Erratum

Estimating energy expenditure of animals using the accelerometry technique: activity, inactivity and comparison with the heart-rate technique

J. A. Green, L. G. Halsey, R. P. Wilson and P. B. Frappell

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Several errors were published in the original online version of *J. Exp. Biol.* **212**, 471-482. These errors occurred in both the PDF and full-text versions of the online article but have now been corrected. The print version is correct.

The captions of Figs 9 and 10 were truncated, and the caption published under Fig. 11 referred to Fig. 10.

Figs 9–11, together with the correct captions, are printed below.

We sincerely apologise to all authors and readers of this article for any inconvenience this has caused.

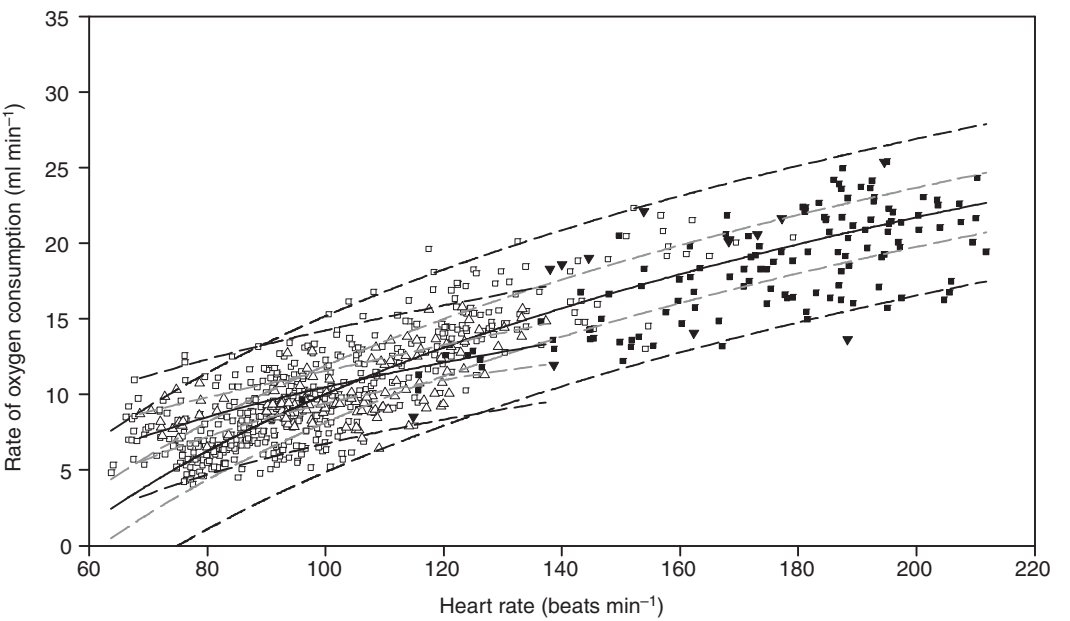

Fig. 9. Rate of oxygen consumption as a function of heart rate in eight bantam chickens. Data were recorded while the chickens walked on a treadmill (filled squares), ate a meal of food pellets (filled triangles), digested the meal of food pellets (open triangles) or thermoregulated (open squares). Also plotted are two best-fit regression lines (solid line) and 95% confidence intervals (black dashed lines) and 95% prediction intervals (grey broken lines). 95% confidence intervals were calculated as if $s\dot{V}_{O_2}$ was estimated from one measurement of heart rate, during one additional behaviour by one additional chicken. 95% prediction intervals were calculated as if $s\dot{V}_{O_2}$ was estimated from 10,000 measurements of heart rate, during four additional behaviours by 100 additional chickens, effectively the smallest possible prediction interval for this model.

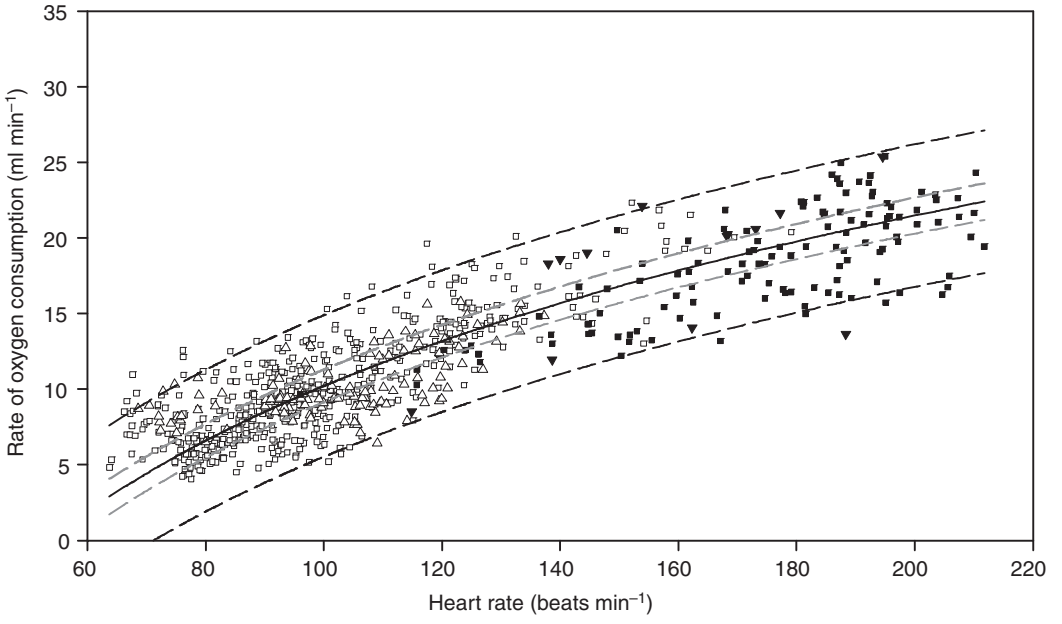

Fig. 10. Rate of oxygen consumption as a function of heart rate in eight bantam chickens. Data were recorded while the chickens walked on a treadmill (filled squares), ate a meal of food pellets (filled triangles), digested the meal of food pellets (open triangles) or thermoregulated (open squares). Also plotted are best-fit regression lines (solid line) and 95% confidence intervals (black broken lines) and 95% prediction intervals (grey broken lines). 95% confidence intervals were calculated as if $s\dot{V}_{O_2}$ was estimated from one measurement of heart rate, during one behaviour by one additional chicken. 95% prediction intervals were calculated as if $s\dot{V}_{O_2}$ was estimated from 10,000 measurements of heart rate, during four additional behaviours by 100 additional chickens, effectively the smallest possible prediction interval for this model.

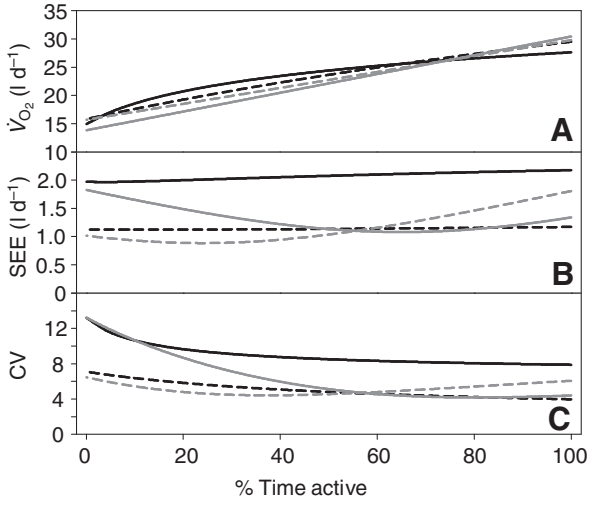

Fig. 11. Simulation showing the effect of model selection on precision and accuracy when estimating the rate of oxygen consumption ($\dot{V}_{O_2}$) in bantam chickens. A day in the life of a chicken was repeatedly simulated where the proportion of time spent 'active' was varied between 0 and 100%. (A) $\dot{V}_{O_2}$, (B) the standard error of the estimate (s.e.e.) and (C) the coefficient of variation (CV=100*s.e.e./Estimate) were calculated for this range of activity using four predictive approaches. Each of the four approaches used either partial dynamic body acceleration in the *x* and *z* axis ($PDBA_{xz}$) or heart rate (f_H) to predict $\dot{V}_{O_2}$. The approaches used were (1) one-model using $PDBA_{xz}$ (black solid lines), (2) two-model using $PDBA_{xz}$ (grey solid lines), (3) one-model using f_H (black broken lines), (4) two-model using f_H (grey broken lines). See text for further details of the four predictive approaches.

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Estimating energy expenditure of animals using the accelerometry technique: activity, inactivity and comparison with the heart-rate technique

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SUMMARY

Several methods have been used to estimate the energy expenditure of free-ranging animals. A relatively new technique uses measures of dynamic body acceleration as a calibrated proxy for energy expenditure and has proved an excellent predictor of energy expenditure in active animals. However, some animals can spend much of their time inactive and still expend energy at varying rates for a range of physiological processes. We tested the utility of dynamic body acceleration to estimate energy expenditure during a range of active (locomotion, eating) and inactive (digesting, thermoregulating) behaviours exhibited by domestic chickens. We also compared this technique with the more established heart-rate method for estimating energy expenditure. During activity, the error of estimation using body acceleration was very similar to that from the heart-rate method. Importantly, our results also showed that body acceleration can be used to estimate energy expenditure when birds are inactive. While the errors surrounding these estimates were greater than those during activity, and those made using the heart-rate method, they were less than those made using interspecific allometric equations. We highlight the importance of selecting a methodology that is appropriate for the life-history of the subject animal. We suggest that, to achieve the greatest possible accuracy and precision when estimating energy expenditure in free-ranging animals, the two techniques should be combined, and both heart rate (f_H) and dynamic body acceleration could be included as covariates in predictive models. Alternatively, measures of acceleration can be used to ascertain which behaviour is being exhibited at each moment and hence which predictive model should be applied.

Key words: accelerometry, heart rate, energetics, chicken, SDA, thermoregulation.

INTRODUCTION

Understanding the energy expenditure of free-ranging animals is fundamental to answering questions in animal biology ranging from life-history decisions (e.g. Stephens and Krebs, 1986) to quantifying population energy budgets (e.g. Winship et al., 2002). In particular, the assignment of energy to specific activities is particularly important when energy is used as a currency of investment in studies of behavioural ecology (e.g. McNamara and Houston, 1996). Over recent years, the use of two techniques has dominated attempts to estimate energy expenditure in free-ranging animals. These techniques, the doubly labelled water and heart-rate methods, each have their strengths and weaknesses (for a review, see Butler et al., 2004).

In addition to these two established methods, a newer method has been developed, in which the overall dynamic body acceleration (ODBA) of an animal is calculated and used as a calibrated proxy for the rate of energy expenditure (Wilson et al., 2006). Using body motion as a proxy for energy expenditure is not a new concept – studies in the medical literature date back to the 1960s (e.g. Cavagna et al., 1963). However, its application to animal biology is a relatively new one, facilitated by the ability of the latest generation of miniature data loggers to record and store acceleration data in three axes at high frequencies (Wilson et al., 2008). However, like all similar techniques, using ODBA has limitations. By definition, this technique relies on recording ATP-fuelled movements of animals and assumes that energy use increases with movement. Energy expended in movement can represent the major portion of the energy budget of mammals and birds (Weibel and Hoppeler, 2005). Thus ODBA should provide a good estimate of energy expenditure during periods of activity.

However, animals can spend hours not moving, yet they can be engaged in energetically expensive physiological processes such as growth, production (e.g. eggs or milk), digestion, thermoregulation or, conversely, energy-saving processes such as torpor. For example, elephants spend less than 20% of their time walking (Shannon et al., 2008), seabirds commonly spend over 50% of their day resting (Falk et al., 2000; Grémillet et al., 1995) even when busy provisioning nestlings, and red deer rest for at least 45% of the day, depending on the time of year (Pépin et al., 2006). So while the relationship between ODBA and energy expenditure has been investigated during activity for a number of species (Fahlman et al., 2008; Halsey et al., 2008a; Halsey et al., 2008b; Halsey et al., 2008c; Wilson et al., 2006), it is well recognised that, during periods of inactivity, estimates of energy expenditure might need to be derived from laboratory studies or allometric estimates (Wilson et al., 2008).

In the present study, we establish whether body acceleration can be used to estimate the rate of energy expenditure across the full range of animal behaviours. As well as making the animals exercise (walk), we investigated whether an accelerometer might detect fine-scale movements associated with thermoregulation such as panting

or shivering or, for example, whether specific dynamic action during the digestion of a meal increases energy expenditure without changing measures of body acceleration. We used the domestic chicken (*Gallus gallus*) as a model species as they are easily maintained in captivity and respond favourably to a variety of experimental manipulations. We also examined the relationship between heart rate and rate of energy expenditure in the same individuals across the same range of behaviours. This enabled the effectiveness of the accelerometry technique during different behaviours to be compared with that of a more established methodology.

Thus we set out to answer the following four questions:

1. Can changes in dynamic body acceleration be used to determine the rate of energy expenditure while eating and during digestion?
2. Can changes in dynamic body acceleration be used to determine the rate of energy expenditure during thermoregulation?
3. Will changes in dynamic body acceleration during these behaviours be similar to the more established pattern found during walking and other active behaviours?
4. Will the predictive power of relationships between dynamic body acceleration and rate of energy expenditure be as great as for relationships between heart rate and rate of energy expenditure?

MATERIALS AND METHODS

Eight Old English bantam chickens (*Gallus gallus*, mean mass ± s.e.m.: 0.78±0.02 kg) were used in the present study. The experimental protocol was carried out with the approval of La Trobe University Ethics Committee [AEC 04/37(L)]. The chickens were housed in a large enclosure (40×50 m) that included a hut with straw-filled boxes and perches where they slept at night. They were provided with food and water *ad libitum* and maintained a constant body mass. The chickens were known to walk consistently and unaided on a variable-speed treadmill. Each bird was used twice in each of three experimental protocols: 'walking', 'eating–digesting' and 'thermoregulation' to mimic four different natural 'behaviours' (locomoting, eating, digesting and thermoregulating). However, the small size of the birds precluded simultaneous recording of the rate of energy expenditure, dynamic body acceleration and heart rate. Instead, during one set of experiments, the rate of energy expenditure and dynamic body acceleration were measured simultaneously, and, during another set of experiments, the rate of energy expenditure and heart rate were measured simultaneously from the same individuals. In all trials, birds were denied food but not water for 14–24 h before experiments and thus could be considered post-absorptive for all measurements.

Respirometry

The rate of oxygen consumption ($\dot{V}_{O_2}$) is an indirect measure of energy expenditure, assuming no anaerobic respiration, and was determined using an open-circuit respirometry system (see Green et al., 2008). During experiments, a bird was placed within a respirometer chamber (470×480×433 mm high) made of clear plastic, which was suspended above a variable-speed treadmill (Australian Treadmill Exerciser). Draught excluders partially sealed the respirometer to the treadmill. Air was drawn from the respirometer using a variable-speed pump (Recipritor) at ~5 l min^{-1}. The flow rate was calculated before and after experiments using a water-displacement technique. A sub-sample of air was drawn from this main flow using a small air pump (Ametek R-1, Applied Electrochemistry), passed through a drying column (Drierite, Hammond), and analysed for the fractional content of O_2 and CO_2 by two gas analysers (Ametek S-3A/I and ADI Instruments ML205). The outputs from the gas analysers and a thermocouple located inside the respirometer were collected at 100 Hz (Powerlab 4SP, ADInstruments) and displayed on a computer using Chart software (ADInstruments). The rate of oxygen consumption was determined from the rate of airflow from the respirometer and the difference in the fractional oxygen concentration between ambient and outflowing air. Instantaneous corrections of the gas concentrations were calculated dry at standard temperature (273 K) and pressure (101.3 kPa) using the method of Frappell and colleagues (Frappell et al., 1989), assuming a first-order linear system (chamber volume=10 l; flow=5 l min^{-1}; tau=17 min, determined from a semi-logarithmic plot of concentration against time following a perturbation, R^2=0.99). $\dot{V}_{O_2}$ was calculated with consideration of RQ-related errors (Frappell et al., 1992). Mean $\dot{V}_{O_2}$ was calculated every 5 min during experiments.

Heart rate and partial dynamic body acceleration

Heart rate (f_H) was monitored using a customised heart-rate transmitter (POLAR a3, Polar Electro Oy, Finland) with largest dimensions 50×20×8 mm and a mass of 21 g. The transmitter was attached to the upper back of the bird with paper tape (see Wilson and Wilson, 1989), and custom-made brass electrodes were inserted under the skin. The transmitter unit had a functional range of ~1 m, and a receiver unit was placed on top of the respirometer to ensure good reception. Outputs from the receiver unit were collected by the Powerlab alongside respirometry data. Acceleration was measured using the same data loggers as Wilson and colleagues (Wilson et al., 2006) and Halsey and colleagues (Halsey et al., 2008c), also attached to the upper back with paper tape. The loggers had largest dimensions of 42×36×13 mm, a mass of 24 g and were set to record tri-axial acceleration (0–6 $\boldsymbol{g}$) at 10 Hz with 22-bit resolution. This recording frequency is sufficiently high when using measures of acceleration as a proxy for energy expenditure in this species (Halsey et al., 2008a).

The absolute values (in $\boldsymbol{g}$) for each axis were calculated [abs(x), abs(y) and abs(z)], to represent the raw accelerometry values. The x axis of the logger measured acceleration laterally (coronal) – that is, wing to wing – and hence sway. The y axis of the logger measured acceleration in the plane at right angles to the x axis, approximately in the anteroposterior dimension of the chicken (sagittal plane) – that is, head to tail – and hence surge. The z axis measured acceleration in approximately the dorsoventral dimension (transverse) and hence heave. From the downloaded logger data, x, y and z, an approximation of absolute $\boldsymbol{g}$, resulting from only dynamic acceleration in that dimension, was extracted (partial dynamic body acceleration, $PDBA_x$, $PDBA_y$ and $PDBA_z$, respectively). However, initial inspection of the data revealed unacceptable noise and interference in the y-axis due to instrument failure, and ODBA was not calculated. Instead, we summed $PDBA_x$ and $PDBA_z$, calculating partial dynamic body acceleration in the x and z axes ($PDBA_{xz}$). $PDBA_{xz}$ is an accurate proxy for energy expenditure in walking chickens (Halsey et al., 2008a).

Eating–digestion protocol

Eating and digesting were examined in a single protocol. Birds were initially introduced to the respirometer and left until calm (<10 min). A small dish containing 30 g of food pellets was then placed into the respirometer. The birds immediately began eating and were left undisturbed for 10 min while their behaviour was recorded. The chickens had usually finished eating by the end of this period, because either the dish was empty or they were satiated. The dish was then removed, the amount of food consumed calculated, the

lights switched off, and the birds left undisturbed for ~2 h to record specific dynamic action (SDA) during digestion. All experiments took place at a constant 18°C.

Thermoregulation protocol

The rate of oxygen consumption and $PDBA_{xz}$ or f_H were measured in the dark at temperatures of: 1, 11, 18, 30 and 36°C. A chicken only experienced a single temperature on any given day to avoid any complications of hysteresis in metabolism, heart rate or body temperature. The temperature range was based on data from larger birds (Meltzer, 1983). In our smaller chickens, we assumed that 1, 11 and 36°C would be outside the thermoneutral zone (TNZ), whereas 18 and 30°C would be close to the lower and upper critical temperatures, respectively. Birds were equipped with a heart-rate transmitter or accelerometer and acclimatised to the experimental temperature in a ventilated box for ~1 h before being transferred to the respirometer for recordings of $\dot{V}_{O_2}$ and $PDBA_{xz}$ or f_H. The birds remained in the respirometer for at least one hour, which allowed f_H and metabolism to settle to a steady level and resting rates to be established.

Walking protocol

Birds, equipped with an accelerometer or heart-rate transmitter, were introduced into the respirometer and allowed to rest for at least 15 min. Birds were then run at randomly assigned speeds between 0.15 and 1.3 km h^{-1} for between 8 and 16 min. The birds were allowed to rest between speed adjustments. To encourage movement, food pellets were placed outside the front of the respirometer; thus, some birds also exhibited pecking behaviours. Indeed, other natural active behaviours were also undertaken, particularly at the lower walking speeds, such as wing flapping and jumping. Thus, the chickens exhibited a range of natural, active behaviours during the trials. Again, experiments were conducted at 18°C. Some of these data on dynamic body acceleration during walking have been reported previously (Halsey et al., 2008a; Halsey et al., 2008c).

Data analysis

Question 1 asked whether $PDBA_{xz}$ would change during eating and digesting. First, 5 min periods were selected where the chicken was eating continuously throughout and mean $\dot{V}_{O_2}$, $PDBA_{xz}$ and f_H were calculated. Second, during digestion, mean $\dot{V}_{O_2}$, $PDBA_{xz}$ and f_H of the first 30 min after eating had finished were selected for analysis as initial visual inspection confirmed that the largest increase in metabolism was seen at this time (e.g. Green et al., 2006). Data during both eating and digestion were compared with the equivalent data obtained at the same temperature (18°C) during the thermoregulation protocol using paired *t*-tests.

To investigate question 2, regarding whether changes in temperature would induce a change in $\dot{V}_{O_2}$, $PDBA_{xz}$ and f_H, the lowest five recordings of $\dot{V}_{O_2}$ were selected for each temperature for each bird, both during trials with the heart-rate transmitter and those with the accelerometer. Two-way analysis of variance with Tukey *post-hoc* comparisons were used to investigate differences in $\dot{V}_{O_2}$, $PDBA_{xz}$ or f_H between temperatures.

During walking, data were usually recorded for more than one 5 min period at each speed for each animal. To determine the relationship between speed and $\dot{V}_{O_2}$, $PDBA_{xz}$ or f_H, a mean was taken for each animal at each speed. A grand mean and s.e.m. of these means was then calculated for each speed, and weighted regression was used to look at relationships between treadmill speed and each variable.

Subsequent analyses examined relationships between either $PDBA_{xz}$ and $\dot{V}_{O_2}$ or f_H and $\dot{V}_{O_2}$ across all behaviours in order to answer question 3. All 5 min periods of data from all protocols were used in these analyses. These data were analysed using general linear models (GLM) in which behaviour was a fixed factor, individual identity was a random factor and $PDBA_{xz}$ or f_H was a covariate. A significant interaction between behaviour and $PDBA_{xz}$ or f_H indicated differences in the slope of the relationship between $PDBA_{xz}$ or f_H and $\dot{V}_{O_2}$ between the different behaviours. Subsequent to this, in some analyses, behaviour was also a random factor.

Predictive relationships were generated from these GLMs and used to answer question 4 and compare the predictive power of relationships between $PDBA_{xz}$ and $\dot{V}_{O_2}$, or f_H and $\dot{V}_{O_2}$. The standard error of the estimates (s.e.e.) made using these equations were calculated following the conventions established by Green and colleagues (Green et al., 2001). As Green and colleagues (Green et al., 2001) outline, the s.e.e. and hence 95% prediction intervals of an estimate made using a predictive equation will depend on the characteristics of (1) the individuals used to derive the predictive relationship and (2) the individuals for which further measurements of the independent variable were made and for whom an estimate is being calculated ('sample' group). In practice, this means that the number of individuals in the 'sample' group and number of measurements taken from those individuals will have a substantial effect on the 95% prediction intervals surrounding an estimate (Green et al., 2001). When dealing with predictive equations, the 95% confidence intervals associated with an estimate are the 95% prediction intervals where a predictive equation is used to make an

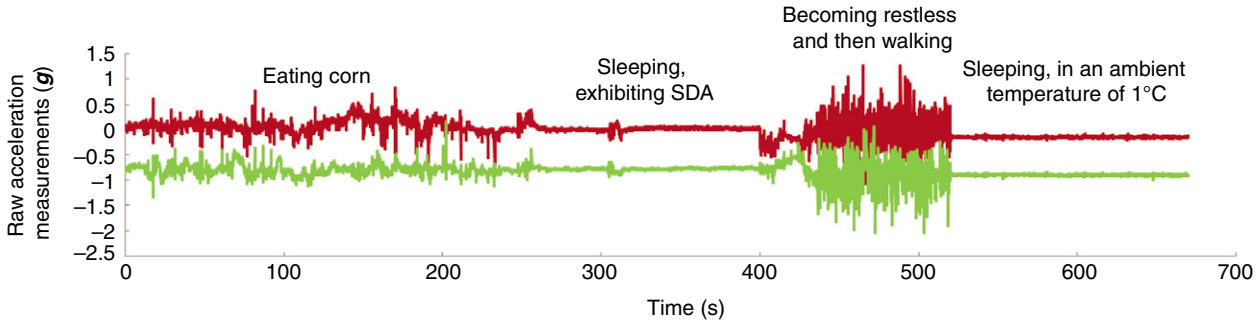

Fig. 1. Measures of acceleration recorded along the *x*-axis (wing to wing, red trace) and *z*-axis (ventral to dorsal, green trace) in a bantam chicken (at 18°C unless otherwise stated). The traces for both axes while the chicken is eating are clearly different to the traces during sleeping and during walking. However, the traces for sleeping while exhibiting an SDA and resting while at an ambient temperature below the thermoneutral zone (TNZ; 1°C) are more difficult to distinguish.

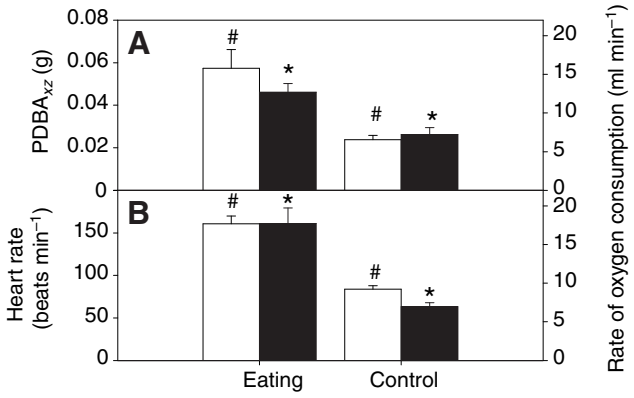

Fig. 2. Mean (±s.e.m.) values of (A) partial dynamic body acceleration in the *x* and *z* axes (PDBA$_{xz}$) and (B) heart rate (white bars, all ± s.e.m.) measured in bantam chickens (*N*=8) while they ate a meal of food pellets or rested at the same constant temperature (18°C) as a control. Concurrently made measurements of the rate of oxygen consumption (black bars, ±s.e.m.) are also shown. Significant differences between control and eating states are indicated by the following symbols: * and #.

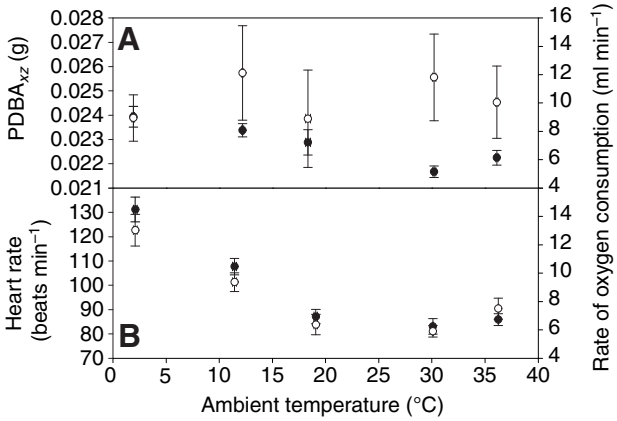

Fig. 4. Mean (±s.e.m.) values of (A) partial dynamic body acceleration in the *x* and *z* axes (PDBA$_{xz}$) and (B) heart rate (white symbols) measured in bantam chickens (*N*=8) at a range of ambient temperature along with concurrent measurements of mean (±s.e.m.) rate of oxygen consumption (black symbols).

estimate based on one further measurement of the independent variable from one further individual. Consequently, the 95% confidence intervals represent the maximum error range around the prediction. *Z*-tests or proximate normal tests (for paired estimates) were used to compare estimates made using predictive equations (Green et al., 2001).

RESULTS

Traces of body acceleration during different behaviours

When the birds undertook 'active' behaviours (walking and eating food pellets), distinctive patterns were observed in the raw acceleration traces in both the *x* and *z* axes (Fig. 1). Traces for different behaviours were distinguishable from each other and also from periods when the chickens were 'inactive' (digesting a meal or thermoregulating outside their TNZ). However, the raw acceleration traces associated with these two inactive behaviours were not easily distinguishable from each other (Fig. 1).

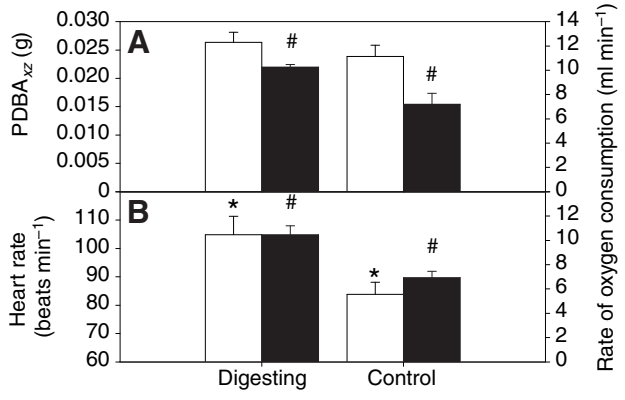

Fig. 3. Mean (±s.e.m.) values of (A) partial dynamic body acceleration in the *x* and *z* axes (PDBA$_{xz}$) and (B) heart rate (white bars, all ±s.e.m.) measured in bantam chickens (*N*=8) while they digested a meal of food pellets or rested while post-absorptive at the same constant temperature (18°C) as a control. Concurrently made measurements of the rate of oxygen consumption (black bars, ±s.e.m.) are also shown. Significant differences between control and digesting states are indicated by the following symbols: * and #.

Body acceleration during eating and digestion

While instrumented with an accelerometer and eating a meal of food pellets placed into the respirometer, the chickens showed a significant increase in both PDBA$_{xz}$ (paired *t*-test; $t_{(7)}$=3.88, P<0.01) and $\dot{V}_{O_2}$ (paired *t*-test; $t_{(7)}$=2.98, P<0.05) when compared with resting quietly at the same temperature (18°C) (Fig. 2). Similarly, when f_H was recorded, both f_H (paired *t*-test; $t_{(7)}$=7.27, P<0.01) and $\dot{V}_{O_2}$ (paired *t*-test; $t_{(7)}$=5.28, P<0.01) were significantly greater during eating. After eating, the magnitude of SDA during digestion was evaluated (Fig. 3). When PDBA$_{xz}$ was recorded, $\dot{V}_{O_2}$ was significantly higher during digestion (paired *t*-test; $t_{(7)}$=3.24, P<0.05), but there was no difference in PDBA$_{xz}$ (paired *t*-test; $t_{(7)}$=1.28, P=0.24). When f_H was recorded, both $\dot{V}_{O_2}$ (paired *t*-test; $t_{(7)}$=3.78, P<0.01) and f_H (paired *t*-test; $t_{(7)}$=3.66, P<0.01) were significantly higher during digestion than while the chickens rested at the same temperature.

Body acceleration during thermoregulation

When exposed to a range of different temperatures, the chickens showed changes in metabolism, suggesting a TNZ with an upper critical temperature around 30°C and a lower critical temperature as low as 20°C (Fig. 4). When instrumented with an accelerometer, the rate of oxygen consumption ($\dot{V}_{O_2}$) was at a minimum at 30°C and was significantly elevated at 1°C and 11°C (two-way ANOVA, with Tukey multiple comparisons; $F_{(4,28)}$=7.29, P<0.001). There were no significant differences in PDBA$_{xz}$ between the different temperatures (two-way ANOVA; $F_{(4,28)}$=0.32, P=0.86). When $\dot{V}_{O_2}$ and heart rate (f_H) were recorded simultaneously, both $\dot{V}_{O_2}$ and f_H were again at a minimum at 30°C, and both were significantly elevated at 1°C and 11°C (two-way ANOVA, with Tukey multiple comparisons; $F_{(4,28)}$=44.3 and 22.7, P<0.001).

Body acceleration during locomotion

The chickens exhibited a range of behaviours as well as walking while undertaking the treadmill experiments. They also pecked the ground, jumped and flapped their wings. During these trials, there

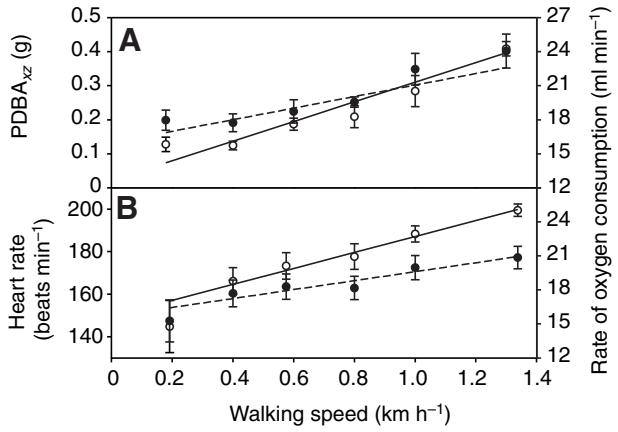

Fig. 5. Mean (±s.e.m.) values of (A) partial dynamic body acceleration in the *x* and *z* axes (PDBA$_{xz}$) and (B) heart rate (white symbols) measured in bantam chickens (N=8) while walking on a treadmill at different speeds along with concurrent measurements of mean (±s.e.m.) rate of oxygen consumption (black symbols). Weighted regression relationships are also shown for heart rate and PDBA$_{xz}$ (solid lines) and rate of oxygen consumption (broken lines).

was a positive, linear relationship between $\dot{V}_{O_2}$ and walking speed in bantam chickens instrumented with an accelerometer (weighted regression; P<0.05, R^2=0.80) (Fig. 5). This was matched by a linear increase in PDBA$_{xz}$ with walking speed (weighted regression; P<0.001, R^2=0.96). Similarly when $\dot{V}_{O_2}$ and f_H were recorded simultaneously, there was a linear relationship between walking speed and both $\dot{V}_{O_2}$ (weighted regression; P<0.01, R^2=0.90) and f_H (weighted regression; P<0.001, R^2=0.97) (Fig. 5).

Predicting the rate of oxygen consumption as a function of body acceleration

The behaviour-specific tests described above suggested that, although PDBA$_{xz}$ might be an effective predictor of $\dot{V}_{O_2}$ during active behaviours such as locomotion or eating, it was likely to be less effective while the chickens were inactive, either thermoregulating or digesting a meal. To investigate this further, $\dot{V}_{O_2}$ was plotted as a function of PDBA$_{xz}$ for each animal (see Fig. 6A for example). Visual inspection confirmed this initial suspicion that PDBA$_{xz}$ was likely to be an accurate predictor of $\dot{V}_{O_2}$ during walking but was unlikely to be so accurate while the chickens were inactive. However, although there was a lot scatter in PDBA$_{xz}$ and $\dot{V}_{O_2}$ during inactivity, these data rarely overlapped with data recorded during activity. Converting PDBA$_{xz}$ to a logarithmic scale suggested that, with a single curvilinear model, it might be possible to predict $\dot{V}_{O_2}$ from PDBA$_{xz}$ across all behaviours (Fig. 6C). An analysis of covariance was conducted to investigate this further.

Analysis of covariance indicated no significant interaction between behaviour and log(PDBA$_{xz}$) ($F_{(3,688)}$=1.69, P=0.17) and thus the slope of the relationship between log(PDBA$_{xz}$) and $\dot{V}_{O_2}$ did not differ between behaviours. Therefore, a single function was used to predict $\dot{V}_{O_2}$ from PDBA$_{xz}$ across all behaviours (Table 1). As both behaviour and individual identity were significant factors in the model, two extra error terms would need to be introduced when

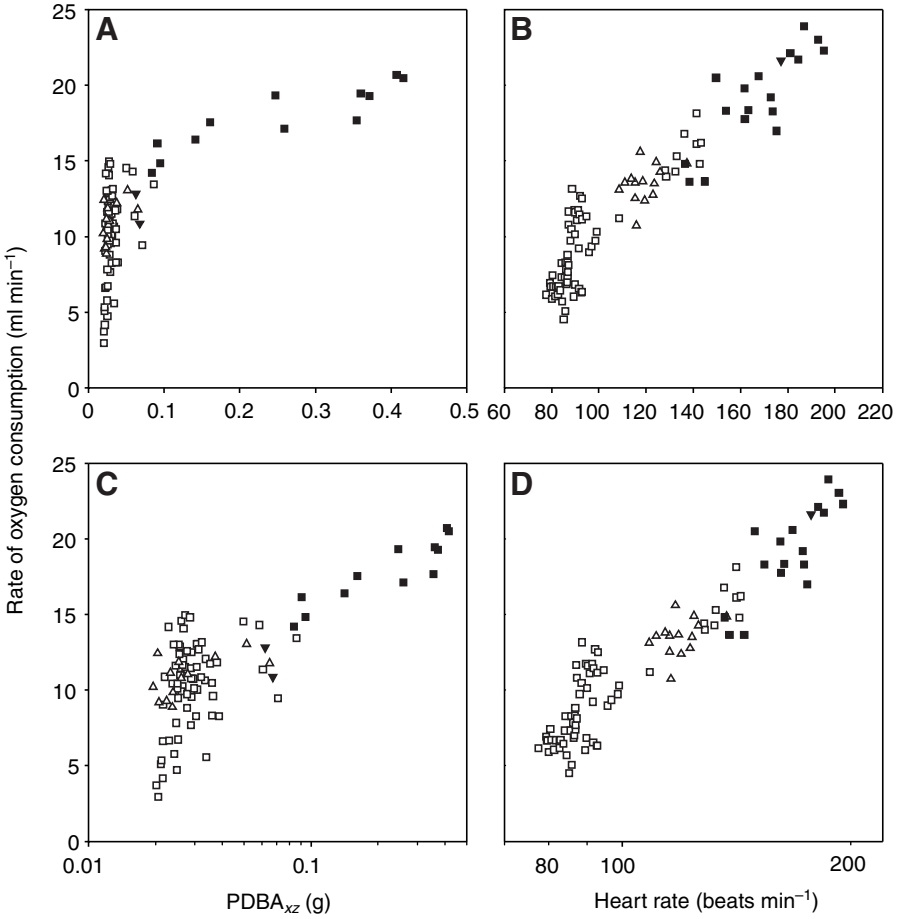

Fig. 6. Rate of oxygen consumption as a function of (A) partial dynamic body acceleration in the *x* and *z* axes (PDBA$_{xz}$) and (B) heart rate across a range of behaviours in a single bantam chicken (ID 73C5). In each case, data were recorded while the chicken walked on a treadmill (filled squares), ate a meal of food pellets (filled triangles), digested the meal of food pellets (open triangles) or thermoregulated (open squares). Data shown in (C) and (D) are the same as in (A) and (B), respectively, but with the *x*-axis displayed as a logarithmic scale.

Table 1. Analysis of variance (ANOVA) of a GLM model to estimate rate of oxygen consumption from partial dynamic body acceleration in the *x* and *z* axes ($PDBA_{xz}$) during all activities

Factor	d.f.	Seq SS	*F*	*P*
Individual	7	893.9	6.15	<0.001
log($PDBA_{xz}$)	1	10413.1	143.85	<0.001
Behaviour	3	314.5	14.50	<0.001
Error	719	5196.8		
Total	730	16818.3		

Data were collected while eight bantam hens undertook four behaviours (eating, digesting, thermoregulating, walking). The model selected was: $\dot{V}_{O_2}$=[8.51 × log($PDBA_{xz}$)]+23.84, R^2=0.69. The significant effects of individual identity and behaviour were incorporated into the standard error of the estimate (s.e.e.) calculated when using the selected model.

Table 2. Analysis of variance (ANOVA) of a GLM model to estimate rate of oxygen consumption from partial dynamic body acceleration in the *x* and *z* axes ($PDBA_{xz}$) while inactive

Factor	d.f.	Seq SS	*F*	*P*
Individual	7	500.8	5.77	<0.001
log($PDBA_{xz}$)	1	549.8	84.69	<0.001
Behaviour	1	161.0	21.52	<0.001
Error	603	4510.8		
Total	612	5722.4		

Data were collected while eight bantam hens undertook two inactive behaviours (digesting, thermoregulating). The model selected was: $\dot{V}_{O_2}$=[8.29 × log($PDBA_{xz}$)]+22.75, R^2=0.21. The significant effects of individual identity and behaviour were incorporated into the standard error of the estimate (s.e.e.) calculated when using the selected model.

calculating s.e.e. values made using this single model. Fig. 7 illustrates this effect and shows how using a single relationship might not be the best approach. Calculation of 95% confidence intervals and 95% prediction intervals shows that using this one-model approach will tend to underestimate $\dot{V}_{O_2}$ during walking. Even if $\dot{V}_{O_2}$ were predicted for a large sample of animals, the error of the estimate would be considerable for estimates made during walking, despite the relatively close relationship during this behaviour.

Further investigation was undertaken with analyses of covariance comparing digestion with thermoregulation ('inactive' states), and walking with eating ('active' states), to investigate the creation of two models. In the case of thermoregulation and digestion, there was again no significant interaction between behaviour and log($PDBA_{xz}$) ($F_{(1,588)}$=2.44, *P*=0.12) and a significant model for inactivity was created (Table 2). While the model was relatively inaccurate (R^2=0.21), the relationship between $PDBA_{xz}$ and $\dot{V}_{O_2}$ during inactivity was statistically significant. Similarly, there was no significant interaction between behaviour and log($PDBA_{xz}$) when eating and walking were compared ($F_{(1,93)}$=0.22, *P*=0.64). Furthermore, there was no significant effect of behaviour in this model ($F_{(1,108)}$=1.95, *P*=0.17), and so a model with only one additional error term was created (Table 3). Plotting predictions made with this two-model approach (Fig. 8) shows that, although the 95% prediction intervals are still relatively large during inactivity, they are substantially smaller during activity than they are in the one-model approach. Furthermore, there is no longer a systematic underestimation of $\dot{V}_{O_2}$ during walking, as in the one-model approach.

Predicting the rate of oxygen consumption as a function of heart rate

Heart rate appeared to be an effective predictor of $\dot{V}_{O_2}$ during all behaviours. To investigate this further, $\dot{V}_{O_2}$ was plotted as a function of f_H during all behaviours for each animal (see Fig. 6B for example). Again, a logarithmic relationship appeared to best fit the data across all behaviours. However, analysis of covariance indicated a significant interaction between behaviour and log(f_H) ($F_{(3,699)}$=5.23,

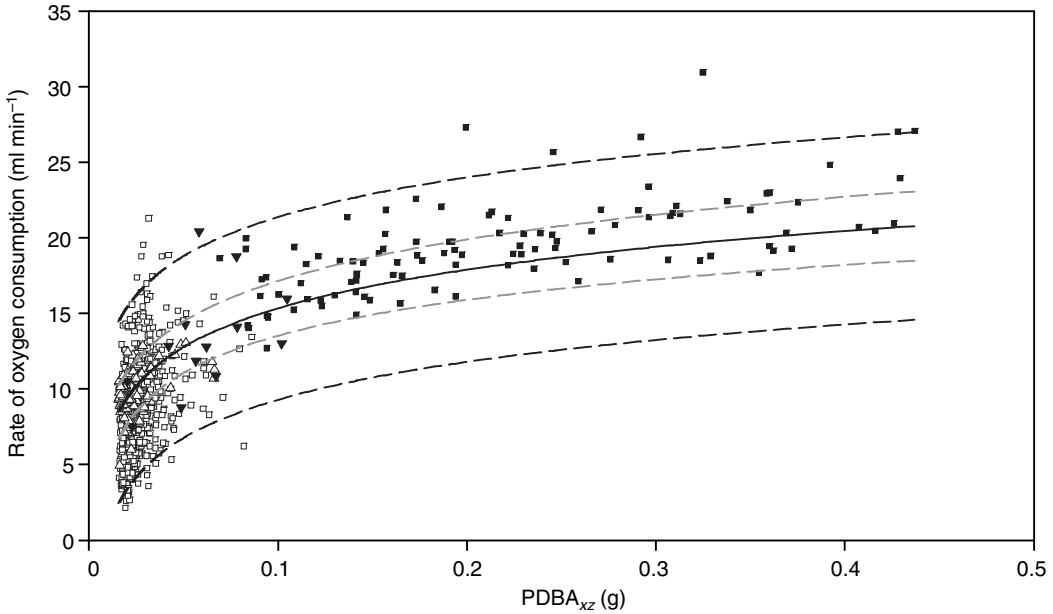

Fig. 7. Rate of oxygen consumption as a function of partial dynamic body acceleration in the *x* and *z* axes ($PDBA_{xz}$) in eight bantam chickens. Data were recorded while the chickens walked on a treadmill (filled squares), ate a meal of food pellets (filled triangles), digested the meal of food pellets (open triangles) or thermoregulated (open squares). Also plotted is a best-fit regression line (solid line) and 95% confidence intervals (black broken lines) and 95% prediction intervals (grey broken lines). 95% confidence intervals were calculated as if s$\dot{V}_{O_2}$ was estimated from one measurement of $PDBA_{xz}$ during one additional behaviour by one additional chicken. 95% prediction intervals were calculated as if s$\dot{V}_{O_2}$ was estimated from 10,000 measurements of $PDBA_{xz}$ of four additional behaviours by 100 additional chickens, effectively the smallest possible prediction interval for this model.

Table 3. Analysis of variance (ANOVA) of a GLM model to estimate rate of oxygen consumption from partial dynamic body acceleration in the *x* and *z* axes ($PDBA_{xz}$) while active

Factor	d.f.	Seq SS	*F*	*P*
Individual	1	309.4	14.46	<0.001
log($PDBA_{xz}$)	7	931.2	279.78	<0.001
Error	109	362.8		
Total	117	1603.3		

Data were collected while eight bantam hens undertook two active behaviours (eating, walking). The model selected was: $\dot{V}_{O_2}$=[10.79 × log($PDBA_{xz}$)]+27.03, R^2=0.77. The significant effects of individual identity were incorporated into the standard error of the estimate (s.e.e.) calculated when using the selected model.

Table 4. Analysis of variance (ANOVA) of a GLM model to estimate rate of oxygen consumption from heart rate (f_H)

Factor	d.f.	Seq SS	*F*	*P*
Individual	7	979.6	37.54	<0.001
log(f_H)	1	13001.3	1423.30	<0.001
Behaviour	3	60.3	5.35	<0.001
Error	730	2741.9		
Total	741	16783.1		

Data were collected while eight bantam hens undertook two inactive behaviours (digesting, thermoregulating). The model selected was: $\dot{V}_{O_2}$=[37.39 × log(f_H)]–64.55, R^2=0.84. The significant effects of individual identity and behaviour were incorporated into the standard error of the estimate (s.e.e.) calculated when using the selected model.

P<0.001). Eliminating each of the behaviours in turn and repeating this analysis revealed that the relationship between f_H and $\dot{V}_{O_2}$ during digestion was significantly different to the relationship between the other three (Fig. 9). Thus, two models were created: one during SDA and the other for all other behaviours. In this two-model approach, there was a considerable overlap between the two relationships (Fig. 9), and so a one-model approach was also investigated. In this case, a single relationship was constructed to enable prediction where behaviour was not known, with behaviour included as an additional error term in the calculation of the s.e.e. (Table 4; Fig. 10). Despite adding this potential for increased uncertainty, there was very little difference in the 95% prediction intervals when comparing the one-model and two-model approaches (Figs 9 and 10).

DISCUSSION

The present study adds more evidence to a growing consensus that measures of dynamic body acceleration can be used to estimate energy expenditure in free-ranging animals (Halsey et al., 2008b; Wilson et al., 2006). That $PDBA_{xz}$ was a good predictor of $\dot{V}_{O_2}$ during activity is not surprising. We reported this result after preliminary analysis of some of the data collected in this study (Halsey et al., 2008a), and the finding matches that from a variety of species with differing gaits and modes of locomotion (Halsey et al., 2008b; Halsey et al., 2008c; Wilson et al., 2006). However, the finding that $PDBA_{xz}$ was also a significant predictor of energy expenditure during periods of inactivity is perhaps unexpected but bodes well for the future application of this technique, particularly given that the model species used exhibited a range of natural behaviours.

Question 1 – dynamic body acceleration during eating and digestion

Comparing $PDBA_{xz}$ and $\dot{V}_{O_2}$ while eating with the same measures during digestion neatly illustrates the potential limitations in using

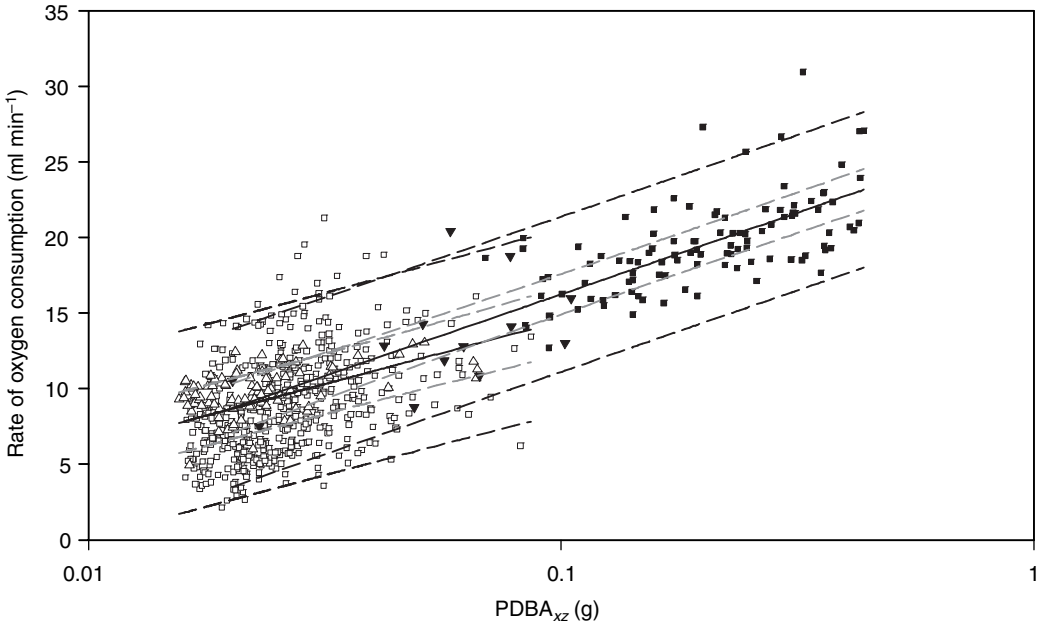

Fig. 8. Rate of oxygen consumption as a function of partial dynamic body acceleration in the *x* and *z* axes ($PDBA_{xz}$) in eight bantam chickens. Data were recorded while the chickens walked on a treadmill (filled squares), ate a meal of food pellets (filled triangles), digested the meal of food pellets (open triangles) or thermoregulated (open squares). Also plotted are two best-fit regression lines (solid line) and 95% confidence intervals (black broken lines) and 95% prediction intervals (grey broken lines). 95% confidence intervals were calculated as if $s\dot{V}_{O_2}$ was estimated from one measurement of $PDBA_{xz}$, during one additional behaviour by one additional chicken. 95% prediction intervals were calculated as if $s\dot{V}_{O_2}$ was estimated from 10,000 measurements of $PDBA_{xz}$, of four additional behaviours by 100 additional chickens, effectively the smallest possible prediction interval for this model. The *x*-axis is displayed on a logarithmic scale to show the clustering of data points while the chickens were inactive (digesting or thermoregulating).

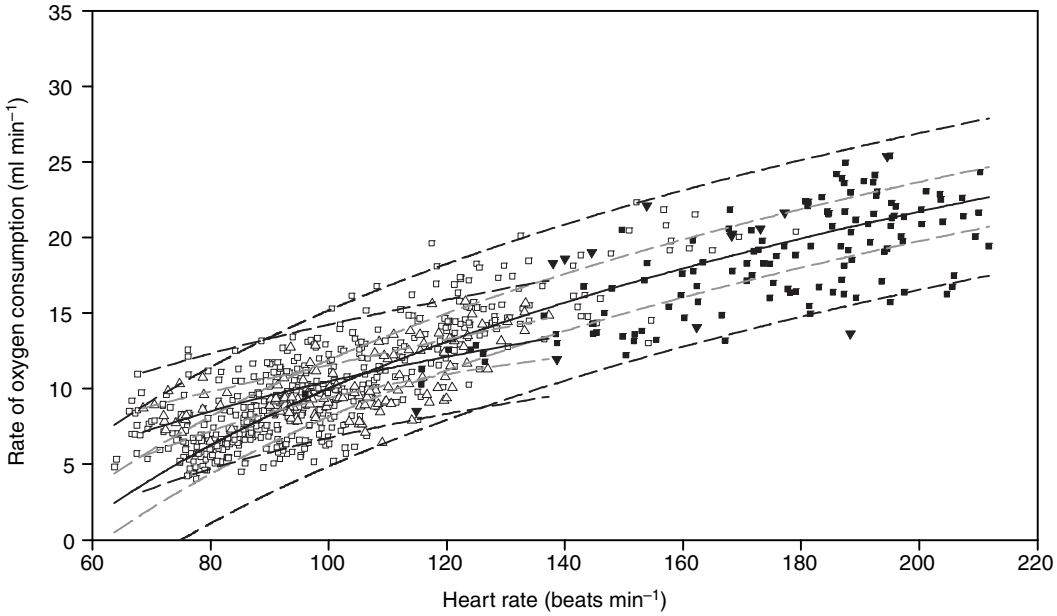

Fig. 9. Rate of oxygen consumption as a function of heart rate in eight bantam chickens. Data were recorded while the chickens walked on a treadmill (filled squares), ate a meal of food pellets (filled triangles), digested the meal of food pellets (open triangles) or thermoregulated (open squares). Also plotted are two best-fit regression lines (solid line) and 95% confidence intervals (black dashed lines) and 95% prediction intervals (grey broken lines). 95% confidence intervals were calculated as if $s\dot{V}_{O_2}$ was estimated from one measurement of heart rate, during one additional behaviour by one additional chicken. 95% prediction intervals were calculated as if $s\dot{V}_{O_2}$ was estimated from 10,000 measurements of heart rate, during four additional behaviours by 100 additional chickens, effectively the smallest possible prediction interval for this model.

$PDBA_{xz}$ to predict $\dot{V}_{O_2}$ for any particular behaviour. While eating, the chickens made repeated pecking movements where the body pivoted from the hip, and the neck extended to reach the food. This induced a 76% increase in $\dot{V}_{O_2}$ and commensurate 141% increase in $PDBA_{xz}$ (Fig. 2), thus the difference was easily detectable – an example of an increase in energy expenditure being well detected by $PDBA_{xz}$ during active behaviours. Furthermore, it should be possible to detect these non-locomotory active behaviours from

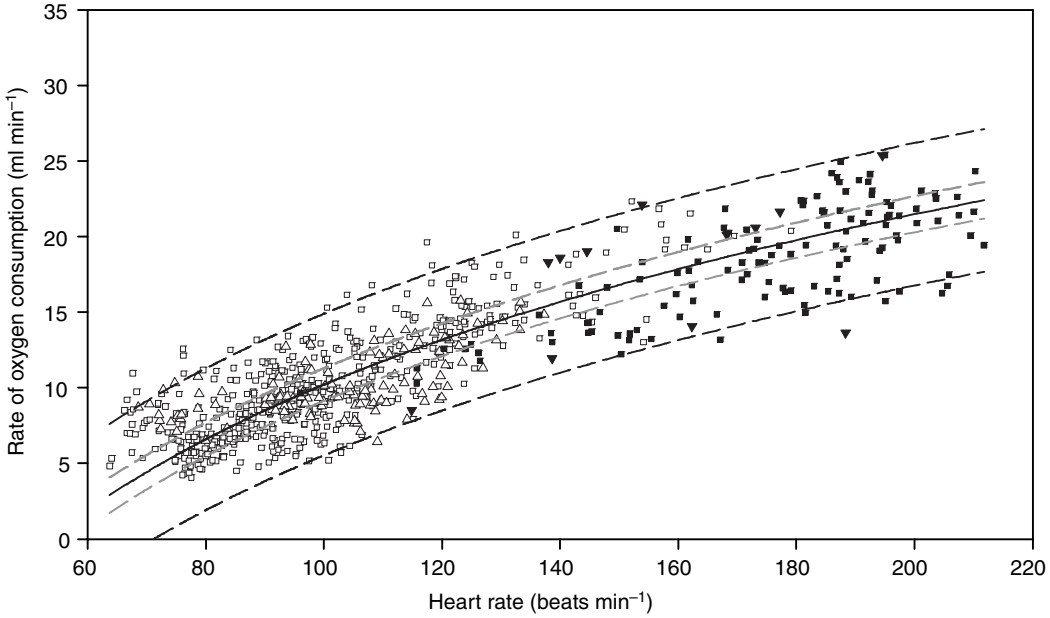

Fig. 10. Rate of oxygen consumption as a function of heart rate in eight bantam chickens. Data were recorded while the chickens walked on a treadmill (filled squares), ate a meal of food pellets (filled triangles), digested the meal of food pellets (open triangles) or thermoregulated (open squares). Also plotted are best-fit regression lines (solid line) and 95% confidence intervals (black broken lines) and 95% prediction intervals (grey broken lines). 95% confidence intervals were calculated as if $s\dot{V}_{O_2}$ was estimated from one measurement of heart rate, during one behaviour by one additional chicken. 95% prediction intervals were calculated as if $s\dot{V}_{O_2}$ was estimated from 10,000 measurements of heart rate, during four additional behaviours by 100 additional chickens, effectively the smallest possible prediction interval for this model.

analysis of characteristics of the acceleration data (Shepard et al., 2008). However, while the birds were digesting, no movement was seen by observers, yet $\dot{V}_{O_2}$ increased by 42% from resting levels. Blaxter (Blaxter, 1989) notes that the increase in metabolism during digestion is attributable predominantly to upregulated biochemical processes, which one would not expect to be reflected in increased body movement. Thus, although the increase in energy expenditure was similar to that found in other birds (Chappell et al., 1997; Green et al., 2006; Kaseloo and Lovvorn, 2003; Sedinger et al., 1992), it was accompanied by an increase in $PDBA_{xz}$ of only 10%, which was not statistically significant.

Question 2 – dynamic body acceleration during thermoregulation

As with digestion, despite clear differences in energy expenditure associated with thermoregulation, there was no significant effect of ambient temperature on $PDBA_{xz}$. In some respects, this is a surprising finding, particularly with regard to ambient temperature below the apparent lower critical temperature of the chickens. In the present study, we did not determine the exact boundaries of the TNZ, but Fig. 4 shows that it would be in the range of 22–30°C, similar to that reported for larger broiler chickens (Meltzer, 1983). Measurements made at 1°C and 11°C were clearly below the lower critical temperature, as shown by the increase in energy expenditure compared with that at 30°C. At 1°C and to a lesser extent at 11°C, $\dot{V}_{O_2}$ was lower while birds were equipped with an accelerometer than when they were equipped with a heart-rate transmitter (Fig. 4). The experiments with the f_H transmitter were conducted first, and, even after repeating the measurements with the accelerometer attached, we achieved the same result. Thus, we attributed this slight difference to the chickens acclimatising to the cooler conditions in their accommodation as the experiments progressed through the winter.

Although the potential for non-shivering thermogenesis has been demonstrated (Toyomizu et al., 2002), the primary mechanism for regulatory thermogenesis in birds is shivering (Dawson and Whittow, 2000); thus, it is surprising that no increase in body movement was registered by the accelerometer. Body tremors during shivering below the LCT have been detected in small mammals and correlate well with ambient temperature and metabolic rate (May, 2003). Presumably, the magnitude of movement or tremors associated with shivering in the chickens was either too small when compared with static elements of acceleration or too low to be detected by the accelerometer. Alternatively, extremely high-frequency movements might not have been detected with our 10 Hz sampling protocol. In our experiments (and most likely applications in birds), the accelerometer was fixed to the feathers with tape (Wilson and Wilson, 1989). It is possible that this might have served as a buffer to the high-frequency movements probably associated with shivering and that movements of the body did not transmit through the feathers to the accelerometer. This could be tested by using a harness attachment, but this is generally not recommended for birds (Phillips et al., 2003). Finally, owing to instrument failure, we could not detect small measures of acceleration in the y axis (surging acceleration) and therefore could not calculate ODBA during our experiments. Although it seems unlikely, it is possible that, during both thermoregulation (and even digestion), noteworthy movement could have occurred exclusively in this plane that we failed to detect.

Question 3 – dynamic body acceleration: comparing inactivity with walking

Both $PDBA_{xz}$ and $\dot{V}_{O_2}$ increased significantly with increasing walking speed (Fig. 5). Coupled with the significant changes in $PDBA_{xz}$ and $\dot{V}_{O_2}$ while eating, $PDBA_{xz}$ was evidently an excellent predictor of $\dot{V}_{O_2}$ during activity. The coefficient of determination (R^2=0.77) (Table 3) of this relationship compared favourably with those from walking great cormorant (*Phalacrocorax carbo*, R^2=0.81) (Wilson et al., 2006), human (R^2=0.91, 0.77) (Halsey et al., 2008b) and coypu (*Myocastor coypus*, R^2=0.91) (Halsey et al., 2008c).

During inactivity (resting during digestion and thermoregulation), we still detected a relationship between $PDBA_{xz}$ and $\dot{V}_{O_2}$ when all the data were considered together. While the individual trials examining digestion and thermoregulation did not detect these effects (see above), when all the data were considered, there must have been small amounts of movement correlated with both $PDBA_{xz}$ and $\dot{V}_{O_2}$. The coefficient of determination was much lower (R^2=0.21) (Table 2) during activity, which can be attributed largely to intra-individual variation in $\dot{V}_{O_2}$ at a given value of $PDBA_{xz}$ and the small range of $PDBA_{xz}$ during inactivity (Fig. 6).

It is perhaps not surprising that $PDBA_{xz}$ predictions are less precise for inactive behaviours than are f_H predictions. Although changes in heart rate can occur without commensurate increases in metabolism (Blix et al., 1974), f_H is still a more direct measure of metabolism. The coefficient of determination of a combined relationship between $PDBA_{xz}$ and $\dot{V}_{O_2}$ was still quite high (R^2=0.69) (Table 1), but this is attributable to the range of $PDBA_{xz}$ during activity being approximately 3–4 times greater than that during inactivity (Fig. 6). Thus, the active data have a disproportionate effect on the fit of the single relationship.

The rate of change of $\dot{V}_{O_2}$ with increasing $PDBA_{xz}$ is far greater during inactivity than activity (Fig. 7). This curvilinear relationship between $PDBA_{xz}$ and $\dot{V}_{O_2}$ across all activities and the large amount of intra-individual error coupled with a truncated range of $PDBA_{xz}$ values during inactivity (see Fig. 7) both have important consequences that must be addressed if predictive relationships based on measures of acceleration are to be used. First, small changes in measurements of $PDBA_{xz}$ while inactive can lead to potentially large changes in estimates of $\dot{V}_{O_2}$; thus, it will be important that calculations of $PDBA_{xz}$ or ODBA made from inactive free-ranging animals are accurate and repeatable (Wilson et al., 2006). Second, the relatively large s.e.e. and prediction intervals around estimates of inactive $\dot{V}_{O_2}$ will mean that statistical comparison of two estimates of inactive $\dot{V}_{O_2}$ are unlikely to detect a significant difference (e.g. Fig. 7). For example, a difference in $PDBA_{xz}$ between resting and digesting could not be detected (Fig. 3). Predictions of $\dot{V}_{O_2}$ made using these $PDBA_{xz}$ estimates were not significantly different either (proximate normal test, z=0.38, P=0.70). However, this should not preclude the application of this method to inactive animals. Applications of body acceleration currently use laboratory measurements and time-budgets to estimate energy expenditure while free-ranging animals are inactive (Wilson et al., 2008). Estimating energy expenditure while inactive from body acceleration might generate large errors around prediction, but these errors are at least quantifiable and depend on fewer assumptions. Certainly, both of these methods are likely to be more accurate than using interspecific allometric formulae as these can have very large errors of prediction, often ignored or obscured by many orders of magnitude in mass data (e.g. Nagy, 2005). For example, an analysis of data presented by McKechnie and colleagues (McKechnie et al., 2006) for captive-bred animals (comparable to the domestic chickens in this study) indicates that an estimate of basal metabolic rate for a body mass would have a coefficient of variation (CV=100*s.e.e./Estimate) of 19.7%. Furthermore, in many cases, using dynamic acceleration to estimate $\dot{V}_{O_2}$ might be the only

practicable method available. A calibrated quantitative estimate is also likely to be more useful than an un-calibrated qualitative measure where only the magnitude of the proxy can be compared (e.g. Weimerskirch et al., 2001).

In the current study, we concentrated on calibrations made over a relatively short timescale (5 min). Preliminary work has shown that, if the timescale over which averages for calibration relationships are constructed is increased, s.e.e. will decrease (D. Thompson and C. Sparling, personal communication). Furthermore, this will tend to reduce the number of data points in the range of $PDBA_{xz}$ found during inactivity, thus reducing the skew that these points currently exert on the one-model relationship. However, predictions from a regression should be used only speculatively to make estimates at a finer timescale than that used in calibration (Green et al., 2001; Halsey et al., 2008a), which could limit the temporal resolution over which $\dot{V}_{O_2}$ can be estimated in the field. This might mean that it is not possible to calculate activity-specific costs, but, for many applications where energy expenditure is predicted on an hourly or daily basis (e.g. Green et al., 2007), this might be the most appropriate approach.

Question 4 – comparing accelerometry with heart rate

Using heart rate to estimate energy expenditure in free-ranging animals is a well-established technique (Butler et al., 2004). Like the use of body acceleration, it requires a laboratory calibration in order to apply it in the field, and precautions should be taken that the calibration procedure encompasses the full range of behaviours undertaken by animals in the field (e.g. Froget et al., 2002; Green et al., 2008; Ward et al., 2002). In the present study, it was easy to calibrate f_H and $\dot{V}_{O_2}$ in the same experimental animals as those used in the calibration of $PDBA_{xz}$ and $\dot{V}_{O_2}$ and hence compare these two proxies of energy expenditure. As in previous studies (Froget et al., 2002; Green et al., 2008), concomitant changes in f_H could be detected alongside changes in $\dot{V}_{O_2}$ during digestion (Fig. 3) and thermoregulation (Fig. 4). The relationship between f_H and $\dot{V}_{O_2}$ during digestion was different to that during walking, whereas, in little penguins (*Eudyptula minor*), there was no difference (Green et al., 2008). By contrast, the relationship between f_H and $\dot{V}_{O_2}$ for thermoregulation was the same as that during walking, whereas, in king penguins (*Aptenodytes patagonicus*), it was different (Froget et al., 2002). These two findings once again highlight the importance of species-specific calibration procedures when applying the f_H technique.

When comparing the two techniques directly, the coefficient of determination of the one-model approach was higher for heart rate (R^2=0.84, Table 4 *vs* R^2=0.69, Table 1). To look at this in more detail, we repeatedly simulated a day in the life of 10 chickens, where $\dot{V}_{O_2}$ and s.e.e. were estimated for 24 h using the one- and two-model approaches for both $PDBA_{xz}$ and f_H. In the simulation, the amount of time spent active by the chicken was varied between 0 and 100%. We assumed that, while inactive, $PDBA_{xz}$ and f_H were equivalent to the mean values for digestion (Fig. 3) and that, while active, $PDBA_{xz}$ and f_H were equivalent to the mean values while walking at 1 km h^{-1} (Fig. 5). We assumed that these measurements had been taken 12 times each hour in each bird. $\dot{V}_{O_2}$ was estimated using the appropriate equations for both the one- and two-model approaches, using both techniques, and s.e.e. and the coefficient of variation (CV=100*s.e.e./Estimate) plotted as a function of the proportion of time spent active (Fig. 11).

The results indicate the importance of choosing both the most appropriate technique and model. It should also be noted that the results and appropriateness of different techniques and models are likely to vary between subject species as a result of the activity

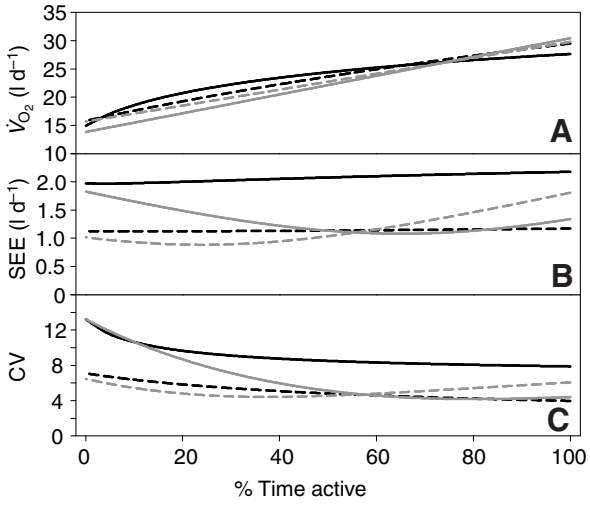

Fig. 11. Simulation showing the effect of model selection on precision and accuracy when estimating the rate of oxygen consumption ($\dot{V}_{O_2}$) in bantam chickens. A day in the life of a chicken was repeatedly simulated where the proportion of time spent 'active' was varied between 0 and 100%. (A) $\dot{V}_{O_2}$, (B) the standard error of the estimate (s.e.e.) and (C) the coefficient of variation (CV=100*s.e.e./Estimate) were calculated for this range of activity using four predictive approaches. Each of the four approaches used either partial dynamic body acceleration in the *x* and *z* axis ($PDBA_{xz}$) or heart rate (f_H) to predict $\dot{V}_{O_2}$. The approaches used were (1) one-model using $PDBA_{xz}$ (black solid lines), (2) two-model using $PDBA_{xz}$ (grey solid lines), (3) one-model using f_H (black broken lines), (4) two-model using f_H (grey broken lines). See text for further details of the four predictive approaches.

levels of the species and the mathematical properties of the predictive relationships. In our example, if a chicken was active for 20% of its life, then using heart rate (two-model) would be the best technique to choose, whereas, if the chicken was active for 80% of its life, using body acceleration (two-model) would provide the more accurate estimate of energy expenditure (Fig. 11). As might be expected given the results thus far in the present study, if chickens are very inactive, then f_H would provide an estimate of $\dot{V}_{O_2}$ with approximately half the error of prediction than that for $PDBA_{xz}$, independent of the model selected. However, the s.e.e. and CV of the $PDBA_{xz}$ estimate is not so low that it could not be used in many applications. For example, CV never exceeds 14%, which is less than the 19.7% calculated earlier for interspecific allometric estimation of resting metabolic rate (McKechnie et al., 2006). As the percentage time active increased, s.e.e. and CV associated with two-model $PDBA_{xz}$ rapidly decreased (Fig. 11b,c). This is an effect of the relatively small error of the active component of the two-model $PDBA_{xz}$ solution (Fig. 8).

Considering different values of the percentage time active highlights the importance of model selection once a technique has been selected. With f_H, the one-model approach provided a better estimate than the two-model approach as the percentage time active increased over the ~50% level. However, with $PDBA_{xz}$, whatever the amount of activity, the two-model approach provided an estimate with less error than the one-model approach. As the percentage time active increased over the 50% level, the s.e.e. and CV of two-model $PDBA_{xz}$ estimates were very similar to the one-model f_H estimates and better than the two-model f_H estimates. This shows once again that, when animals are fairly active, using body acceleration can provide estimates of $\dot{V}_{O_2}$ with a similar accuracy to those made using heart rate (Halsey et al., 2008b; Halsey et al., 2008c).

Combining heart rate and accelerometry

The simulation shows the importance of selecting the best model available, whichever proxy is being used. Crucially, this entails knowing at any particular time what behaviour(s) the subject animals were undertaking. With the heart-rate method, this can prove to be difficult unless the data logger recording heart rate can store additional pertinent information and/or observations are made of behaviour. Thus, in many applications using the heart-rate method, a one-model approach has been recommended even if it means a decrease in the precision of predictions (e.g. Green et al., 2008). However, acceleration data can provide detailed information on behaviour as well as being a proxy for energy expenditure. Different active behaviours produce different characteristic traces in raw acceleration data (Wilson et al., 2006) (Fig. 1), and thus analyses of these data can reveal a minute-by-minute record of the behaviours undertaken by animals (Gómez Laich et al., 2008; Tsuda et al., 2006; Yoda et al., 1999). Analyses thus far have tended to focus on active behaviours (Gómez Laich et al., 2008), but future work should also concentrate on subtle changes in acceleration traces that might be present during inactivity. For example, during thermoregulation, animals will change their posture in order to minimise or maximise heat loss (Dawson and Whittow, 2000), and this change in position should be detectable by multi-axis accelerometers (Wilson et al., 2008; Yoda et al., 1999). Indeed, this is likely to explain the apparent change in the baseline of the raw accelerometry traces while sleeping inside and outside the TNZ (Fig. 1). A more crude method would be simply to use dynamic body acceleration measures rather than more-complex acceleration analyses. For instance, in the present study, a threshold value of $PDBA_{xz}$ of 0.08 $\boldsymbol{g}$ could be used to delineate activity and inactivity reliably (Fig. 8), thus allowing selection of the appropriate equation from a two-model approach. For example, both Green and colleagues (Green et al., 2008) and Froget and colleagues (Froget et al., 2002) report that the relationship between f_H and $\dot{V}_{O_2}$ during thermoregulation or inactivity was different to that obtained during exercise in penguins. However, in their studies, these authors did not suggest how this difference in behaviour might be detected, because, as in the present study (Fig. 9), the ranges of f_H in these behaviours overlapped substantially. Where Green and colleagues (Green et al., 2008) recommended a one-model approach as a solution to this, accelerometry could easily have detected whether birds were active or inactive.

The inevitable conclusion is that the best way to estimate energy expenditure accurately from free-ranging animals would be to record both heart rate and three-axis acceleration simultaneously in the same animal. This approach has been used in human studies (Brage et al., 2005) – but with only one-axis acceleration. The combination of heart rate and body acceleration would facilitate the choice of both technique and model to match any circumstance encountered by study animals. Future studies should include both f_H and $PDBA_{xz}$ in predictive models of $\dot{V}_{O_2}$.

LIST OF ABBREVIATIONS

f_H	heart rate
$ODBA_x$	overall dynamic body acceleration
$PDBA_x$	partial dynamic body acceleration in the *x*-axis
$PDBA_y$	partial dynamic body acceleration in the *y*-axis
$PDBA_z$	partial dynamic body acceleration in the *z*-axis
$PDBA_{xz}$	partial dynamic body acceleration in the *x* and *z* axes
s.e.e.	standard error of the estimate
$\dot{V}_{O_2}$	rate of oxygen consumption

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REFERENCES

Blaxter, K. (1989). *Energy Metabolism in Animals and Man.* Cambridge: Cambridge University Press.

Blix, A. S., Stromme, S. B. and Ursin, H. (1974). Additional heart rate – an indicator of psychological activation. *Aerosp. Med.* **45**, 1219-1222.

Brage, S., Brage, N., Franks, P. W., Ekelund, U. and Wareham, N. J. (2005). Reliability and validity of the combined heart rate and movement sensor Actiheart. *Eur. J. Clin. Nutr.* **59**, 561-570.

Butler, P., Green, J., Boyd, I. and Speakman, J. (2004). Measuring metabolic rate in the field: the pros and cons of the doubly-labelled water and heart rate methods. *Funct. Ecol.* **18**, 168-183.

Cavagna, G. A., Saibene, F. and Margaria, R. (1963). External work in walking. *J. Appl. Physiol.* **18**, 1-9.

Chappell, M. A., Bachman, G. C. and Hammond, K. A. (1997). The heat increment of feeding in house wren chicks: magnitude, duration and substitution for thermostatic costs. *J. Comp. Physiol. B* **167**, 313-318.

Dawson, W. R. and Whittow, G. C. (2000). Regulation of body temperature. In *Sturkie's Avian Physiology* (ed. G. C. Whittow), pp. 343-390. San Diego: Academic Press.

Fahlman, A., Wilson, R., Svärd, C., Rosen, D. A. S. and Trites, A. W. (2008). Activity and diving metabolism correlate in Steller sea lion Eumetopias jubatus. *Aquat. Biol.* **2**, 75-84.

Falk, K., Benvenuti, S., Dall'Antonia, L., Kampp, K. and Ribolini, A. (2000). Time allocation and foraging behaviour of chick-rearing Brünnichs Guillemots *Uria lomvia* in high-arctic Greenland. *Ibis* **142**, 82-92.

Frappell, P. B., Blevin, H. A. and Baudinette, R. V. (1989). Understanding respirometry chambers: what goes in must come out. *J. Theor. Biol.* **138**, 479-494.

Frappell, P. B., Lanthier, C., Baudinette, R. V. and Mortola, J. P. (1992). Metabolism and ventilation in acute hypoxia: a comparative analysis in a small mammalian species. *Am. J. Physiol.* **262**, R1040-R1046.

Froget, G., Handrich, Y., Le Maho, Y., Rouanet, J.-L., Woakes, A. J. and Butler, P. J. (2002). The heart rate/oxygen consumption relationship during cold exposure of the king penguin: a comparison with that during exercise. *J. Exp. Biol.* **205**, 2511-2517.

Gómez Laich, A., Wilson, R. P., Quintana, F. and Shepard, E. L. C. (2008). Identification of imperial cormorant *Phalacrocorax atriceps* behaviour using accelerometers. *Endanger. Species Res.* (in press).

Green, J. A., Butler, P. J., Woakes, A. J., Boyd, I. L. and Holder, R. L. (2001). Heart rate and rate of oxygen consumption of exercising macaroni penguins. *J. Exp. Biol.* **204**, 673-684.

Green, J. A., Frappell, P. B., Clark, T. D. and Butler, P. J. (2006). Physiological response to feeding in little penguins. *Physiol. Biochem. Zool.* **79**, 1088-1097.

Green, J. A., Boyd, I. L., Woakes, A. J., Green, C. J. and Butler, P. J. (2007). Feeding, fasting and foraging efficiency during chick-rearing in macaroni penguins. *Mar. Ecol. Prog. Ser.* **346**, 299-312.

Green, J. A., Frappell, P. B., Clark, T. D. and Butler, P. J. (2008). Predicting rate of oxygen consumption from heart rate while little penguins work, rest and play. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **150**, 222-230.

Grémillet, D. J. H., Schmid, D. and Culik, B. M. (1995). Energy requirements of breeding great cormorants *Phalacrocorax carbo sinensis*. *Mar. Ecol. Prog. Ser.* **121**, 1-9.

Halsey, L. G., Green, J. A., Wilson, R. P. and Frappell, P. B. (2008a). Accelerometry to estimate energy expenditure during activity: best practice with data loggers. *Physiol. Biochem. Zool.* (in press).

Halsey, L. G., Shepard, E. L. C., Hulston, C. J., Venables, M. C., White, C. R., Jeukendrup, A. E. and Wilson, R. P. (2008b). Acceleration versus heart rate for estimating energy expenditure and speed during locomotion in animals: Tests with an easy model species, *Homo sapiens*. *Zoology* **111**, 231-241.

Halsey, L. G., Shepard, E. L. C., Quintana, F., Gomez Laich, A., Green, J. A. and Wilson, R. P. (2008c). The relationship between oxygen consumption and body acceleration in a range of species. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* (in press).

Kaseloo, P. A. and Lovvorn, J. R. (2003). Heat increment of feeding and thermal substition in mallard ducks feeding voluntarily on grain. *J. Comp. Physiol. B* **173**, 207-213.

May, E. L. (2003). Application of piezoelectric sensor for measuring shivering in small marsupial. *J. Therm. Biol.* **28**, 469-475.

McKechnie, A. E., Freckleton, R. P. and Jetz, W. (2006). Phenotypic plasticity in the scaling of avian basal metabolic rate. *Proc. Biol. Sci.* **273**, 931-937.

McNamara, J. M. and Houston, A. I. (1996). State-dependent life histories. *Nature* **380**, 215-221.

Meltzer, A. (1983). Thermoneutral zone and resting metabolic rate of broilers. *Br. Poult. Sci.* **24**, 471-476.

Nagy, K. A. (2005). Field metabolic rate and body size. *J. Exp. Biol.* **208**, 1621-1625.

Pépin, D., Renaud, P.-C., Dumont, B. and Decuq, F. (2006). Time budgets and 24-h temporal rest-activity patterns of captive red deer hinds. *Appl. Anim. Behav. Sci.* **101**, 339-354.

Phillips, R. A., Xavier, J. C. and Croxall, J. P. (2003). Effects of satellite transmitters on albatrosses and petrels. *Auk* **120**, 1082-1090.

Sedinger, J. S., White, R. G. and Hauer, W. E. (1992). Heat increment of feeding and partitioning of dietary energy in the yearling black brant. *Can. J. Zool.* **70**, 1047-1051.

Shannon, G., Page, B. R., MAckey, R. L., Duffy, K. J. and Slowtow, R. (2008). Activity budgets and sexual segregation in African elephants (*Loxodonta africana*). *J. Mammal.* **89**, 467-476.

Shepard, E. L. C., Wilson, R. P., Quintana, F., Gómez Laich, A., Liebsch, N., Albareda, D. A., Halsey, L. G., Gleiss, A., Morgan, D. T., Myers, A. E. et al. (2008). Identification of animal movement patters using tri-axial accelerometry. *Endanger. Species Res.* (in press).

Stephens, D. W. and Krebs, J. R. (1986). *Foraging Theory*. Princeton, NJ: Princeton University Press.

Toyomizu, M., Ueda, M., Sato, S., Seki, Y., Sato, K. and Akiba, Y. (2002). Cold-induced mitochondrial uncoupling and expression of chicken UCP and ANT mRNA in chicken skeletal muscle. *FEBS Lett.* **419**, 313-318.

Tsuda, Y., Kawabe, R., Tanaka, H., Mitsunaga, Y., Hiraishi, T., Yamamoto, K. and Nashimoto, K. (2006). Monitoring the spawning behaviour of chum salmon with an acceleration data logger. *Ecol. Freshw. Fish* **15**, 264-274.

Ward, S., Bishop, C. M., Woakes, A. J. and Butler, P. J. (2002). Heart rate and the rate of oxygen consumption of flying and walking barnacle geese (*Branta leucopsis*) and bar-headed geese (*Anser indicus*). *J. Exp. Biol.* **205**, 3347-3356.

Weibel, E. R. and Hoppeler, H. (2005). Exercise-induced maximal metabolic rate scales with muscle aerobic capacity. *J. Exp. Biol.* **208**, 1635-1644.

Weimerskirch, H., Martin, J., Clerquin, Y., Alexandre, P. and Jiraskova, S. (2001). Energy saving in flight formation. *Nature* **413**, 697-698.

Wilson, R. P. and Wilson, M.-P. (1989). Tape: a package attachment technique for penguins. *Wildl. Soc. Bull.* **17**, 77-79.

Wilson, R. P., White, C. R., Quintana, F., Halsey, L. G., Liebsch, N., Martin, G. R. and Butler, P. J. (2006). Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant. *J. Anim. Ecol.* **75**, 1081-1090.

Wilson, R. P., Shepard, E. L. C. and Liebsch, N. (2008). Prying into the intimate details of animal lives: use of a daily diary on animals. *Endanger. Species Res.* **4**, 123-137.

Winship, A. J., Trites, A. W. and Rosen, D. A. S. (2002). A bioenergetic model for estimating the food requirements of Steller sea lions *Eumetopias jubatus* in Alaska USA. *Mar. Ecol. Prog. Ser.* **229**, 291-312.

Yoda, K., Sato, K., Niizuma, Y., Kurita, M., Bost, C.-A., Le Maho, Y. and Naito, Y. (1999). Precise monitoring of porpoising behaviour of Adélie penguins determined using acceleration data loggers. *J. Exp. Biol.* **202**, 3121-3126.