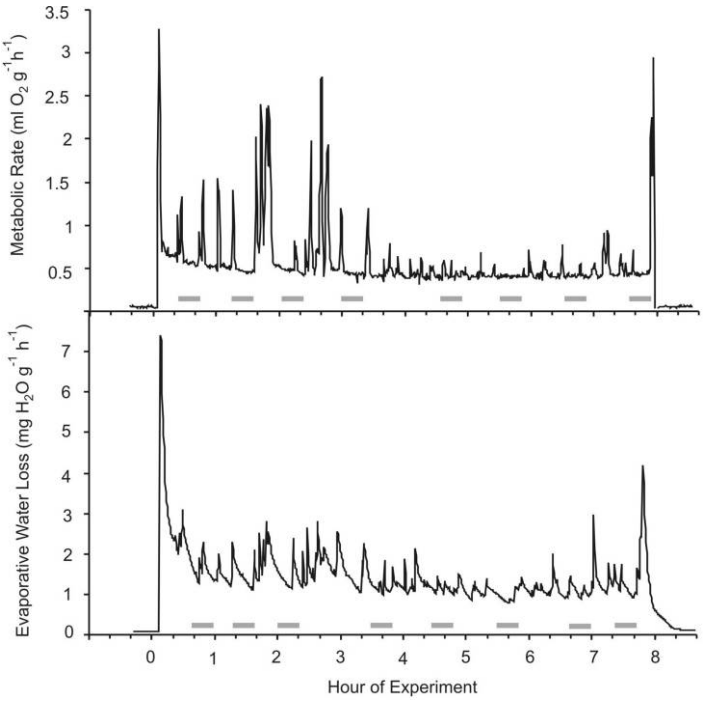

Figure 1. Experimental time course for metabolic rate and evaporative water loss of a little red kaluta (*Dasykaluta rosamondae*). Gray bars indicate hourly minima.

SPSS (ver. 11.0), and a custom-written macro in Excel. Species and measurement duration (time) effects for each variable were determined with two-way ANOVAs and Student-Newman-Keuls (SNK) post hoc tests using the rank of the percentage values from highest to lowest (equivalent to a nonparametric Kruskal-Wallis test; Boos and Brownie 1995). The effect of measurement duration (time) for each species was further examined by ANOVA (also using ranked data) with simple a priori contrasts (comparing each hour in turn with the final hour). Values are presented as mean ± SE for each species unless stated otherwise.

Results

There were significant effects of species ($F_{5,257} = 11.7$, $P < 0.001$) and time ($F_{8,257} = 63.8$, $P < 0.001$) on the percentage of experimental minima attained at each hourly minimum for $\dot{V}O_2$ by two-way ANOVA, and there was a significant interaction between time and species ($F_{34,257} = 1.93$, $P = 0.002$). Ningauis and hairy-footed dunnarts had higher mean rankings for the percentage of minimum $\dot{V}O_2$ than the other four species (SNK, $P < 0.05$). For $\dot{V}CO_2$, there was also a significant effect of both species ($F_{5,257} = 23.3$, $P < 0.001$) and time ($F_{8,257} = 61.4$, $P < 0.001$) on the percentage of experimental minima attained at each hourly minimum. Again, there was a significant interaction between time and species ($F_{34,257} = 2.7$, $P < 0.001$). Post hoc tests separated the species into three groups (SNK $P < 0.05$): pygmy possums had the lowest mean rank; kalutas, mouse opossums, and fat-tailed dunnarts had intermediate mean ranks; and ningauis and hairy-footed dunnarts had the highest mean ranks. A significant effect of both species ($F_{5,257} = 27.7$, $P < 0.001$) and time ($F_{8,257} = 58.4$, $P < 0.001$) was found for EWL along with a significant interaction between species and time ($F_{34,257} = 3.1$, $P < 0.001$). Post hoc tests separated the species into four groups (SNK $P < 0.05$) with the order of mean ranks from lowest to highest as follows: pygmy possums, mouse opossums and fat-tailed dunnarts, fat-tailed dunnarts and kaluta, and ningauis and hairy-footed dunnarts.

We conducted one-way ANOVAs individually for each species because of significant differences between species and the significant interaction terms between species and time (which occurs as the initial species effect is significant but minima converge on 100% for all species). The significant effect of measurement duration on the measurement of minimal $\dot{V}O_2$, $\dot{V}CO_2$, and EWL for all six species remained (Table 1; Fig. 2). For all species, the hourly minimum metabolic rate ($\dot{V}O_2$ and $\dot{V}CO_2$) and EWL declined sharply over the first 2 h and from then either remained constant or continued to decline more gradually (Fig. 2). Hourly minimum metabolic rate remained significantly higher than the overall experimental minimum for between 2 (ningaui, hairy-footed dunnart) and 5 h (kaluta, fat-tailed dunnart), while the hourly minimum EWL remained higher than the experimental minimum for between 3 (pygmy possum, fat-tailed dunnart) and 5 h (kaluta, hairy-footed dunnart; Table 1). Hourly minimum $\dot{V}O_2$ values were statistically indistinguishable from the experimental minimum by 3 (ningaui, hairy-footed dunnart) to 6 (fat-tailed dunnart) h but, although not statistically different, were still 100% ± 0.5% to 104% ± 3.5% of the actual experimental minimum. Hourly

Table 1: Effect of measurement duration on the measurement of minimal $\dot{V}O_2$, $\dot{V}CO_2$, and EWL for six species

	Cercatetus concinnus	*Dasykaluta rosamondae*	*Gracilinanus agilis*	*Ningaui yvonneae*	*Sminthopsis crassicaudata*	*Sminthopsis hirtipes*
Mass g ± SE (*n*)	13.6 ± .7 (6)	38.3 ± 1.7 (8)	33.5 ± .7 (6)	6.5 ± .15 (6)	11.8 ± 5.6 (6)	13.4 ± 1.0 (7)
$\dot{V}O_2$:						
Effect of duration:						
F	15.4	22.0	7.5	3.5	20.9	7.5
df	7, 40	6, 49	6, 34	7, 40	8, 45	6, 34
P	<.001	<.001	<.001	.005	<.001	<.001
Hours different (contrast):						
Hours	1–4	1–4	1–3	1–2	1–5	1–2
P	≤.004	≤.016	≤.035	≤.026	≤.005	<.001
First hour not different						
(% of minimum)	5 (102 ± 1.1)	5 (104 ± 3.5)	4 (103 ± 1.7)	3 (104 ± 1.6)	6 (100 ± .5)	3 (101 ± 1.1)
$\dot{V}CO_2$:						
Effect of duration:						
F	15.4	12.5	19.9	3.3	11.3	7.7
df	7, 40	6, 49	6, 34	7, 40	8, 45	6, 34
P	<.001	<.001	<.001	<.007	<.001	<.001
Hours different (contrast):						
Hours	1–3	1–5	1–4	1–2	1–4	1–3
P	≤.012	<.001	≤.008	≤.002	<.001	<.001
First hour not different						
(% of minimum)	4 (100 ± .3)	6 (101 ± .8)	5 (101 ± .6)	3 (109 ± 6.0)	5 (104 ± 1.8)	4 (108 ± 7.6)
EWL:						
Effect of duration:						
F	26.2	12.5	32.0	13.1	3.2	7.4
df	7, 40	6, 49	6, 34	7, 40	8, 45	6, 34
P	<.001	<.001	<.001	<.001	<.002	<.001
Hours different (contrast):						
Hours	1–3	1–5	1–4	1–5	1–3	1–5
P	<.001	<.001	≤.024	<.001	≤.24	<.001
First hour not different						
(% of minimum)	4 (101 ± 1.0)	6 (106 ± 3.7)	5 (100 ± .02)	6 (102 ± 1.7)	4 (118 ± 16.8)	6 (106 ± 3.8)

Note. Shown are body mass, statistical effect of measurement duration, hours of measurement during which the hourly mean metabolic rate or EWL were significantly higher than the experimental minimum, and the first hour that was not significantly different from the minimum for oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and evaporative water loss (EWL) of six species of small marsupial.

minimum $\dot{V}CO_2$ values had become statistically indistinguishable from the experimental minimum by 3 (ningaui) to 6 (kaluta) h but were still 100% ± 0.3% to 109% ± 6% of the the actual experimental minimum (Table 1). For EWL, hourly minimum values had become statistically indistinguishable from the experimental minimum by 4 (pygmy possum, ningaui) to 6 (kaluta, ningaui) h but were still 100% ± 0.02% to 118% ± 16.8% of the actual experimental minimum.

Random reassortment (10,000 times) of hourly $\dot{V}O_2$ minima indicated that measured experimental means were significantly higher ($P < 0.05$) than randomized means for all species in the first hour, except mouse opossums, and remained higher for up to 3 h (kalutas; Fig. 2). For $\dot{V}CO_2$ minima, measured experimental means were all significantly higher ($P < 0.05$) than randomized means for the first hour and remained higher for up to 5 h (kalutas; Fig. 2). For EWL minima, measured experimental means were all significantly higher ($P < 0.05$) than randomized means for the first 3 h and remained higher for up to 7 h (hairy-footed dunnarts; Fig. 2).

Discussion

The current working definition of BMR states that among other criteria, the animal must be at rest (McNab 1997). However, animals, especially wild-caught individuals not familiar with being handled or held in an artificial environment, may require some time to achieve a resting state during metabolic experiments. Here, we have demonstrated a significant influence of experimental duration on the determination of BMR and standard EWL of six small marsupial species.

Hayes et al. (1992) demonstrated for wood mice that the lowest 15-min mean $\dot{V}O_2$ after 30 min of measurement was 165% of the lowest 15-min mean in the sixth hour of measurement. They attributed this elevated metabolism during the early part of an experiment to the animal's response to handling. The effect of time on the measurement of metabolic rate for voles was less than for wood mice but still significant, with the lowest 15-min mean $\dot{V}O_2$ in the first 30 min of the experiment being 113% of that in the sixth hour (Hayes et al. 1992).

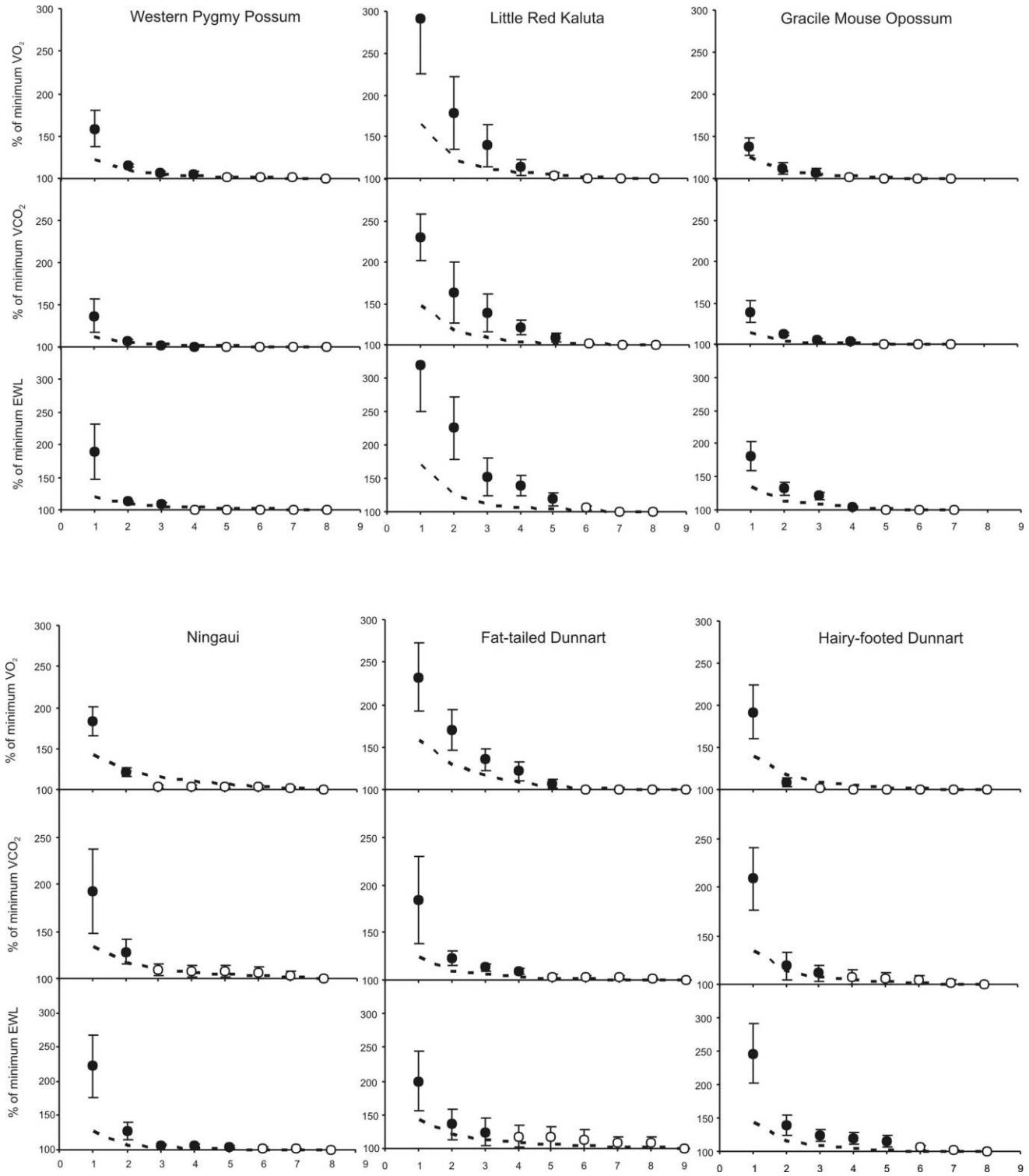

Figure 2. Relationship between experimental duration (h) and the percent of experimental minimum oxygen consumption ($\dot{V}o_2$), carbon dioxide production ($\dot{V}co_2$), and evaporative water loss (EWL) for six small marsupials: western pygmy possum (*Cercartetus concinnus*, $n = 6$), little red kaluta (*Dasykaluta rosamondae*, $n = 8$), gracile mouse opossum (*Gracilinanus agilis*, $n = 6$), ningaui (*Ningaui yvonneae*, $n = 6$), fat-tailed dunnart (*Sminthopsis crassicaudata*, $n = 6$), and hairy-footed dunnart (*Sminthopsis hirtipes*, $n = 7$). Solid symbols are significantly different from the experimental minimum; open symbols are not significantly different from the experimental minimum. The dashed line is the mean percent of the experimental minimum for 10,000 random reallocations of hourly minimum $\dot{V}o_2$, $\dot{V}co_2$, and EWL values. Values are mean ± SE.

Table 2: Body mass, basal metabolic rate (BMR), and measurement duration for 21 species of small (≤40 g) marsupial (taken from Withers et al. 2000, 2006)

Species	Mass (g)	BMR (mL O_2 g^{-1} h^{-1})	Duration (h)	Reference
Planigale maculata	13.1	1.01	1	Morton and Lee 1978
Acrobates pygmaeus	14	1.08	1	Fleming 1985
P. maculata	8.5	1.26	2	MacMillen and Nelson 1969
Sminthopsis crassicaudata	14.5	1.67	2	MacMillen and Nelson 1969
S. crassicaudata	19	1.88	2	Kennedy and MacFarlane 1971
Antechinus stuartii	22.1	1.53	2	MacMillen and Nelson 1969
Antechinomys laniger	24.2	.98	2	MacMillen and Nelson 1969
Monodelphis brevicaudata	40	.92	2	McNab 1978
S. crassicaudata	15.6	1.85	3.5	Hinds et al. 1993
Sminthopsis macroura	16.7	1.26	3.5	Hinds et al. 1993
Planigale gilesi	9.5	1.28	5	Dawson and Dawson 1982
Marmosa microtarsus	13	1.44	10	Morrison and McNab 1962
Sminthopsis murina	19	1.13	18	Geiser et al. 1984
P. gilesi	8.3	1.43	24	Geiser and Baudinette 1988
Tarsipes rostratus	10	2.9	24	Withers et al. 1991
Ningoui yvonneae	11.6	1.35	24	Geiser and Baudinette 1988

Note. For species with measurement durations of 1–3.5 h, the durations were probably too short to have obtained BMR. For species with measurement durations of 5–24 h, the durations were probably sufficient to attain BMR.

Gallivan (1992) also found that allowing animals to become familiar with the metabolic system and increasing measurement duration resulted in lower estimates of metabolic rate for harp seals and manatees. Steffensen et al. (1994) and Steffensen (2002) attributed the elevated metabolic rates of several fish species after introduction into a metabolic system as a response to the presence of the researcher and handling stress. This effect lasted several hours and increased metabolic rate by as much as eight times resting values.

The decline in hourly minimum metabolic rate or EWL over time can result from random sampling effects and/or a change in metabolic rate or EWL as the animal attains basal conditions after handling and adjusts to its novel environment. Even if an animal was accustomed to handling and immediately adjusted to a metabolic chamber with random periods of nonrest (e.g., alertness, activity, grooming), repetitive sampling over time would result in successively lower hourly minimum measurements converging on 100%. Comparison of the mean percentage of minima for randomly sampled hourly minima with our actual measured data (see Fig. 2) indicates that all species' metabolic and EWL measurements differed from random for at least the first hour of measurement (except $\dot{V}O_2$ measurements for gracile mouse opossums) and for up to 7 h of measurement. This indicates that there is an effect of handling and/or introduction into an unfamiliar environment on the animals that contributes to an elevated metabolic rate in the early part of an experiment and that this, as well as sampling effects, contributes to the temporal effects on measurement of physiological variables that we have demonstrated.

Overall, mean experimental durations of 4.3 ± 0.49 h for $\dot{V}O_2$, 4.5 ± 0.43 h for $\dot{V}CO_2$, and 5.2 ± 0.40 h for EWL were required for the six marsupial species to attain levels statistically indistinguishable from basal/standard values. Substantial overestimations of BMR and standard EWL result from shorter experimental periods (Table 2). These estimates of experimental duration are for wild-caught individuals that were not accustomed to a metabolic chamber, as field-fresh individuals are usually the desired target of studies attempting to determine BMR/standard EWL for a species rather than long-term captive individuals. Required experimental durations may differ for tame, long-term captive animals or for individuals that are familiar with the experimental procedure.

There were substantial interspecific differences in the time required to measure metabolic rate for different small marsupial species. Ningauis and hairy-footed dunnarts quickly reached a metabolic rate that was statistically indistinguishable from minimal after only 3 h of measurement. However, other species required much longer experimental periods. Kalutas and pygmy possums required 5 h to attain metabolic rates that were statistically indistinguishable from minimal rates, and fat-tailed dunnarts required 6 h. For these species, measurement for 3 h would overestimate BMR by 6% ± 2.5%, 40% ± 24.8%, and 35% ± 12.8%, respectively. Because of this variability between species, it is necessary to ensure that initial measurements for a previously unmeasured species are conducted over a long period of time (e.g., 7–8 h) to ensure that the experimental period is sufficient to attain BMR for that species.

There were also substantial interspecific differences in the time required to attain standard rates of EWL. Two species required 1 h less (pygmy possums and hairy-footed dunnarts), while the others required between 1 and 3 h longer than for BMR to attain standard EWL. The shortest time required was 4 h (pygmy possums and hairy-footed dunnarts), while the longest time required was 6 h (ningauis, hairy-footed dunnarts

and kaluta). For these last three species, EWL would have been 6% ± 2.2%, 40% ± 15.7%, and 21% ± 8.8%, respectively, higher than the minimal value if they had only been measured for 4 h. Once again, given the interspecific variability in required measurement duration, it is clear that any previously unstudied species must be measured for a substantial period to ensure that measurement duration is sufficient to attain minimal rates of EWL. The measurement period required to attain BMR is not necessarily sufficient to attain standard EWL.

Previous studies have also observed species differences for the effect of experimental time on achieving a minimal measurement. Substantial interspecific differences in the effect of measurement duration on metabolic rate for wood mice and voles were attributed to the docility of voles, which appeared to remain calm during handling and did not show any apparent fright response (despite being recently captured from the field), unlike the more active wood mice (Hayes et al. 1992). We did not, however, notice any obvious relationship between a species' overall demeanor and activity level and the time required to attain minimal metabolic rate. In fact, the most docile species involved in this study, the western pygmy possum, was one of the species that took the longest to attain a minimal rate (5 h), while the more active and aggressive ningauis only required 3 h to attain metabolic rates statistically indistinguishable from minimal. Therefore, it is important to directly measure the time required to attain minimal metabolic rates and not estimate these based on the apparent demeanor of a species.

Rates of EWL often took longer to approach minimal values than $\dot{V}O_2$ or $\dot{V}CO_2$. EWL is generally correlated with metabolic rate, as the increase in respiratory ventilation necessary to accommodate a higher metabolic rate results in increased respiratory water loss due to increased respiratory frequency and/or tidal volume. Activity may also increase cutaneous EWL by reducing the evaporative boundary layer and increasing body temperature, therefore increasing the water vapor pressure differential between the animal and the ambient air. However, measurement of EWL may become uncoupled from the measurement of metabolic rate, as the washout of water vapor from the metabolic chamber can be slower than for O_2 or CO_2 because of the "stickiness" of water vapor, especially if there are plastic components in the metabolic system. Our technique of measuring EWL, where the incurrent air is used to dry any urine or faeces produced during the experiment, would also uncouple metabolic rate and EWL if the animal urinates or defecates during the experiment. In this situation, EWL will be higher than expected from metabolic rate until the incurrent air dries the urine/faeces. Consequently, it is not unexpected that we found that the time required to reach an EWL value statistically indistinguishable from minimal was on average longer than the time required to attain BMR.

To assess the likely validity of current small marsupial BMR data, we examined the marsupial BMR data set (see Withers et al. 2000, 2006) for small (≤40 g) marsupials and referred to the original papers to determine measurement duration for these species. Of the 29 measurements for small species, measurement duration to attain BMR was provided for 16. Of these, only six studies had measurement durations longer than 4 h (the mean minimal duration required to achieve a metabolic rate statistically indistinguishable from the minimal metabolic rate), while 10 studies had measurement durations of 4 h or less (Table 2). Therefore, it is likely that over half of the current small marsupial data set overestimates BMR. Two species for which BMR data and measurement duration are available in the literature were measured during this study (fat-tailed dunnart and ningaui). For the fat-tailed dunnart, the two previous BMR estimates of 1.85 and 1.67 mL O_2 g^{-1} h^{-1}, obtained with experimental durations of 2 h (MacMillen and Nelson 1969; Kennedy and MacFarlane 1971), were 128% and 115% of our estimate of BMR (1.45 mL O_2 g^{-1} h^{-1}). For the ningaui, the previous BMR estimate of 1.35 mL O_2 g^{-1} h^{-1}, determined with an experimental duration of 24 h (Geiser and Baudinette 1988) was 65% of our estimate of BMR (2.09 mL O_2 g^{-1} h^{-1}). Although it is difficult to make direct comparisons between studies because of differences in T_a, body mass, body temperature, and time in captivity, it does appear that a longer experimental duration is consistent with a lower estimate of BMR.

For small marsupials (≤40 g), EWL data are available only for four species (see Cooper et al. 2005; Withers et al. 2006): the brown antechinus (*Antechinus stuartii*), the common planigale (*Planigale maculata*) the fat-tailed dunnart (*Sminthopsis crassicaudata*), and the stripe-faced dunnart (*Sminthopsis macroura*). The first three species came from a single study (Hinds and MacMillen 1986) with an experimental duration of only 2 h. Thus, these EWL values are likely to be overestimated by around 45%. Indeed, for the one species common to this study and Hinds and MacMillen's (1986) study, their value of 91.3 mg H_2O h^{-1} for the fat-tailed dunnart was 30% higher than our mean minimum EWL of 70 ± 1.1 mg H_2O h^{-1}. However, it is difficult to directly compare these studies, because Hinds and MacMillen (1986) used a gravimetric method to measure EWL for 1-h periods, whereas we used near instantaneous measures of excurrent chamber relative humidly using a thin-film capacitance hygrometer and calculated the minimal 20-min average. It is not clear if the difference in values for the fat-tailed dunnart results from differences in measurement duration, measurement technique, or both. The EWL for the stripe-faced dunnart was measured over a period of 24 h using the same technique used in this study, and it was very low, at 36 mg H_2O h^{-1}(Cooper et al. 2005), almost half that of the fat-tailed dunnart. Whether this difference is a species difference or results from a long 24-h measurement duration is unclear.

We have thus far addressed the question of how long is long enough for the mean BMR/EWL of a group of individuals to not significantly differ from the long-term minimum, that is, to measure BMR and standard EWL. The mean percentage of minima for six small marsupial species that did not differ significantly from 100% was 102.3 ± 0.6 (range 100.5–104.2) for $\dot{V}O_2$, 103.7 ± 1.5 (range 100.9–108.6) for $\dot{V}CO_2$, and 105.3 ± 2.8 (range 100.0–118.2) for EWL. Therefore minimal measurement durations based on this statistical "no difference" approach would overestimate BMR by 2%–4% and standard EWL by 5%. The artifact of an insufficiently long experimental

duration will always overestimate BMR/standard EWL; that is, if the minimum 20-min average is calculated during an experimental period, then extending that measurement period can only result in an equal or lower estimate of BMR/standard EWL (Hayes et al. 1992)

Even with an extended experimental duration (e.g., 6–8 h), it is possible that an individual (or individuals) might not reach their BMR or standard EWL; that is, a lower metabolic rate/EWL value would have been measured had the experiment been longer. In this study, $\dot{V}O_2$, $\dot{V}CO_2$, and EWL has usually reached 100% of the minimal value a few hours before the end of the experiment, suggesting that little further decrease would have occurred had the experimental period been extended. Extending the experimental duration will minimize the probability of not attaining the lowest measurement of metabolic rate and EWL and hence the best estimate of BMR/standard EWL. Thus, 24-h duration experiments are likely to achieve the best estimates of BMR/standard EWL because they would include the entire rest phase. However, such long-term experiments are typically designed to examine torpor, and BMR is obtained under the same experimental conditions for comparison (e.g., Geiser and Baudinette 1987, 1988; Cooper et al. 2005). Much shorter measurement durations are routinely used when the primary aim of the experiment is to attain BMR/standard EWL (e.g., Geiser 1986; Geiser and Baudinette 1987). A disadvantage of extending the experimental period is that the animal will increase activity and metabolic rate/EWL as its active phase approaches. This would compromise measurement of body temperature (typically done at the end of the experiment using a thermocouple) and other related parameters (e.g., tidal volume and minute volume). Thus, it is usually desirable to conclude an experiment while the animal is still in a state of BMR/standard EWL, and experimental duration may often be a tradeoff between a sufficiently long experimental period to attain BMR/standard EWL and not extending the experimental period into the animal's active phase.

We have shown that appropriate experimental duration is an important component of the measurement protocol for both BMR and standard EWL and needs to be rigorously observed in future studies. There were species differences in the actual time required to achieve basal/standard values, although in general, O_2 consumption rate reached basal after 4.3 h, CO_2 production after 4.5 h, and standardized EWL was reached after 5.2 h. Measurement durations shorter than these are common in the literature, and thus for many small marsupials, BMR and standardized EWL may not have been attained, rendering values invalid for comparison with correctly measured BMR/standardized EWL values.

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