Does Personality Correlate with Energetics in Little Brown Bats (*Myotis lucifugus*)?

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A thesis submitted in partial fulfillment of the Honours Thesis Course (05.4111/6)

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2010

Abstract

Basal metabolic rate (BMR), the minimum energy expenditure required to maintain an animal at rest, is highly variable within and between species and understanding this variation is a central theme of evolutionary physiology. The expression of energy-saving torpor or heterothermy by endotherms (i.e., mammals and birds) is also highly variable. One possible correlate of this energetic variation is animal "personality". Personality can be defined as a set of behavioural responses of an individual that are consistent over time and/or across situations. Recent work suggests that personality should correlate with BMR and I predicted that it should also correlate with tendency to express torpor. I tested for these relationships by measuring personality, BMR and torpor expression in juvenile individuals from two populations of little brown bats (*Myotis lucifugus*). Exploratory behaviour was measured twice for each individual, using an ecologically relevant, novel-environment test designed to mimic how bats might explore potential roost cavities in the wild. For the same individuals I used open-flow respirometry to measure BMR and quantify torpor expression. I found that some behaviours were repeatable over time and behavioural traits clustered into three categories (i.e., activity, exploration and anxiety) in a way that was consistent with past studies of rodents, birds and fish. Contrary to my hypothesis I did not find strong evidence of a relationship between personality and BMR but various aspects of personality were related to torpor expression. My results provide preliminary evidence that, within species, variation in energetics does correlate with individual variation in personality.

Acknowledgements

I would like to thank Dr. C. Willis for his accessible brainpower, unrelenting patience and infinite help throughout this whole experience. I also thank Dr. M. Timonin for her kindness, her guidance and for the infinite hours of her assistance with behavioural recording, without complaint. I would also like to thank the other bat lab members for their advice, inspiration and friendship both in the field and in the "real world". Thank you to Dr. M. Wiegand for coordinating the Honours thesis course and providing valuable feedback, along with my committee members Dr. S. Forbes and Dr. J. Franck. Funding for this project was provided by the University of Winnipeg, Natural Sciences and Engineering Research Council (NSERC) Undergraduate Student Research Award, and Discovery Grant to Dr. C. Willis as well as grants from the Canada Foundation for Innovation and the Manitoba Research and Innovation Fund. In addition, I thank the residents of Altamont, MB and Misipawistik First Nation for access to my study animals and Manitoba Conservation for logistical support and for allowing me to do my research. Last, but certainly not least, I would like to thank my family and friends for their constant support, encouragement and numerous distractions, when necessary!

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1.0 Introduction

Maintaining a balance among energy acquisition and consumption is essential for survival in animals (Frappell and Butler 2004). Metabolic rate (MR) reflects the rate at which animals take up resources from the environment, convert them into usable forms of energy and allocate them to fitness-related processes important for maintenance, growth and reproduction (Brown *et al.* 2004). The minimum amount of energy required to sustain life is known as basal metabolic rate (BMR) and is highly standardized allowing for comparison among many animal species (Speakman *et al.* 2004). BMR is also viewed as an important trait in animal ecology because it correlates with other traits important for fitness (e.g., field metabolic rate, maximum metabolic rate, resting heart rate; White and Seymour 2004) but can be measured much more easily. As a measure of energy requirements, it helps indicate the demands for resources an individual will place on its environment.

BMR is highly variable both within and among species (Speakman *et al.* 2004). Among species, over a broad range of taxonomic groups, body mass accounts for most of this variability (White and Seymour 2004) but, after the influence of body mass is controlled for, many other factors also contribute such as muscle mass (Raichlen *et al.* 2010), organ size (Konarzewski and Diamond 1995), diet (Bozinovic *et al.* 2007, Mueller and Diamond 2001, McNab 1988), climate (Lovegrove 2003), and latitude (Lahti *et al.* 2002). Within species, the relationship between BMR and body mass is typically much weaker or even non-significant (Careau *et al.* 2008, Mueller and Diamond 2001, Hayes *et al.* 1992). For example, Unruh (2009) demonstrated that in meadow voles (*Microtus*

pennsylvanicus) ranging in size from 14-38 g, that body mass only explains about 43% of the inter-individual variability in BMR.

Another energetic trait that is highly variable in the wild is expression of facultative heterothermy or torpor. Torpor is a controlled reduction of body temperature, metabolic rate and other physiological functions used by many species of endotherms (i.e., mammals and birds) to save large amounts of energy (Willis 2007, Geiser 2004). Small endotherms face distinct challenges in terms of their energy budgets due to their large surface area to volume ratio and rapid rates of heat loss (Speakman 1997). Prolonged periods of high metabolic rate require high energy intake (Geiser 2004.) Thus, if conditions are unfavourable (e.g., reduced food availability or low ambient temperature), many small endotherms employ torpor to facilitate survival (Frappell and Butler 2004). As for BMR, small mammals and birds exhibit differences in their patterns of torpor expression both within and among species, and again, a large portion of the variability is accounted for by body mass, as well as phylogeny (Geiser 2004, 1998, French 1985). Many other factors also contribute to individual differences in torpor expression such as reproductive status (Lausen and Barclay 2003, Chruszcz and Barclay 2002), sex (Dietz and Kalko 2006, Cryan and Wolf 2003, Geiser 1988), diet (Munro *et al.* 2005, Frank 1992), time of year (Hiebert 1993, French 1989, Heldmaier and Steinlechner 1981) and weather (Norquay 2009, Turbill 2008).

One factor that could influence both BMR and torpor expression in heterothermic endotherms is animal temperament or personality. Personality is defined as a suite of correlated behavioural traits that are consistently expressed by an individual over time and across different situations (Réale *et al.* 2007). These inter-individual differences in

behaviour are heritable across generations and repeatable over an individual's lifetime (Cockrem 2007, Réale *et al.* 2007, Dingemanse and Réale 2005, Dingemanse *et al.* 2002). Through various behavioural tests, five ecologically relevant dimensions of personality have been well defined (Réale *et al.* 2007). These include reaction to risky situations (i.e., shyness/boldness; Brown *et al.* 2007, Réale and Festa-Bianchet 2003), reaction to novel objects or situations (i.e., exploration/avoidance; Carere *et al.* 2005 , Dingemanse *et al.* 2002, Verbeek *et al.* 1993), activity levels (Boon *et al.* 2008, Sih *et al.* 2003), agonistic reactions to conspecifics (i.e., aggressiveness; Garamszegi *et al.* 2009, Bell 2005, Lahti *et al.* 2002) and non-aggressive reactions to the presence or absence of conspecifics (i.e., sociability; Favre *et al.* 2008). Recent studies have shown that, via relationship to resource acquisition and reproductive ability, some of these traits can be linked to life-history and fitness (Careau *et al*. 2009, Biro and Stamps 2008, Boon *et al.* 2007, Dingemanse *et al.* 2004). For example, high quality exploration may improve foraging ability (Dingemanse and de Goede 2004, Verbeek *et al.* 1993) which will affect the amount of energy an individual can obtain. Animals live in environments that vary in time and space, resulting in changing selection pressures and trade-offs that may help to maintain multiple personality traits within a single population (Sih *et al.* 2004). For example, in Great Tits (*Parus major*) fast exploration/high activity increased the competitive ability of individuals to find food and acquire breeding territories, leading to greater weight gain of adults and larger clutch sizes (Réale *et al.* 2007). However, slow exploring, less active individuals had increased nest success and larger offspring (Both *et al.* 2005).

Dimensions of personality are often classified along a continuum with the two definitive behavioural traits placed at either extreme (Réale *et al.* 2007). The "proactive/reactive" continuum categorizes individuals based on their response to a novel, presumably stressful situation (Cockrem 2007). It incorporates aspects of exploration and curiosity, fearfulness, aggression and the physiological (i.e., corticosteroid) stress response (Sih *et al.* 2004, File and Wardill 1975). A range of novel environment tests have been developed to assess these aspects of personality (Boon *et al.* 2008, Martin and Réale 2008, Carere *et al.* 2005, Verbeek *et al.* 1993) and these behaviours are thought to reflect curiosity and fear (File and Wardill 1975). Using these measures, proactive and reactive personality types have been well-defined. Proactive individuals tend to be fast, superficial explorers, have an active response characterized by high aggression, high sympathetic nervous system reactivity (i.e., a strong "fight-or-flight" response) and low corticosterone release (Careau *et al*. 2009, Koolhaas *et al.* 1999). Reactive individuals are characterized by immobility (fear-induced "freezing" behaviour), low aggression, high parasympathetic reactivity (i.e., a "rest-and-digest" response) and high corticosterone release (Koolhaas *et al.* 1999). They tend to be slow, thorough explorers (Careau *et al.* 2009). Evidence of this range of coping strategies has been found in rodents (Koolhaas *et al.* 1999), birds (Cockrem 2007, Carere *et al.* 2005) and fish (Brelin *et al.* 2005, Schjolden *et al.* 2005), suggesting mechanisms which are consistent across many species and groups reflecting fundamental links between physiology and behaviour via the corticosterone stress responses (Cockrem 2007).

Given the apparently high energy lifestyles of proactive individuals and lower energy lifestyles of reactive ones, Careau *et al*. (2008) hypothesized that there might also

be a link between personality and metabolism. In a subsequent paper, Careau *et al*. (2009) found evidence for this hypothesis as a negative correlation between exploration thoroughness and BMR among multiple species of rodents after controlling for body mass and phylogeny. They argued that exploration thoroughness is inversely related to boldness and aggressiveness which are both highly energetically demanding. Thus, proactive species with lower exploration thoroughness had higher BMR presumably because of their high energy lifestyles characterized by aggression, boldness and high activity levels, whereas reactive species had lower BMR because of their lower energy lifestyles (Careau *et al.* 2009). However, in the only intra-specific study of the relationship between personality and BMR to date, no significant correlation was found in meadow voles (*Microtus pennsylvanicus*), although the sample size of this study was relatively small (Unruh 2009).

Addressing the relationship between personality and energetics has significant implications for evolutionary ecology. BMR (Brown *et al.* 2004, McNab 1980), torpor expression (Audet and Fenton 1987, Racey and Swift 1981) and personality (Biro and Stamps 2008, Smith and Blumstein 2008) are all known to be independently related to life-history. Thus, ecological and physiological factors which have, in the past, largely been considered in isolation as separate entities may be closely related and co-evolve within populations as correlated suites of traits. Since consistent phenotypic differences in both personality (Dingemanse *et al.* 2002) and BMR (Boratynski and Koteja 2008) are heritable and repeatable, these traits are potentially subject to natural selection. It is possible that the maintenance of various life-history traits, seen across many animal taxa, has been shaped by selection for competing coping strategies. Thus, the patterns of

behaviour or the physiological traits expressed by free-living individuals within populations may be constrained by this potential link between personality and physiology (Carere *et al.* 2005).

My objective was to test Careau *et al*'s (2008) hypothesis that personality (i.e., exploration, activity) is correlated with BMR in little brown bats (*Myotis lucifugus*). Given the clear importance of heterothermy for survival and fitness in many small endotherms, I also aimed to test the hypothesis that personality relates to the expression of torpor. To date there have been no behavioural studies of personality variation in bats so an additional objective of my study was to determine if similar patterns of personality variation can be detected in bats, the second largest order of mammals, as in rodents, birds and fish.

2.0 Materials and Methods

2.1 Field Sites

All procedures were approved by the University of Winnipeg Animal Care Committee and conducted in compliance with guidelines of the Canadian Council on Animal Care. Bats were captured from two field sites in Manitoba. The first was a maternity colony in the community centre of Altamont, Manitoba (49°21'N 98°35'W) in the southwest corner of the province. The second was a hibernaculum called Microwave Cave (McRitchie and Monson 2000) near Grand Rapids, Manitoba (53º12'N 99º19'W) at the north end of Lake Winnipeg. Work was carried out in Altamont from 9–19 August 2009 and in Grand Rapids from 24-31 August 2009. To reduce the potential for age effects, behaviour and metabolic rates were measured for juvenile little brown bats only.

2.2 Capture

Bats were caught at each site using harp traps. Traps were set at the entrance of the maternity roost in Altamont and at the entrance of Microwave cave during pre-hibernation mating swarms. Traps were opened after dusk and remained open until a sufficient number of bats were obtained (3-6 per night). Captured individuals were sexed, weighed (to the nearest 0.1g), and aged (adult or young-of-the-year based on the degree of ossification of the finger joints, Anthony 1988). I also measured forearm length for calculation of body condition index (BCI) (i.e., calculated by dividing body mass (g) by forearm length (mm) (Speakman and Racey 1986)) and then placed each bat in an individual cloth bag. Bats were then transported to a field laboratory 10 – 40 km from the site of capture where they were housed for up to 3 days in a quiet room at room temperature under natural photoperiod. All bats were provided water with an eyedropper

before and after each behavioural and metabolic trial. All individuals were fed mealworms (*Tenebrio molitor*) following behavioural testing and metabolic trials.

2.3 Personality Measurements

To quantify personality I designed a novel environment test modeled after the rodent "hole-board test (File and Wardill 1975, Nolan and Parkes 1973) but modified to be more ecologically relevant for bats by encouraging behaviours a bat might exhibit while selecting from potential tree roost cavities. The test chamber was constructed from a plastic storage container (57 x 42 x 14 cm) outfitted with a plexiglass top. To facilitate climbing by the bats, the test was constructed to hang vertically and plastic window screening was attached along the back surface for grip. To provide openings for bats to explore, four circular holes (2 cm deep by 3 cm diameter) were drilled into this surface. As in hole-board tests used to assess exploration propensity and fearfulness of rodents (Martin and Réale 2008), I varied the distances of the holes from the edges (i.e., walls) of the test. Two were positioned closer to the centre (holes 1 and 2; 15 cm from left or right wall of the test) and two were positioned closer to the corners of the box (holes 3 and 4; 5.5 cm from the left or right wall). Investigation of the holes closer to the centre of the test would potentially indicate less anxiety or fear (Ohl *et al.* 2001). At the bottom of the test, I attached a smaller (14.5 x 14.5 cm) plastic "start box" to hold each bat prior to its release into the test chamber. A sliding door separated the bat from the test until behavioural recording began.

Behavioural testing was started one hour after returning to the field laboratory each night. Prior to being tested, to ensure bats were normothermic and active, I measured each individual's body temperatures by inserting a 1 mm diameter

thermocouple probe 3 mm into the rectum and bats were not tested until their body temperature exceeded 30°C. Once they were normothermic, each bat was placed in the start box for two minutes and then I opened the entrance to the test. Bats were given a maximum of one minute to enter the test on their own and if they did not enter voluntarily I gently pushed them out of the start box and into the testing chamber. The start box was then blocked off with the sliding door to ensure that bats could not return to it once they had entered the test. To simulate natural conditions, all trials were run in a darkened room at night and the behaviour of each bat was recorded for 10 minutes using a miniature infrared video camera (Bat House Spy Cam, Bat Conservation International, Austin TX) mounted on a tripod. After 10 minutes individuals were retrieved from the test, offered food (mealworms) and water before being returned to their cloth bag until morning. Any fecal pellets in the test box were counted and, to eliminate olfactory cues, the test was cleaned using mild, unscented dish detergent and water after every trial.

Videos were scored for behaviours that I anticipated would reflect exploration propensity, activity and anxiety. These included proportion of time spent walking or crawling along the back surface (i.e., scuttling), proportion of time spent attempting to scale the side walls of the test (i.e., climbing), frequency of flight attempts (i.e., flight), the proportion of time during which wing movement occurred (i.e., flapping), the proportion of time spent echolocating (i.e., echolocating), number head dips in each hole (i.e., frequency of head dips), the latency until the first head dip in each hole (i.e., latency to head dip), the length of time it took to enter the test (i.e., latency to exit the start box), the proportion of time spent grooming (i.e., grooming) and the number of fecal pellets collected (i.e., defecation). I combined latency to first head dip in holes 1 and 2 (i.e., the

two holes that were placed closer to the walls of the test) into a single variable because both holes were the same distance from the edge of the test. I did the same for holes 3 and 4. For behavioural traits to be characterised as "personality" they must be repeatable within individuals across time (Réale *et al.* 2007). Ideally, I would have recaptured individuals at a later date to see if behaviours were repeatable. However, as bats are typically difficult to recapture in the wild, this was not possible for my study. Therefore, to confirm within-individual repeatability, two trials were conducted, separated by an interval of approximately 24-hours. The second test for each individual was conducted under identical conditions the night following their first behavioural test after their respirometry trial had been completed. The order of testing for a group of individuals in captivity at any one time was identical to ensure that the time between subsequent behavioural tests was consistent for all individuals. Some behavioural traits were excluded including flapping (which was perfectly correlated with flying), and frequency of head dipping (which was strongly negatively correlated with latency to head dip).

I used Principal Components Analysis (PCA) to reduce the number of measured traits into a smaller number of composite, synthetic variables or principal component (PC) scores. I used the Kaiser-Guttman criterion (i.e., eigenvalues > 1) to choose which principal components to retain (Kaiser 1991). The PC scores were then used as representative behavioural values in subsequent analyses.

I used intra-class correlation (Fleiss and Cohen 1973) to test for the repeatability of behavioural PC scores within individuals between the two trials and to test for repeatability of the individual behaviours that comprised each of the PC scores. I also

used intra-class correlation to test for the reliability of behavioural scores between two observers (myself and an assistant).

2.4 Metabolic Measurements

Metabolic trials were conducted during the day (i.e., rest phase), between the two sets of behavioural trials. I used open-flow respirometry to measure oxygen consumption (VO_2) and carbon dioxide production (VCO_2) for calculation of MR. Before each trial each bat was weighed and offered water but was not fed to ensure it was post-absorptive during recording. Each bat was sealed in a 235 ml cylindrical metabolic chamber (5 cm in diameter and 12 cm long) which was airtight except for an inlet and outlet tube. The chamber contained a piece of plastic window screening on which the bat could sit, hang and climb, and was large enough to allow bats to move freely. The chamber was hung vertically in a temperature-regulated cabinet and an infrared video camera (Bat House Spy Cam, Bat Conservation International, Austin TX) was positioned in the cabinet to record behaviours of the bats throughout metabolic trials. The cabinet was maintained at 35ºC, which is within the thermoneutral zone of little brown bats (Herreid 1963), for the first 90 minutes of each trial and then reduced to 20ºC for the remainder of each 3.5 hr trial. Measurements obtained during the first hour of the trial were discarded to ensure that bats had become accustomed to the metabolic chamber before measurements were taken. Airflow through the metabolic chamber was maintained at a constant rate of 250ml/min using mass flow controllers (GFC17, Aalborg Instruments Inc., Orangeburg NY). This flow rate ensured that oxygen content never dropped below approximately 20.2% and CO_2 content never rose above approximately 0.7% in the chamber (Willis and Cooper 2009). Sub-sampling was alternated between two separate airstreams: an outside-

air reference airstream and an “animal” airstream using a computer controlled respirometry multiplexer (Intelligent Multiplexer V3, Sable Systems International, Las Vegas NV). Every 15 minutes throughout the metabolic trials, the outside-air channel was sampled for 2 minutes, to provide a baseline measurement of O_2 and CO_2 concentration, and then the animal channel was sampled for the next 13 minutes. Water vapour was removed from the incoming and excurrent airstreams with anhydrous calcium sulphate (Drierite Drying Agent; W.A. Hammond Drierite Co. Ltd., Ohio) to account for the dilution effect of water vapour in the air. A sub-sample of air was drawn (at a flow rate of 90 ml/min) from the excurrent airstream and first past the CO_2 sensor of the gas analyser (Foxbox, Sable Systems International, Las Vegas NV USA), and subsequently past the O_2 sensor. Metabolic recordings were stored on a laptop using ExpeData (v. 1.0.24, Sable Systems International). Flow controllers were calibrated prior to the study using a NIST-traceable soap bubble flowmeter (Bubble-o-meter, LLC., Dublin, OH; Levy 1964). Gas analyzers were calibrated weekly using pure N_2 to zero O_2 and CO_2 sensors, 1% CO_2 in pure N_2 to span the CO_2 analyser, and outside air to span the O_2 sensor since atmospheric air has a stable oxygen concentration (20.95%).

After metabolic trials, I removed bats from the chamber and measured their body temperature and body mass. Bats were provided food and water prior to being returned to their cloth bag in the holding room until their second behavioural trial, which began approximately 24 hours after their first. At the end of the second set of behavioural trials, bats were released at their site of capture. To ensure that I could identify recaptured individuals, each bat was implanted with a passive inductive transponder (PIT tag) prior to release.

I used ExpeData to correct metabolic data for analyzer drift based on baseline recordings from the reference airstream. I also corrected for the timelag between CO_2 and O_2 measurements (i.e., the time it took for air to travel from one sensor to the next). Values for VCO_2 and VO_2 were then calculated using equations 10.6 and 10.7 of Lighton (2008). Values of BMR were identified from within each individual's 2.5 hr metabolic trace as the minimum, stable metabolic rate averaged over a 13-min sampling period, at 35°C. I assumed a linear decrease in body mass throughout metabolic trials to determine each individual's mass at the time its BMR was recorded. I also confirmed that bats were inactive during of the time period that I designated them at BMR, using videos recorded during the respirometry trials.

Due to variable conditions in the field laboratory (especially barometric pressure with passing storm fronts), signals from the O_2 analyser drifted significantly throughout many of the trials. Therefore, I calculated energetic equivalents from VCO_2 values (which were much more stable). Since the bats were not fed prior to metabolic trials I assumed they were metabolising fat and converted values of VCO_2 to milliwatts (i.e., J/sec) using 1L CO_2 = 27.8 KJ. I used least squares regression to determine the relationship between body mass and metabolic rate (mW) and saved the mass-independent residuals from this regression for subsequent analyses.

From graphs of VCO_2 over the duration of each respirometry trial, it was possible to determine which bats entered torpor once the chamber temperature was reduced to 20ºC (Figure 1). Torpid individuals exhibited a clear and abrupt decline in MR once the temperature was reduced while individuals who remained normothermic (i.e., defended a warm body temperature) exhibited a clear increase in MR. I also used measurement of

rectal temperature at the end of metabolic trials to confirm the expression of torpor. I included body condition in an ANCOVA to control for possible effects of body condition on torpor expression but found no significant effect so I excluded body condition and used a Student's t-test to compare behavioural measures (PC scores and individual variables) between individuals that entered torpor and those that did not. I did not adjust for multiple comparisons since, due to a small sample size, I had very low statistical power and in this case, a Bonferroni correction would be too conservative and increase the chance of type II errors (i.e., false rejection of H_o; Nakagawa 2004).

I used two-tailed T-tests to determine if either personality measures or BMR-residuals varied between populations. There was a significant effect of location on both personality and BMR, so I performed separate analyses on bats from Altamont and Microwave Cave. In the population from Altamont I was only able to capture one male so I was unable to test for a sex-effect. However, in the population from Microwave cave, I found that sex had no significant effect on either BMR or personality. Moreover, all bats I tested were juveniles which should have further reduced the potential for sex differences. Therefore, I pooled data from males and females for further analysis. I tested for normality of all variables using Kolmogorov-Smirnov tests. For statistical tests other than PCA (which is not sensitive to deviations from normality; Timm 2002) I log-transformed variables that were not normally distributed. Significance for all tests was assessed at an alpha level of 0.05. I used Minitab 12 (Minitab Software Inc. State College, PA USA) to do PCA and normality tests while all other statistical analyses were done using Systat 11 (Systat Software Inc., Chicago IL USA).

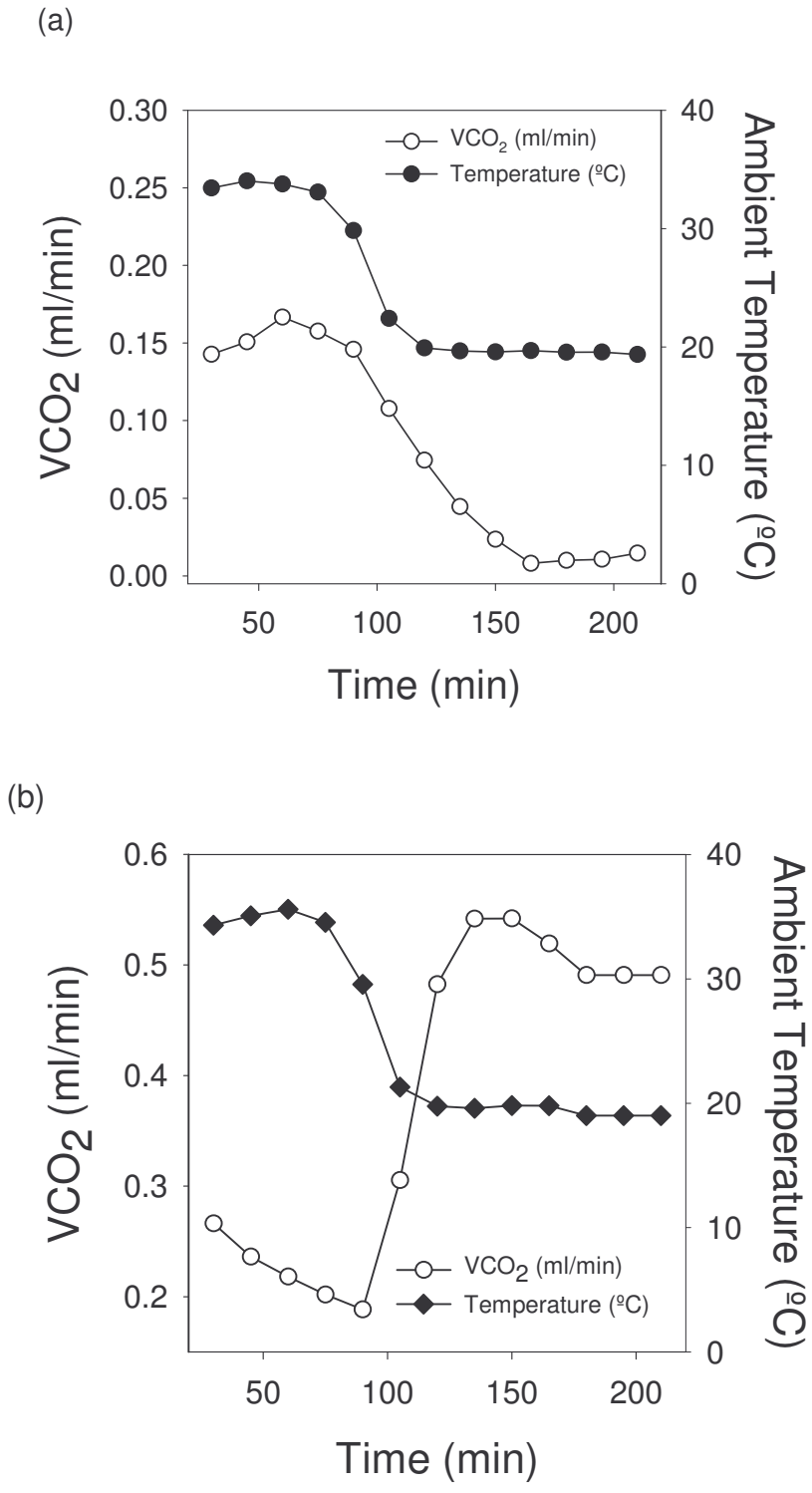

Figure 1: Representative time courses of VCO_2 throughout metabolic trials for ML37 (a) and ML32 (b). ML37 was considered to have entered torpor due to the obvious decline in VCO_2 as ambient temperature was reduced. ML32 remained normothermic based on the increase in VCO_2 as ambient temperature was reduced.

3.0 Results

Behaviours and MRs were measured for 10 individuals from Altamont (1 male, 9 female) and 18 from Microwave Cave (9 males, 9 females) between 9-30 August (Appendix 1).

3.1 Behaviour

Based on the PCA, I retained three components that explained over 60% of the total variance in the original behavioural scores (Table 1). Additional components had eigenvalues smaller than one and each accounted for only a negligible proportion of the variation in the dataset. Principal Component 1, accounted for 28.8% of the variation in the data and included behaviours associated with activity and locomotion with scuttling (i.e., rapid scurrying), number of flights and echolocation (which is closely associated with flight and activity in bats) contributing most heavily (Table 1). Thus, individuals with high scores for PC1 tended to fly, scurry and echolocate more often than those with lower PC1 scores. The second principal component accounted for 21.7% of the variation in the dataset and included behaviours which appeared to reflect exploration including climbing (i.e., scaling the walls of the test), latency to head dip in the holes closest to the walls of the test and latency to enter the test from the start box. Individuals with high scores for PC2 head-dipped earlier and spent more time scaling the walls of the test but were later to enter the test compared to those with low PC2 scores. Principal component 3 accounted for 11.8% of the variation in my behavioural data and appeared to reflect anxiety. This was represented most strongly by defecation and grooming but also included latency to head dip (Table 1; holes 1 and 2). Individuals with high PC3 scores

groomed and defecated more often and took longer to head-dip in the exposed holes closest to the middle of the test, which may suggest higher levels of anxiety.

Sex of bats from Microwave Cave had no significant effect on any of the PC scores (PC1 $t = -0.94$, d.f. = 14, $p = 0.36$; PC2 $t = -0.95$, d.f. = 14, $p = 0.36$; PC3 $t = -0.59$, d.f. = 14, $p = 0.57$). Location did have a significant effect on PC1 as scores were significantly higher for Microwave then they were for Altamont (Figure 2, $t = -5.67$, d.f. = 23, $p < 0.001$). Therefore, I considered individuals from these two field sites separately for subsequent analyses.

Table 1: Summary of results for Principal Component Analysis of behavioural responses of 28 juvenile little brown bats in the open-field test. Principal Components retained met the Kaiser-Guttman criterion (i.e., eigenvalues > 1, Kaiser 1991). Bolded eigenvectors represent factors with loadings > 0.4 which were considered to have contributed significantly to a particular component (Martin and Réale 2008)

Variables	Components		
	1	2	3
Scuttling	**0.525**	0.034	-0.055
Climbing	0.185	**0.488**	-0.251
Echolocation	**0.525**	-0.159	0.065
Flight	**0.471**	-0.001	0.211
Latency to Enter	0.006	**-0.499**	-0.285
Latency to Head Dip in Holes 1+2	-0.336	0.364	**0.404**
Latency to Head Dip in Holes 3+4	-0.022	**0.432**	-0.360
Grooming	-0.110	-0.276	**0.525**
Defecation	0.265	0.302	**0.485**
Standard Deviance (Eigenvalues)	2.59	1.95	1.06
% of total variance	28.81	21.70	11.78
Cumulative Variance	28.80	50.51	62.29

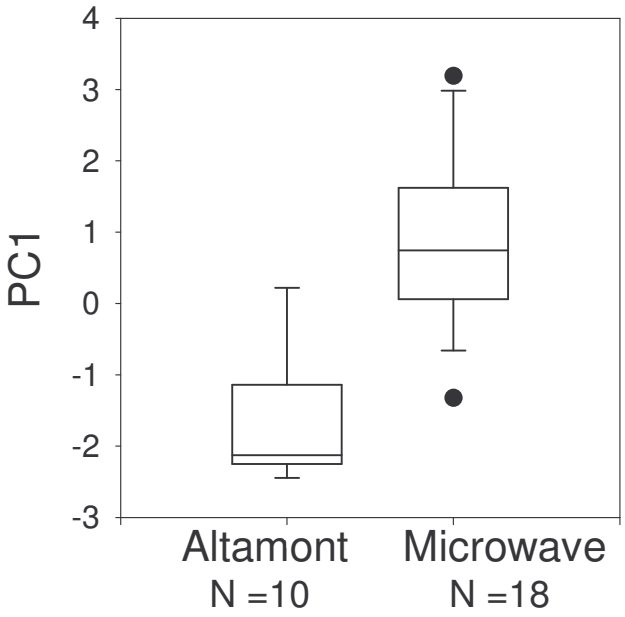

Figure 2: Differences in PC1 scores (i.e., activity levels) of 10 little brown bats from Altamont MB and 18 little brown bats from Microwave Cave near Grand Rapids MB.

3.2 Repeatability of Behavioural Scores

Scores for PC1 was significantly repeatable from test 1 to test 2 ($F = 2.51$, d.f. = 27, $p = 0.01$, Figure 3a, Appendix 2) and when I analysed individual behaviours that loaded strongly on PC1 (Table 1) I found that flight was highly repeatable (Figure 4a, $F = 4.51$, d.f. = 27, $p < 0.001$), echolocation approached significance ($F = 1.88$, d.f. = 27, $p = 0.053$) but scuttling was not significantly repeatable (Figure 4b, $F = 1.73$, d.f. = 27, $p = 0.08$). PC2 and PC3 were not significantly repeatable between trials (Figure 3b). All three principle component scores were also highly repeatable between the two observers (Figure 5a, PC1, $F = 33.04$, d.f. = 27, $p < 0.001$; PC2, $F = 53.36$, d.f. = 27, $p < 0.001$; PC3, $F = 4.14$, d.f. = 27, $p < 0.001$), as were all of the individual behavioural scores (e.g., Figure 5b, Appendix 2c).

3.3 Basal Metabolic Rate

Mean body mass at the time I measured BMR was 7.77 ± 0.71g and mean basal metabolic rate for all individuals was 92.63 ± 14.73 mW (0.20 ± 0.03 mlCO_2/hr). There was a significant relationship between body mass and BMR for bats from Altamont (Figure 6a, $r^2 = 0.55$, $t = 2.15$, d.f. = 1, $p = 0.05$) and Microwave Cave (Figure 6b, $r^2 = 0.25$, $t = 4.00$, d.f. = 1, $p < 0.01$). Residuals from these regression analyses were used as mass-corrected BMR for subsequent analysis. Sex of bats from Microwave Cave had no significant effect on mass-BMR residuals ($t = -0.23$, d.f. = 14, $p = 0.82$) but location did have an effect with individuals from Altamont exhibiting significantly higher mass-BMR residuals than bats from Microwave (Figure 7, $t = 2.06$, d.f. = 23, $p = 0.05$). I did not find a significant difference between body mass ($t = 1.04$, d.f. = 23, $p = 0.31$) or body

condition ($t = 0.68$, d.f. = 21, $p = 0.50$) of the two populations showing that this effect appears after controlling for size and physical condition of the bats that I caught.

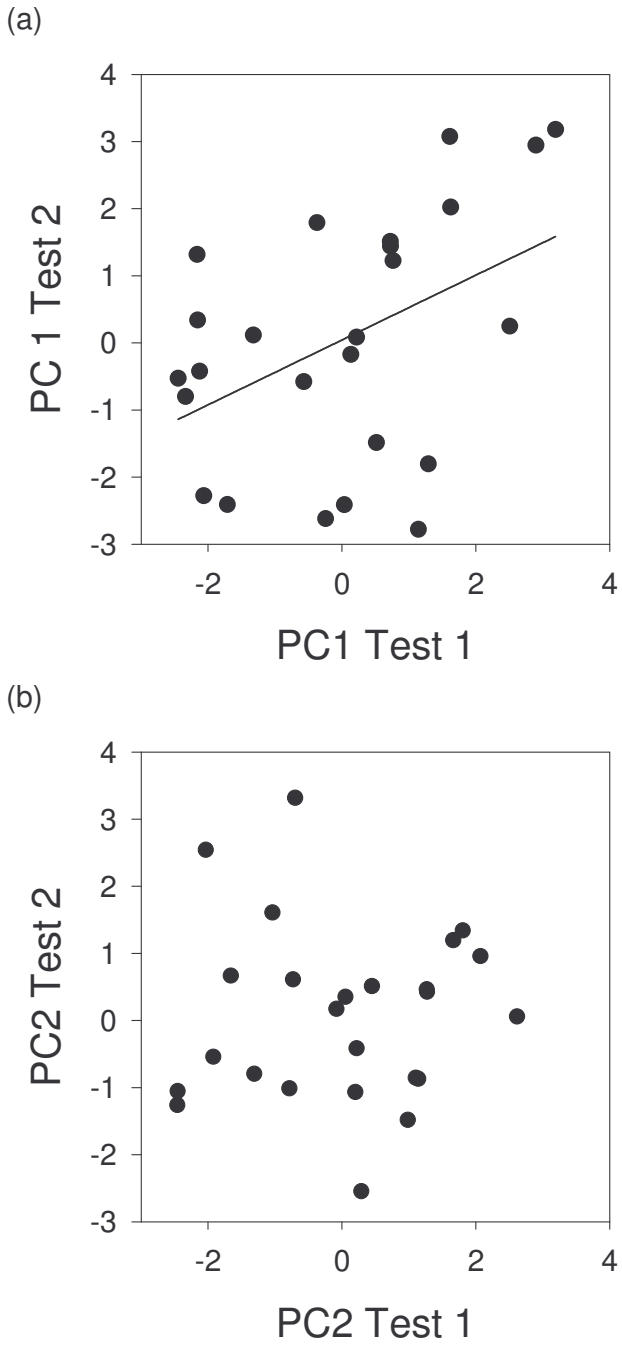

Figure 3: Scatterplots demonstrating repeatability of PC scores between behavioural trials 1 and 2 for 28 little brown bats in an open-field test. PC1 (a) was significantly repeatable and PC2 (b) was not repeatable.

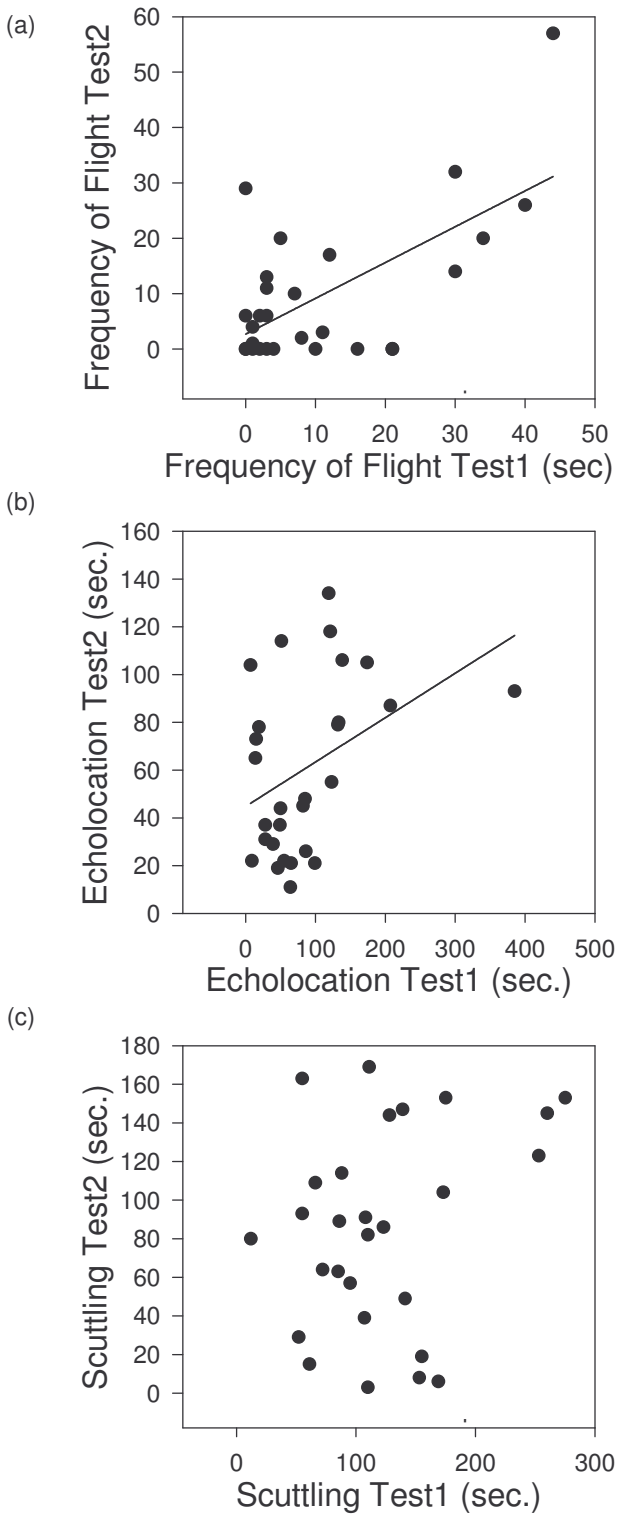

Figure 4: Scatterplots demonstrating repeatability of scores for behavioural Trials 1 and 2 for 28 little brown bats in an open-field test. Flight (a) was significantly repeatable, repeatability of echolation (b) approached significance and scuttling (c) was not repeatable.

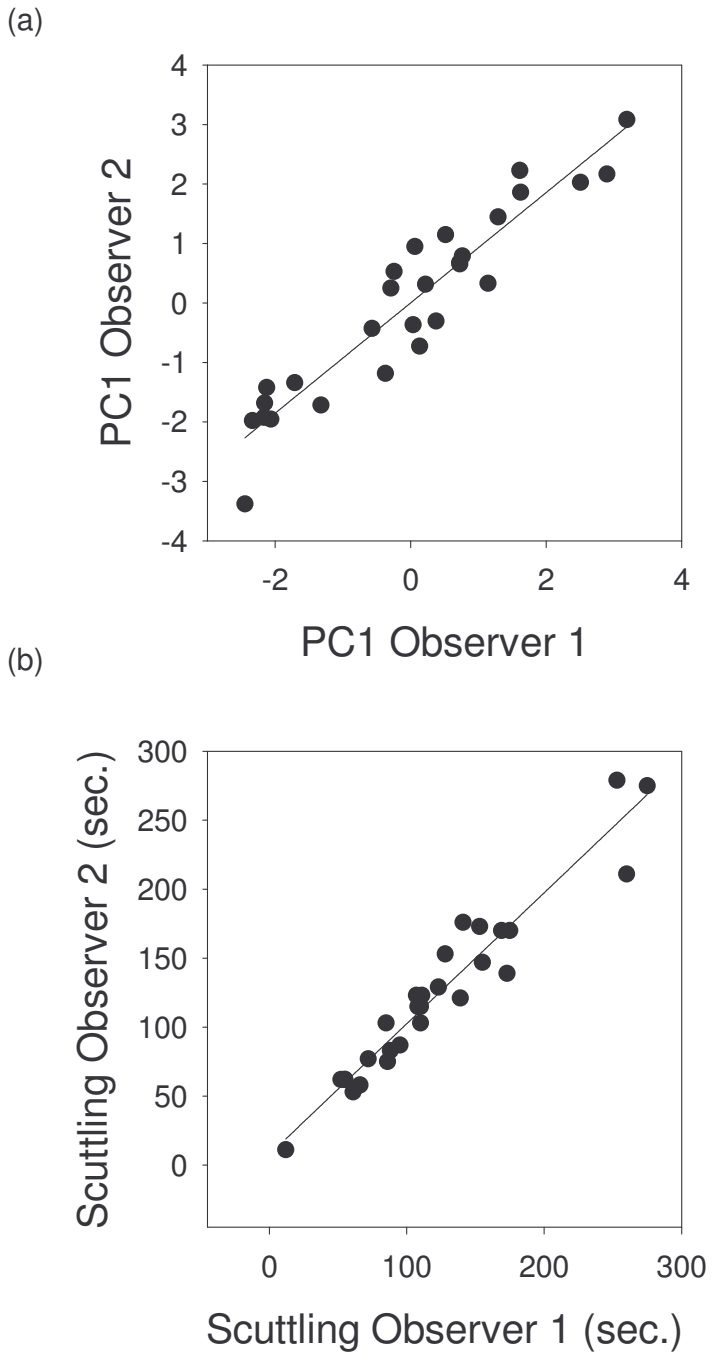

Figure 5: Scatterplots illustrating the repeatability of scores obtained by 2 independent observers for one of the principal components (i.e., PC1, (a)) and one of the individual behavioural variables (i.e., scuttling, (b)) recorded during open field trials for 28 little brown bats. All PC scores and behavioural variable scores were significantly repeatable between the observers.

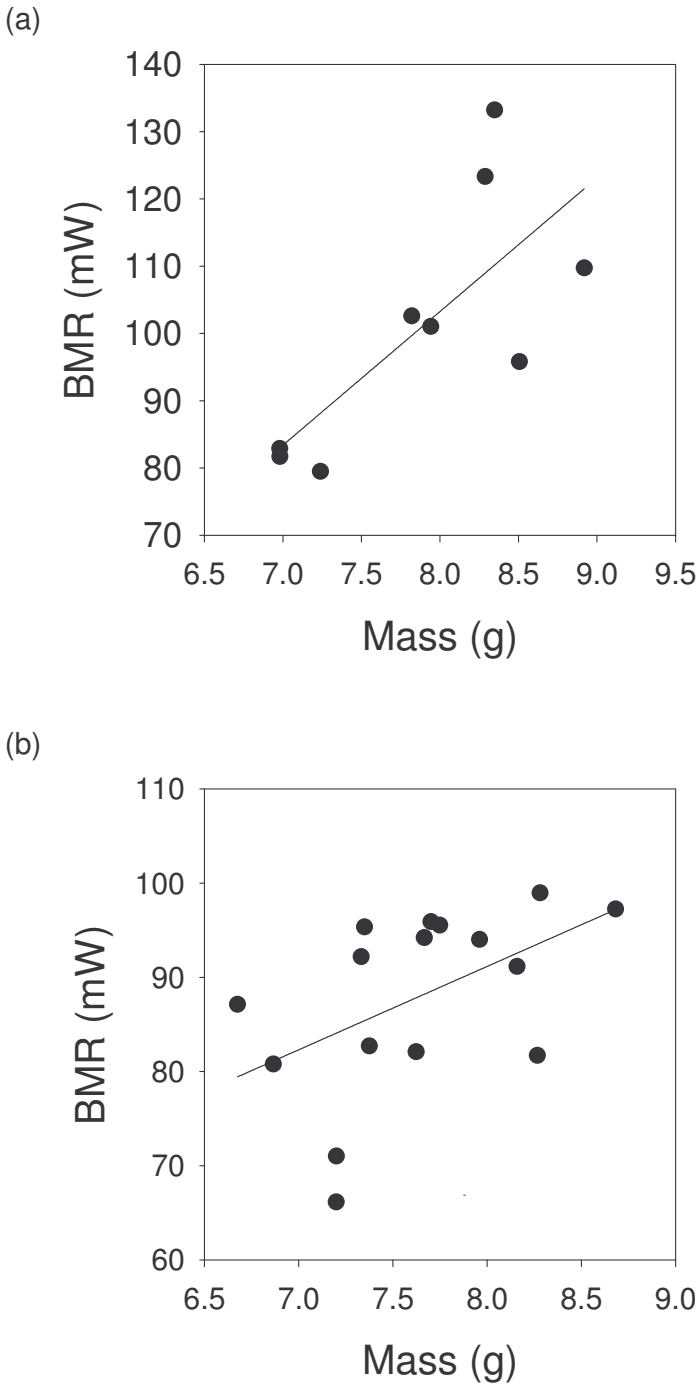

Figure 6: Relationship between body mass and BMR for 9 little brown bats from Altamont (a) and 16 little brown bats from Microwave Cave (b)**.**

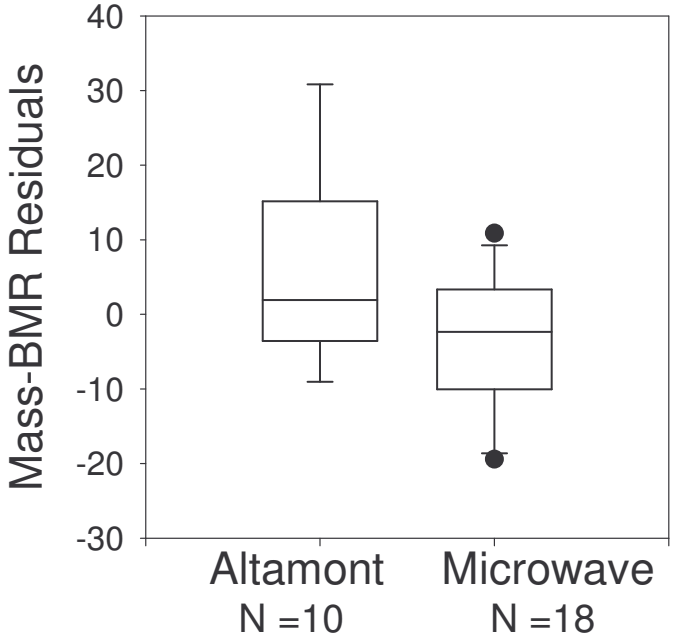

Figure 7: Difference in mass-BMR residuals of 9 little brown bats from Altamont MB and 16 little brown bats from Microwave Cave near Grand Rapids MB. Basal Metabolic Rate (BMR) was defined as the lowest, stable, 13-minute average value of VCO_2 recorded during each metabolic trial

3.4 Relationship between Behavioural Scores and Energetics

I used a series of least-squares regression analyses to test for relationships between Mass-BMR residuals and each of the Principle Components as well as individual behavioural scores. For individuals from Altamont there was no effect of any of the PC scores on BMR (Appendix 3; PC1: $F = 1.36$, d.f. = 1, $p = 0.28$; PC2: $F = 0.65$, d.f. = 1, $p = 0.45$; PC3: $F = 0.33$, d.f. = 1, $p = 0.58$). Similarly most of the behaviours had no significant effect (Appendix 3a) but latency to enter the test did have a significant effect. Individuals that entered the test more quickly had higher BMRs than individuals that were slower to enter (Figure 7; $F = 6.85$, d.f. = 1, $p = 0.04$). There was no effect of any of the behavioural measures (PC scores or individual behaviours) on BMR for individuals from Microwave (Appendix 3b; PC1, $F = 0.01$, d.f. = 1, $p = 0.92$; PC2, $F = 0.24$, d.f. = 1, $p = 0.63$; PC3, $F = 1.43$, d.f. = 1, $p = 0.25$).

About half of the individuals I tested entered torpor during the metabolic trials, based on an obvious decline in their MR when I reduced the temperature in the metabolic chamber (Figure 1a), while the other half remained normothermic, based on a clear increase in their MR at the reduced temperature (Figure 1b). Moreover, there was no significant difference between the pre-trial body temperatures of the torpid (33.36 ± 2.23°C) vs. normothermic individuals (33.78 ± 1.09°C; $t = 0.63$, d.f. = 22, $p = 0.54$). However, the post-trial body temperatures of individuals that I determined were normothermic during their metabolic trials (29.85 ± 4.80°C) were significantly higher than those that I determined to be torpid (24.06 ± 4.62°C; $t = 2.90$, d.f. = 22, $p < 0.01$). Thus, I compared PC scores and behavioural scores of the individuals which entered torpor during metabolic trials to those which remained normothermic using a two-sample

T-test**.** There was no significant relationship between behavioural measures and torpor expression in bats from Altamont (Appendix 4a) but there was a significant effect on some behavioural variables for the bats from Microwave Cave (Appendix 4b). Scores for PC1, PC2 and PC3 were not significantly affected by torpor expression but flight (one of the activity related behaviours from PC1) was significantly higher in bats that entered torpor (Figure 9a, Appendix 4). Grooming (one of the anxiety related variables from PC3) also appeared to be related to the tendency to enter torpor. Individuals who spent more time grooming during behavioural trials were less likely to use torpor, although the difference was not significant after a Yates correction for small sample size (Figure 9b; X^2= 4.36, d.f. = 1, p = 0.037; Yates Corrected Chi-Square = 2.35, p = 0.126). Interestingly, however all of the individuals that went torpid during their metabolic trials, never groomed during behavioural trials.

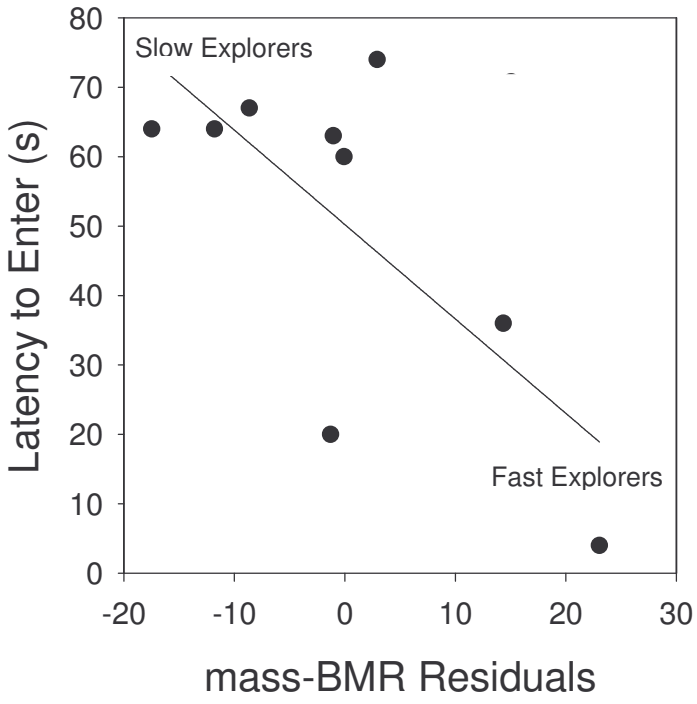

Figure 8: Relationship between BMR and latency to enter the open field test for 9 little brown bats from Altamont Manitoba

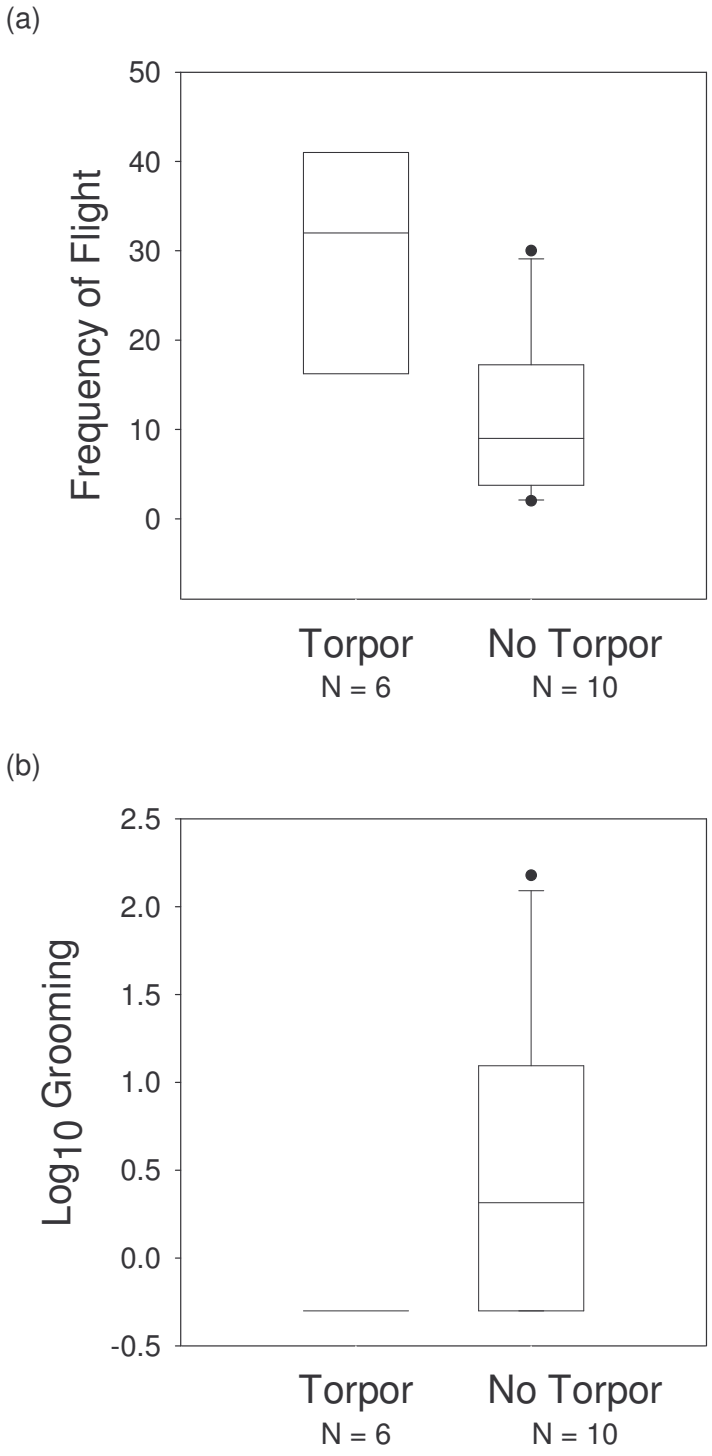

Figure 9: Comparison of values for number of flights (a) and time spent grooming (b) for little brown bats that went into torpor vs. individuals that did not. Values are for individuals from Microwave Cave only. The difference in grooming behaviour between the two groups was not significant after a Yates correction for small sample size

4.0 Discussion

4.1 Behaviour

This study is the first to quantify any measure of personality in bats. Based on my novel environment test, I detected similar dimensions of personality in bats compared to patterns previously identified in rodents (e.g., Martin and Réale 2008), songbirds (e.g., Both *et al.* 2005, Dingemanse *et al.* 2002, Verbeek *et al.* 1993) and fish (e.g., Schjolden *et al.* 2005, Yoshida *et al.* 2005, Warren and Callaghan 1975). In particular, behaviours separated into those associated with activity (i.e., PC1), especially flight and echolocation which are clearly linked to locomotion in bats, as well as exploration (i.e., PC2) based on latency to enter the test and head-dipping, both of which are thought to reflect exploration tendencies (File and Wardill 1975). Importantly, PC2 established a relationship between latency to enter the test and latency to head dip in holes closest to the walls of the test (i.e., holes 3 and 4). A key feature of the hole-board test is that holes are spread out so individuals can be active without coming into contact with a hole and, therefore contact with these structures is likely to reflect exploration (File and Wardill 1975). I also found that latency to head dip was strongly negatively correlated with frequency of head dips (i.e., individuals that head dipped more quickly also head-dipped more often), which is why I included only latency in my analysis. Consistent with the proactive-reactive classification of personality, my results indicate that exploration thoroughness (i.e., head-dips) was negatively related to exploration speed (i.e., latency to enter). Individuals who took longer to enter the test took less time to visit the first hole and explored the holes more often (slow, thorough explorers). Climbing up the walls of the test also contributed to PC2. I predicted that this trait would reflect activity, but it is possible that it relates to

exploration aimed at finding an exit from the test. The "hole-board" test was initially developed to help separate activity components of personality from exploration components in rodents, which is something that the standard novel environment test (i.e., without holes to investigate) failed to do (File and Wardill 1975). My findings suggest that this test also works to isolate activity from exploration in bats.

In addition to activity and exploration, I also identified behaviours that seem to represent anxiety. PC3 was comprised largely of grooming and defecation and grooming was positively associated with higher rates of defecation. Although more work is needed to confirm behavioural indicators of anxiety in bats, in rodents bursts of grooming as well as defecation both indicate anxiety (Kalueff and Tuohimaa 2005). Latency to head dip in the exposed holes closest to the middle of test (i.e., holes 1 and 2) was also captured in PC3 and this may also reflect anxiety as longer latency times for these holes could indicate apprehension to venture away from the walls of the test. Thus, the behaviours I measured for bats in the hole-board test divided out into distinct components which are consistent with past studies of rodents and birds.

4.2 Repeatability of Behavioural Scores

In order to be defined as personality, behavioural traits must be repeatable over time (Réale *et al.* 2007). Importantly, the general pattern of behaviour I observed was highly repeatable across trials 1 and 2, with the same 3 principal components, comprised of nearly all the same variables, dividing out for both tests. In addition, individual scores for some aspects of behaviour and some of the individual behaviours were also significantly repeatable. Scores for PC1 (i.e., activity) were consistent across behavioural trials and individuals that were more active during the first test were more likely to be

active during the second. Within PC1, repeatability was driven by echolocation and number of flights which were both repeatable across trials.

Although activity was repeatable between trials, behaviours associated with exploration (PC2) and anxiety (PC3) were not. One explanation for this could be habituation to the testing environment. An important aspect of this kind of behavioural test is that the animal perceives the environment as unfamiliar (i.e., novel) and therefore experiences a stress response (Martin and Réale 2008). If an individual becomes habituated to the environment it can change the expression of certain behaviours across tests, including those associated with exploration and anxiety (Martin and Réale 2008, Dingemanse *et al*. 2002). This could explain why PC2 and PC3 were not significantly repeatable over time in my study. To avoid habituation, for future studies of personality in bats, I recommend the use of longer intervals between tests, if possible, or the use of a larger number of behavioural tests. This would help to preserve the novelty of each test and which would ensure a similar stress response to each trial (Réale *et al.* 2007). Unfortunately these approaches were not possible for my study because I could only keep bats in captivity for a short time, and recapturing the same individual, free-ranging bats from these sites was not possible. Another possible explanation for the lack of repeatability of behaviours associated with exploration and anxiety is that the open-field test fails to capture exploration/avoidance and fear/aggression as well as it quantifies activity. The tests that are more commonly used for these dimensions of personality are novel objects tests (i.e., latency to approach novel object, percent of time spent in contact with object), predator/conspecific presentation tests (i.e., avoidance or inspection of

predator/conspecific) and reaction to handling (i.e., latency to move/bite) (Réale *et al.* 2007).

I found a significant difference in scores for some aspects of behaviour between my two study populations. Activity levels, in particular, were higher for bats from Microwave Cave compared to those from Altamont. Due to my sampling schedule it is not possible to determine if this reflects a difference between two populations of bats or a slight difference in age between juvenile bats from the two study sites. Although I only studied juveniles that had reached volancy, the individuals from Altamont were still at their summer natal roost and were possibly still growing and learning to fly while individuals from Microwave had already travelled to fall mating swarms and thus were presumably further in their development and preparing for hibernation. I also found a small but significant difference in mass-corrected BMR between the two populations which, again, could be due to environmental or age effects. Northern environments can select for lower BMR as an adaptation to greater seasonal change in energy availability (Lahti *et al.* 2002) but, alternatively, bats from Altamont may have exhibited higher metabolic rates because they were still growing (Brown *et al.* 2004). However, the fact that I found no difference in body mass or BCI between the two populations suggests that volant juveniles from both populations had reached adult size. This supports the population effect hypothesis. More work with larger sample sizes of bats from both maternity colonies and hibernation sites in northern and southern locations would be useful to tease apart the effects of age from location. To ensure these effects did not influence my results, however, I analysed behavioural and metabolic data for each population individually.

Despite the location/age effect, there was no influence of sex on energetics or personality for bats from Microwave cave. This is not surprising given that all individuals were juveniles and likely not exhibiting mating behaviour in their first year, and that past studies using open-field and novel environment tests have not found sex differences in activity, exploration propensity or anxiety (Martin and Réale 2008, Dingemanse *et al.* 2002 etc.). It is possible that other aspects of personality, such as dominance and aggression toward conspecifics would differ between sexes since they are linked to social interactions related to reproduction but my open-field test did not examine social aspects of personality.

4.3 Relationship between Behavioural Scores and Energetics

I found only weak support for Careau *et al*'s (2008) hypothesis that BMR and personality are correlated. In the population from Altamont, I found that only one behavioural trait, latency to enter the test, was correlated with BMR. This relationship was consistent with predictions of Careau *et al*'s (2008) hypothesis as individuals that took longer to enter the test (i.e., slow explorers) had lower mass-corrected BMR than fast explorers. This provides some support for the hypothesis that the low energy lifestyle of reactive individuals is associated with a lower BMR, and vice versa. However, the fact that no other behavioural variables for either population correlated with BMR, suggests that, any effect, if it exists, is relatively weak or, at least not strong enough to be detected with my sample size. It is also possible that metabolic measurements may have been affected by a stress response for some, but not all individuals. By definition, over the time period a BMR measurement is recorded, the animal should be resting and relaxed and I used video-recordings to confirm that bats were inactive at point in the metabolic time

course that I designated as their BMR. However, reactive individuals can sometimes exhibit a prolonged "freezing" response when faced with stressful stimuli (Cockrem 2007) so the absence of activity does not necessarily indicate a relaxed state. Excluding individuals based on higher activity levels might be too simplistic and may not prevent inflated measures for BMR. In an attempt to reduce this effect I discarded the measurements from the first hour of each metabolic trial when individuals were adjusting to the metabolic chamber but it is possible that some individuals exhibited heightened MR due to stress for longer periods during the test. One alternative which would be useful for future studies would be to run multiple respirometry trials for each individual allowing the animals to habituate to the metabolic chambers (Careau *et al.* 2008). However, this would increase the amount of time each individual was held captive which can induce changes in behaviour (McPhee 2003) and physiology (i.e., hormonal changes (Landsman 1991) and torpor expression (Körtner and Geiser 2009)). I found support for my hypothesis that personality differences may correlate with consistent differences in torpor expression. Although there was no relationship between any of the composite PC scores and use of torpor, when I looked at individual behaviours for bats from Microwave Cave, I found a greater tendency to express torpor in individuals that flew more often during their behavioural trials. The effect of torpor expression on flight for bats from Altamont was not significant, which could be due to a small sample size or could reflect an age difference for juveniles from the two sites. If bats from Altamont were still learning to fly at the time they were captured, it is possible that the pattern I found in the Microwave Cave population had not yet fully developed in the younger Altamont individuals. This finding is consistent with predictions about how natural selection might

act on variation in both personality and energetics in populations. To balance their energy budgets, more active (i.e., proactive) individuals with higher energy lifestyles might need to use torpor more often than reactive individuals which likely require less energy in the wild. The strong relationship between flight and torpor expression, at least for bats that were full grown and preparing for hibernation, could reflect the importance of both flight activity and torpor for energy balance and survival in little brown bats, especially during the critical pre-hibernation fattening period. A large proportion of a bat's activity and exploration obviously relies on flight which is an energetically costly form of locomotion (Butler *et al.* 1977, Tucker 1966) known to exert an enormous influence on energy balance in free-ranging bats. Thus, given the importance of both flight and torpor for energy balance and survival in bats (Turbill and Geiser 2005, Geiser 2004, Lausen and Barclay 2003, Speakman and Rowland 1999), it makes sense that these traits would be strongly correlated and that flight would have a larger, more consistent impact on energy balance for bats than other behavioural variables that I examined.

Interestingly, I also found preliminary evidence of a relationship between torpor expression and grooming, a behaviour which can reflect anxiety in other mammals and was consistent with anxiety in my study. Although when I corrected results of my chi-square analysis for a small sample size, the relationship was non-significant, there was nonetheless a noticeable difference in grooming between bats that used torpor (which never groomed) and those that did not use torpor (which often groomed). Again this finding is consistent with the hypothesis that proactive individuals, which are predicted to live relatively high energy lifestyles and exhibit low responsive to environmental stress, rely more heavily on torpor to help them maintain energy balance in the wild. If

grooming reflects anxiety, it is possible that a heightened physiological stress response may have prevented reactive bats from using torpor. Given the limited sample sizes, my tests had low statistical power, thus further work with a larger sample size would be beneficial. Also, measuring personality traits and quantifying torpor expression in free-ranging individuals using temperature radio-telemetry could shed light on whether these relationships are important in the wild.

The relationships I found between these behavioural variables and torpor expression fits with predictions based on the proactive-reactive continuum of personality. This continuum also describes reactions to environment stressors in terms of hormonal responses (Careau *et al.* 2009, Cockrem 2007, Koolhaas *et al.* 1999). It is thought that the fear of a stressful environment elicits a neuroendocrine response which affects both metabolism and various behaviours (Cockrem 2007). Thus, individual differences in hormonal responses to stress may lead to consistent individual differences in behaviours suggesting personality, metabolism and torpor expression might be correlated with the magnitude of the glucocorticoid stress response. Among mammals bats represent a potentially important model for studies of personality, particularly for studies of both torpor and those incorporating measures of physiological stress. Bats were advantageous for this study due to their ability to use torpor. However, another benefit of addressing these questions in bats vs. rodents is that they can be caught using continuously monitored nets and traps, which allows for rapid blood sampling to establish baseline levels of stress hormones before these levels begin to rise in the circulation (Reeder *et al*. 2003). Paired with conventional behavioural measurements, stress hormone analysis

would allow for a multi-dimensional definition of personality in bats that may not be possible when studying other mammals which have to be captured in remote traps.

5.0 Conclusions

[1] I was able to identify individual differences in personality traits in bats. Activity and, more specifically, flight were repeatable across two tests. Further studies, using multiple behavioural tests, could better measure other dimensions of personality. However, high inter-observer reliability for all of the PC scores and all of the individual behaviours lends confidence to the behavioural measures I analyzed and suggests that they will be useful for future studies of personality in bats.

[2] I found a difference in activity levels and BMR but not body mass or BCI between bats from my two study sites. Further work isolating age and location, along with a larger sample size, is needed to determine the cause of this difference.

[3] Although weak, I found some support for Careau *et al's* (2008) hypothesis that personality correlates with BMR and my observations with consistent with the between-species pattern reported for rodents by Careau *et al.* (2009).

[4] I found support for my hypothesis that torpor expression is influenced by personality. Individuals that flew more often in the behavioural test showed a greater tendency to use torpor in the metabolic trial. I also found an interesting trend relating grooming and torpor expression. Further studies on free-ranging individuals could help determine the existence of this relationship in the wild.

[5] Further studies addressing the relationship between personality and energetics are important because it is possible that they might have a shared influence on life-history and, ultimately, survival and fitness in the wild.

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7.0 Appendix

Appendix 1: Summary of capture data, behavioural test results and metabolic measurements for 28 little brown bats from Altamont MB and Microwave Cave near Grand Rapids MB. PC scores reflect composite scores of behaviours scored from the novel environment test. Basal Metabolic Rate (BMR) was the lowest, stable measurement of metabolism from each metabolic trial.

Bat ID	Location	Sex	Mass (g)	Forearm (mm)	BCI (g/mm)	PC1	PC2	PC3	BMR (mW)	Torpid
ML7	Alt	F	6.98	38.81	0.18	-0.569	1.804	-1.037	81.71	YES
ML8	Alt	M	6.98	38.62	0.18	-2.126	-2.035	2.334	82.89	YES
ML9	Alt	F	7.24	38.54	0.19	-2.164	-1.038	-0.096	79.49	NO
ML10*	Alt	F	9.25	37.72	0.25	-0.293	0.862	0.374	173.13	NO
ML11	Alt	F	8.29	39.10	0.21	-2.156	-0.700	0.312	123.32	-
ML13	Alt	F	8.51	38.52	0.22	-2.064	-0.786	-0.247	95.81	NO
ML14	Alt	F	8.92	38.28	0.23	0.217	-2.461	-0.410	109.72	NO
ML15	Alt	F	8.35	39.00	0.21	-2.335	0.051	0.171	133.21	NO
ML17	Alt	F	7.82	-	-	-2.448	-1.312	0.006	102.60	YES
ML18	Alt	F	7.94	-	-	-1.712	-0.082	-0.499	101.04	NO
ML24	Mic	M	7.20	38.03	0.19	1.625	-1.922	-0.723	71.01	YES
ML26	Mic	F	7.38	38.72	0.19	-0.245	0.218	-0.381	82.69	YES
ML27	Mic	M	7.62	38.23	0.20	-1.323	2.067	-0.862	82.09	NO
ML28	Mic	F	8.28	38.93	0.21	0.722	0.982	0.585	98.95	NO
ML29	Mic	F	7.70	39.78	0.19	2.508	-2.454	-2.122	95.90	NO
ML30	Mic	M	6.87	37.58	0.18	3.190	0.447	1.582	80.78	YES
ML31	Mic	M	7.33	37.56	0.20	0.132	2.614	-0.032	92.17	NO
ML32	Mic	M	7.75	38.21	0.20	0.763	1.140	1.392	95.53	NO
ML33	Mic	F	7.67	38.44	0.20	0.036	1.267	0.679	94.20	NO
ML34	Mic	M	8.16	37.82	0.22	2.896	-0.733	-0.489	91.15	YES
ML36*	Mic	M	7.52	38.12	0.20	0.063	0.036	-0.834	210.24	NO
ML37	Mic	F	7.20	38.04	0.19	0.515	0.199	-0.726	66.14	YES
ML38*	Mic	M	6.28	39.44	0.16	0.374	-0.824	-0.970	190.61	YES
ML39	Mic	M	6.68	38.60	0.17	0.724	1.270	1.054	87.12	NO
ML40	Mic	F	8.27	38.38	0.22	1.610	0.288	0.904	81.70	YES
ML41	Mic	F	8.68	38.76	0.22	-0.373	1.661	-1.639	97.24	NO
ML42	Mic	M	7.96	38.04	0.21	1.142	1.101	-0.105	94.01	NO
ML43	Mic	F	7.35	38.21	0.19	1.290	-1.662	1.777	95.34	NO

Alt = Altamont, Mic = Microwave Cave, F = Female, M = Male, BCI = Body Condition Index, PC = Principal Component

* Individuals that were removed due to activity during BMR measurements

Appendix 2: Summary of data and results from intra-class correlation. a) Three PC scores retained for each observer. b) Results for PC scores and behaviours between test 1 and test 2. c) Results for PC scores and behaviours between observer 1 and observer 2.

(a)

Observer 1						Observer 2					
Test 1			Test 2			Test 1			Test 2		
PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
-0.569	1.804	-1.037	-0.578	1.341	-1.256	-0.428	0.723	-0.436	-0.542	0.445	0.174
-2.126	-2.035	2.334	-0.422	2.542	0.164	-1.424	-2.246	1.544	-0.670	2.557	-0.495
-2.164	-1.038	-0.096	1.317	1.608	-0.541	-1.923	-1.248	0.078	0.755	1.076	0.791
-0.293	0.862	0.374	-0.013	-1.366	-1.288	0.248	0.913	0.721	0.136	-1.654	0.167
-2.156	-0.700	0.312	0.341	3.318	1.069	-1.685	-0.974	0.732	0.142	3.123	-0.697
-2.064	-0.786	-0.247	-2.278	-1.011	0.476	-1.955	-1.145	0.020	-2.365	-1.095	0.163
0.217	-2.461	-0.410	0.084	-1.258	1.872	0.313	-2.363	0.050	-0.010	-0.142	2.732
-2.335	0.051	0.171	-0.800	0.353	0.314	-1.981	-0.478	0.865	-0.148	-0.057	-0.591
-2.448	-1.312	0.006	-0.528	-0.792	1.927	-3.381	-0.399	-1.834	-0.593	-0.873	1.161
-1.712	-0.082	-0.499	-2.408	0.172	-0.036	-1.345	-0.249	0.330	-2.503	1.378	0.260
1.625	-1.922	-0.723	2.021	-0.540	1.020	1.860	-1.594	-2.697	2.131	-0.169	0.471
-0.245	0.218	-0.381	-2.619	-0.414	-0.903	0.528	-0.009	0.991	-2.415	-0.830	-0.340
-1.323	2.067	-0.862	0.116	0.958	2.070	-1.718	1.869	-0.670	0.227	0.654	-1.996
0.722	0.982	0.585	1.510	-1.480	-0.538	0.674	0.981	1.113	1.637	-1.289	-1.397
2.508	-2.454	-2.122	0.247	-1.052	-0.348	2.026	-2.230	-1.659	0.511	-0.450	-1.535
3.190	0.447	1.582	3.181	0.513	-2.158	3.081	0.701	0.176	3.397	0.279	1.347
0.132	2.614	-0.032	-0.172	0.059	0.973	-0.729	2.706	-0.995	-0.381	-0.080	-0.378
0.763	1.140	1.392	1.226	-0.868	-0.061	0.790	1.383	0.906	1.319	-0.666	0.924
0.036	1.267	0.679	-2.410	0.463	-1.441	-0.368	1.559	0.953	-2.728	0.225	1.447
2.896	-0.733	-0.489	2.945	0.611	-0.447	2.164	-0.241	-1.118	2.699	0.668	0.214
0.063	0.036	-0.834	0.501	-0.674	0.479	0.945	-0.261	1.279	0.797	-1.054	-0.823
0.515	0.199	-0.726	-1.486	-1.065	0.168	1.145	0.171	0.029	-1.239	-1.455	-0.407
0.374	-0.824	-0.970	-1.499	-0.317	0.815	-0.306	-0.489	-0.068	-1.489	0.077	-0.143
0.724	1.270	1.054	1.440	0.428	0.450	0.655	1.420	0.561	1.602	1.257	1.331
1.610	0.288	0.904	3.075	-2.544	-0.535	2.227	0.358	0.439	3.019	-2.219	-0.186
-0.373	1.661	-1.639	1.790	1.197	0.202	-1.185	1.353	-1.567	1.631	1.115	-1.762
1.142	1.101	-0.105	-2.777	-0.852	-0.435	0.327	1.214	-0.987	-2.766	-1.250	-0.563
1.290	-1.662	1.777	-1.803	0.669	-2.013	1.444	-1.423	1.242	-2.155	0.428	0.128

(b)

Variable	Intra-Class Correlation	F value	p
PC2	0.01	1.02	0.48
PC3	-0.36	0.49	0.97
Climbing	-0.08	0.83	0.69
Latency to Enter	0.25	1.67	0.10
Latency to Head Dip 1+2	0.05	1.10	0.40
Latency to Head Dip 3+4	-0.09	0.82	0.69
Grooming	-0.04	0.92	0.58
Defecation	-0.28	0.57	0.92

(c)

Variable	Intra-Class Correlation	F value	p
Scuttling	0.96	51.70	< 0.001
Climbing	0.96	68.62	< 0.001
Echolocation	0.54	5.29	< 0.001
Flights	0.99	138.79	< 0.001
Latency to Enter	1.00	-	< 0.001
Latency to Head Dip 1+2	0.99	226.71	< 0.001
Latency to Head Dip 3+4	0.78	8.08	< 0.001
Grooming	0.94	33.92	< 0.001
Defecation	1.00	-	< 0.001

Appendix 3: Summary of Results from least squares regression analyses relating behavioural variable scores to BMR for individuals from Altamont (a) and Microwave (b).

(a)

	Altamont		
	F Value	d.f.	p
Scuttling	0.09	1	0.77
Climbing	0.03	1	0.86
Echolocation	2.51	1	0.16
Flights	0.27	1	0.62
Latency to Head Dip	0.05	1	0.83
Grooming	0.16	1	0.70

(b)

	Microwave		
	F Value	d.f.	p
Scuttling	1.09	1	0.32
Climbing	0.42	1	0.53
Echolocation	0.94	1	0.35
Flights	2.79	1	0.18
Latency to Enter	0.50	1	0.49
Latency to Head Dip	0.29	1	0.60
Grooming	2.98	1	0.11
Defecation	2.31	1	0.15

Appendix 4: Summary of results for paired T-test comparing PC scores and individual behaviours to torpor expression for individuals. Significant results are in bolded.

(a)

	Altamont		
	t value	d.f.	p
PC1	0.14	6	0.90
PC2	-0.33	6	0.75
PC3	-0.87	6	0.42
Scuttling	-0.62	6	0.56
Climbing	-0.56	6	0.60
Echolocation	0.33	6	0.75
Flights	-1.47	6	0.19
Latency to Enter	0.05	6	0.96
Latency to Head Dip	0.01	6	0.99
Grooming	-1.12	6	0.31

(b)

	Microwave		
	t value	d.f.	p
PC1	-1.75	14	0.10
PC2	1.46	14	0.17
PC3	0.07	14	0.94
Scuttling	-0.76	14	0.46
Climbing	1.15	14	0.27
Echolocation	-1.04	14	0.32
Flights	**-2.88**	**14**	**0.01**
Latency to Enter	0.43	14	0.67
Latency to Head Dip	1.49	14	0.16
Defecation	1.00	14	0.33