529 **Behaviour, temperature and terrain slope impact estimates of energy**

530 **expenditure using oxygen and dynamic body acceleration**

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Abstract

547 The energy used by animals is influenced by intrinsic (e.g. physiological) and extrinsic (e.g.

548 environmental) factors. Accelerometers within biologging devices have proven useful for

549 assessing energy expenditures and their behavioural context in free-ranging animals. However,

550　certain assumptions are frequently made when acceleration is used as a proxy for energy

551　expenditure, with factors, such as environmental variation (e.g. ambient temperature or slope

552　of terrain), seldom accounted for.

553　To determine the possible interactions between behaviour, energy expenditure and the

554　environment (ambient temperature and terrain slope), the rate of oxygen consumption ($\dot{V}O_2$)

555　was measured in pygmy goats (*Capra hircus aegarus*) using open-flow indirect calorimetry.

556　The effect of temperature (9.7 to 31.5˚C) on resting energy expenditure was measured. The

557　relationship between $\dot{V}O_2$ and dynamic body acceleration (DBA) was measured at different

558　walking speeds (0.8 to 3.0 km.h^{-1}) and on different inclines (0, +15˚, -15˚). The daily behaviour

559　of individuals was measured in two enclosures: enclosure A (level terrain during summer) and

560　enclosure B (sloped terrain during winter) and *per diem* energy expenditures of behaviours

561　estimated using behaviour, DBA, temperature, terrain slope and $\dot{V}O_2$.

562　During rest, energy expenditure increased below 22˚C and above 30.5˚C. $\dot{V}O_2$ (ml.min^{-1})

563　increased with DBA when walking on the level. Walking uphill (+15˚) increased energetic

564　costs three-fold, whereas walking downhill (-15˚) increased energetic costs by one third. Based

565　on these results, although activity levels were higher in animals in enclosure A during summer,

566　energy expenditure was found to be significantly higher in the sloped enclosure B in winter

567　(means of enclosures A and B: 485.3 ± 103.6 kJ.day^{-1} and 744.5 ± 132.4 kJ.day^{-1}).

568　We show that it is essential to account for extrinsic factors when calculating animal energy

569　budgets. Our estimates of the impacts of extrinsic factors should be applicable to other free

570　ranging ungulates.

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Introduction

At the core of understanding an animal's survival and reproductive fitness is calculating the energetic costs of the ecological processes involved [1,2]. Variation in energy expenditure associated with the extents and intensities of different behaviours impact the fitness and survival of individuals. Part of this relates to efficient foraging [3-5]. Multiple intrinsic (i.e. physiological, such as body mass) and extrinsic (e.g. environmental, such as temperature and terrain) factors influence behaviour and therefore energy expenditure [6-8]. Extrinsic factors are variable, and may be exacerbated climate change [9] which in turn will bring about changes in behaviour, movement, and associated energy expenditure [10,11]. Indeed, it is becoming increasingly clear to what extent environmental variation *per se* affects animal power-use and this is immensely important in understanding the consequences of this variation in a changing world [12-14] A rigorous examination of the methods used to determine energy expenditure within this context is necessary for future work.

Extrinsic factors that influence metabolic rate in animals are associated with variation in environmental conditions, such as ambient temperature (T_a) that changes with latitude, season, elevation as well as the time of day [1,15,16]. The majority of mammals are homeotherms and as a consequence must invest energy into maintaining core body temperature (T_b) when the T_a is on either side of the thermoneutral zone [8,17]. Mammals occurring in seasonal environments may need to invest excess energy into thermoregulation [10,18], or they may evolve physiological, morphological or behavioural traits to moderate the energetic costs of thermoregulation [16,19,20].

Movement is a fundamental part of the ecology of many animals and it necessitates travel through a heterogenous landscape which determines the energetic costs of movement according to the physical characteristics of the 'energy landscape' [21,22]. Specifically, the

597 physical characteristics of the environment, such as aspect, the substrate (e.g. rock *vs* sand) and

598 the 'superstrate' (e.g. grass or snow), affect the energy cost of locomotion [22-24]. Generally,

599 the cost of transport increases when animals travel on sloped terrain; however, the amplitude

600 of this effect varies both within and between species [25]. Energy expenditure during

601 locomotion is also be influenced by limb length [26], gait (i.e. walking, running) [27], and

602 travel speed [28]. Energy expended in locomotion, for resource acquisition or other reasons

603 (e.g. reproduction; Betts *et al.*, 2008), contributes to a large proportion of an animal's energy

604 requirements or daily energy expenditure (DEE) [29,30]. For example, locomotion in North

605 American pumas (*Puma concolor*) was estimated to contribute 14% of the DEE (MJ day^{-1})

606 despite individuals being active for only 4.7% of the time [13].

607 Tri-axial accelerometers in animal-attached tags have been deployed extensively on

608 individuals to measure behaviour and movement [31-33], and can be used to determine the

609 relative cost of energy expended during activity [5,34,35]. Specifically, measures of

610 acceleration such as 'overall dynamic body acceleration' (ODBA) or 'vectorial dynamic body

611 acceleration' (VeDBA), generally referred to as DBA [5,36], have been corroborated with

612 simultaneous measurements of the rate of oxygen consumption (VO_2) using indirect

613 calorimetry [35,37] or the doubly labelled water technique [38,39]. The particular utility is that

614 calibrations of energy expenditure, for example from measured $\dot{V}O_2$ against DBA, allows for

615 an estimate of a free-roaming animal's behaviour-specific power (J s^{-1}) and DEE (kJ day^{-1})

616 (e.g. [13,40]), and furthermore how these tie in with variation in the environment.

617 In this study, we aimed to describe the interaction between temperature and terrain

618 slope using a caprid (the African pygmy goat, *Capra hircus aegarus*) as a model species, by

619 simultaneous measurements of $\dot{V}O_2$ using indirect calorimetry and body movement with tri-

620 axial accelerometers. We aimed to examine how behaviour and energy expenditure measured

using biologging tools (tri-axial accelerometers) are predicted to vary in free-ranging individuals depending on the aspect of their environment (i.e., T_a and terrain slope). Pygmy goats are small ruminants (mean $\pm$ SD = 25.9 $\pm$ 6.3 kg) able to adapt to a range of climates and can endure particularly arid conditions [41,42]. We suggest that, since this species can be exposed to a range of conditions in a captive setting, it would be s a good model to use in order to relate to other wild caprids of conservation or management concern, including bezoar goats (*Capra aegagrus*), Alpine ibex (*Capra ibex*) and Siberian ibex (*Capra sibirica*).

The aims of the study were to: 1) measure the resting energy expenditure (REE) of individuals at different temperatures (9.7 to 31.5 ˚C); 2) measure the relationship between DBA and $\dot{V}O_2$ when individuals are resting and walking at different speeds (0.8 to 3.0 km/h; increments of 0.1 km.h^{-1} at temperatures between 11 and 28 ˚C) and to test how this relationship varies with terrain slope (level = 0, positive = +15˚, negative = -15˚); 3) classify behaviours from accelerometry data; 4) and using the measured energy expenditure and daily tri-axial acceleration data to estimate the daily behaviour and DEE of individuals allowed to roam freely in two different enclosures; enclosure A (level terrain) during summer and enclosure B (sloped terrain) during winter. For logistical reasons it was not possible to study the goats in both enclosures during multiple seasons. General linear models (GLM) or generalized linear mixed effects models (GLMM) were used to test the relationship and models were ranked using An Akaike's Information Criterion (AIC). Behaviour was classified using random forest models. Outcomes from these models were then used to estimate daily behaviour and DEE from daily acceleration data, which was then compared between the two enclosures.

Results

Resting energy expenditure

645   The mean ($\pm$ SD) $\dot{V}O_2$ at rest or REE, measured when goats were standing on the treadmill

646   (n=7), was 69.38 ($\pm$ 23.02) ml.min^{-1}. Measured $\dot{V}O_2$ at rest (ml min^{-1}) significantly increased

647   with individual body mass (Table 1). Body mass and age were highly correlated (Pearson's r =

648   7.58, p < 0.001). There was a significant effect of temperature on measured $\dot{V}O_2$ tested using

649   a quadratic function (values derived from predicted values; Figure 1a; Table 1). $\dot{V}O_2$ at rest

650   was highest at the lowest temperature (97.13 $\pm$ 18.36 ml.min^{-1} at 9.72˚C) and decreased with

651   increasing temperature. Between 22˚C and 30˚C, $\dot{V}O_2$ at rest did not change with temperature

652   but increased by 0.03 ml min^{-1} from 30.5˚C (the upper critical limit) to the maximum recorded

653   temperature, 31.5˚C. $\dot{V}O_2$ at rest did not change with humidity ($F_{7,8}$= 0.13, p = 0.069).

654   During measurements, food was provided to individuals while standing in the chamber;

655   therefore, some movement was expected during measurements. $\dot{V}O_2$ at rest increased with

656   body movement (DBA) measured by tri-axial accelerometers (Table 1; Figure 1b).

657

658   **Figure 1:** (a) Measured $\dot{V}O_2$ at rest (ml.min^{-1}) of standing pygmy goats at different ambient

659   temperatures (˚C) using a quadratic model and (b) dynamic body acceleration (DBA, *g*) using

660   a linear regression. The lines show the predicted relationships, the shaded areas show the 95%

661   confidence interval.

662

663   *Walking on level, positive and negative slopes of terrain*

664   The relationship between $\dot{V}O_2$ and DBA was measured on the level (0 ˚, n = 4), and a positive

665   slope (+15˚, n = 3) and negative slope (-15˚, n = 3) while walking at different speeds on a

666   treadmill (Additional file 2). When walking on the level *g*- specific $\dot{V}O_2$ (i.e. $\dot{V}O_2$ at a specific

667   acceleration value) was 76.4 $\pm$ 14.05 ml.min^{-1} at 0.093 g DBA. As DBA increased, $\dot{V}O_2$

668   increased by 6.42 $\pm$ 8.70 ml.min^{-1} per 0.01 g acceleration (Table 1; Figure 2a). The relationship

669 between DBA and $\dot{V}O_2$ was significantly affected by slope (Table 1; Figure 2a). When waking

670 on a positive slope *g*- specific $\dot{V}O_2$ was higher (183.4 ± 17.85 ml.min^{-1} at 0.14 g) and the

671 relationship between $\dot{V}O_2$ and DBA was steeper; $\dot{V}O_2$ increased by 17.93 ± 17.04 ml.min^{-1} per

672 0.01 g acceleration. On a negative slope, by contrast, $\dot{V}O_2$ was also higher than on the level

673 terrain when considered as *g*- specific $\dot{V}O_2$ (110.8 ± 18.98 ml.min^{-1} at 0.14 g acceleration) but

674 the relationship between $\dot{V}O_2$ and DBA did not differ significantly (5.71 ± 15.85 ml.min^{-1} per

675 0.01 g acceleration; Figure 2a). DBA increased linearly with speed (Pearson's r = 0.904, p <

676 0.0001) while goats were walking on the treadmill at different speeds and slopes (Figure 2b).

677 Walking measurements obtained over a range of temperatures (11.7 to 27.8 ˚C) which was

678 accounted for in the model.

Table 1: Top model set explaining energy expenditure associated with; (a) standing at different temperatures, (b) walking at different speeds on the level and (c) walking at different speeds for each slope, with all simpler nested models within $\Delta AIC_c = 6$ retained for inference.

Model	DBA	Temp	Temp^2	Weight	Sl:DBA	df	R^2	ΔAIC_c	Weight
(a) *Resting energy expenditure (REE)*	186.3	-7.96	0.15	3.11	NA	7	0.758	0.00	0.985
(b) *Effect of activity: walking*	455.5		-0.11	7.92	NA	5	0.806	0.00	0.509
	455.8	-4.32		7.87	NA	5	0.800	1.63	0.226
(c) *Effect of terrain: Walking on different slopes*	431.4		-0.11	8.90	+	9	0.874	0.00	0.491
	432.6	-4.42		8.88	+	9	0.875	0.84	0.323

Sl = terrain slope. The degrees of freedom (df), amount of variation explained (R^2), AICc's and Akaike model weights are shown for each model.

679

Figure 2: (a) Predicted relationship between $\dot{V}O_2$ (ml.min^{-1}) and DBA (*g*) while pygmy goats were walking on a treadmill at three different slopes; level (0˚), negative slope (-15˚), and positive slope (+15˚). The shaded area shows the 95% confidence interval. (b) The correlation between DBA (*g*) and walking speed (km.s^{-1}), while goats are walking on a treadmill at different speeds and different slopes.

Behavioural classification

To classify behaviour, random forest models were built for data collected in each enclosure using a subset of data with behavioural observations (Additional file 1). Classification accuracy to identify five behaviours in enclosure A (level) was 93.2% and to identify five behaviours including the slope of terrain in enclosure B (sloped) for two locomotion behaviours was 80.4% (for further information see Additional file 2).

Estimated daily behaviour and energy expenditure of animals in outdoor enclosures

Overall, standing accounted for the largest proportion of time (0.45 ± 0.30), followed by resting (0.38 ± 0.34) and eating (0.21 ± 0.17; Table 2). Locomotory behaviours made up the least amount of time (walking = 0.019 ± 0.03, running = 0.0011 ± 0.002). A total of 867 seconds were marked as unknown behaviour, which accounted for 0.028% of total time. Using predictions from measured $\dot{V}O_2$, energy expenditure for each behaviour was estimated using models shown in Table 3 (Table 2; Figure 3; Additional file 1).

The proportion of time spent active (eating, walking or running) was higher in the level enclosure during summer (enclosure A) than the sloped enclosure during winter (enclosure B) and was not explained by mean daily temperature (Table 3; Figure 4a). There was no interaction

703 between enclosure and temperature. Mean estimated daily energy expenditure was 620.3 ± 119

704 $kJ.day^{-1}$. Daily energy expenditure was higher in the sloped terrain during winter than the level

705 enclosure during summer and with lower mean daily temperatures (Table 3; Figure 4b). The

706 effect of temperature was stronger than the effect of enclosure, which relates to the slope of

707 enclosure B. Temperature and daily energy expenditure were not correlated (Pearson's r = 1.02,

708 p = 0.31).

709

Table 2: Description of the energetic costs for each behaviour, the acceleration range observed for each behaviour and the line equations used to calculate the energetic costs of each behaviour.

Behaviour	Description of energetic cost	Acceleration range (g)	Equation
Standing	VO_2 at rest measured in this study at different temperatures and accounting for DBA.	0 - 1.22	$y = 66.95 + 0.15T_a^2 + -7.97T_a + W + A$
Resting	Lying down has been measured to use 29% less energy than standing for goats (Dailey and Hobbs, 1989).	0 - 0.434	$y = (66.95 + 0.15T_a^2 + -7.97T_a + W + A) *0.29$
Eating	$\dot{V}O_2$ at rest measured in this study at equivalent DBA.	0.218 - 1.01	$y = 66.95 + 0.15T_a^2 + -7.97T_a + W + A$
Walking	Walking $\dot{V}O_2$ measured in this study at different speeds and terrain slopes at equivalent DBA.	0.105 - 0.786	$y = -75.622 + \alpha_j + (642.345+\gamma_i) *A + T_a + W$
Running	The additional cost of this gait was estimated as 2.5 time the energetic cost of walking (Parker, Robbins and Hanley, 1984).	0.786 - 1.67	$y = -75.622 + \alpha_j + (642.345+\gamma_i) *A + T_a + W) *2.5$ $\alpha_l = 0, \gamma_l = 0$ $\alpha_p = -88.393, \gamma_p = 2072.780$ $\alpha_n = 11.853, \gamma_n = 227.434$
Other	Energetic cost not included.		

T_a = Ambient temperature, A = DBA, W = body weight, α_j = terrain slope (α_l = level, α_p = positive, α_n = negative), γ_i = terrain slope*DBA (γ_l = level, γ_p = positive, γ_n = negative)

710

711

Table 3: Top model set explaining the (a) proportion of time spent active and (b) daily energy expenditure, with all models within $\Delta AIC_c = 6$ and lower than all simpler nested models.

Model	Variables retained	K	R^2	AIC_c	ΔAIC_c	Weight
(a) Proportion Time Active	Enclosure + (ID) + (Date)	5	0.64	-430.7	0	0.82
	(ID) + (Date)	4	0.63	-427.6	3.09	0.18
(b) Daily Energy Expenditure	Enclosure x Temp + (ID) + (Date)	7	0.82	1264.7	0	0.966

The degrees of freedom for each model (df), amount of variation explained (R^2), AICc and Akaike model weights are shown for each model.

712

713

714 **Figure 3:** Predicted energy expenditure for pygmy goats in each enclosure (A or B) for five

715 behaviours in relation to the strongest predictor for the respective model used: (a) Resting, (b)

716 Standing, (c) Eating, (d) Walking and (e) Running. Dashed lines represent prediction intervals.

717

718 **Figure 4:** (a) Proportion of time spent active and (b) mean daily energy expenditure in each

719 enclosure (A or B) for pygmy goats in addition to; (c) mean daily energy expenditure at

720 different temperatures in each enclosure.

721

722 **Discussion**

723 Biologging devices are frequently used to quantify behaviour and estimate the energy

724 expenditure of free-roaming animals [5,32,33,43]). In fact, the real importance of these devices

725 is that they seemingly have the potential to examine the intimate details of animal lives

726 remotely (e.g. [34,44]), including measurements of aspects of the environment that the

727 individuals experience [22]. It is therefore useful to think of these devices as an approach that

728 allows easy access to the relationship between activity and power use. However, given the

729 inherent complexities of measuring energy expenditure [7,5], quantifying behaviour [45,46]

730 and the interactions between these and the environment, including the role of power in incline

731 movement [25], superstrate [22]and thermal substitution [47], investigating and controlling for

732 limitations in the method is clearly essential.

733 Although the use of acceleration as a proxy for energy expenditure has been shown to

734 be a valuable tool after an association between DBA and energy expenditure was found in

735 cormorants [43], our study indicated that energy expenditure in these pygmy goats was higher

736 in was higher in the sloped terrain during the winter (enclosure B), than on the level terrain

737 during the summer months (enclosure A), despite animals having higher activity in A. This

738 could be explained both by the topography of the enclosures, because enclosure B was sloped,

739 and the temperatures, which were lower in enclosure B. A dramatic illustration is the way in

740 which g- specific energy expenditure varied in our studies with slope (Figure 2) which suggests

741 that when using DBA metrics to estimate power, aspect and gradient of the substrate must at

742 least be included.

743 The response of the pygmy goats to temperature (Figure 1a) is typical of that found in

744 mammals [8] and broadly reflect the characteristic U-shape of the power *versus* temperature

745 curve displaying the thermo-neutral zone at its lowest point [48]. In short, the increase in energy

746 expenditure at colder temperatures was explained by the energy cost of thermoregulation

747 [49,50], which requires individuals to spend more energy to maintain their core body

748 temperature to cope with the increasing rate of heat loss to the environment. Energy

749 expenditure also increases with higher temperatures [51,52] as was observed (Figure 1a), since

750 animals must engage in energy demanding processes to lose heat when the rate of heat transfer

751 between themselves and the environment is inadequate to maintain body temperature. Pygmy

752 goats were domesticated in Africa where temperatures are generally higher than in this study

753 and are able to tolerate high temperatures [41]. In our study, an increase in energy expenditure

754 was observed at the warmest temperatures measured. Thus, data from this study supports a

755 "thermo-neutral zone" between about 22 and 30.5 ˚C. This is in agreement with a previously

756 measured upper critical temperature measured by Luiting *et al.*, [53], but is higher than the

757 lower critical temperature of 9˚C found for feral goats [8], although reasons for this are unclear.

758 Acclimation may play a role in observed TNZ, where the TNZ is dependant on the conditions

759 experienced by individuals prior to measurement [54].

760 The relationship between $\dot{V}O_2$ and temperature that we measured for animals at rest

761 (while standing) would, under normal conditions, also be affected by activity, with higher

762 activity, for example, displacing the characteristic power *versus* temperature curve to the left.

763 This is because muscular activity generates considerable heat [55] and this can mitigate what

764 would otherwise have to be paid for by inactive thermogenesis [56]. This thermal substitution

765 has been recognised as important in diving animals (e.g. [47]) and obviously complicates

766 estimates of energy expenditure of wild animals and in turn would apply to the pygmy goats in

767 this study that were predominantly in the outside enclosures. The details of how the process

768 would affect our overall estimates of energy expenditure in the two environments are

769 problematic to assess. The best approach would have been to adopt experimental protocols

770 with varying temperatures and varying degrees of activity to see how exercise-mediated $\dot{V}O_2$

771 affects the power *versus* temperature curve.

772 The increase in energy expenditure calculated for pygmy goats in enclosure B can also

773 be attributed to the sloped terrain, which increases the energetic costs of locomotion for animals

774 both ascending and descending the slopes compared to goats on the level terrain (Figure 2a).

775 Indeed, as temperature was accounted for (but see thermal substitution above), the main

776   difference between the two enclosures was the presence of a slope in enclosure B as well as a

777   seasonal effect. Energy expenditure during locomotion in caprids has been measured in a few

778   studies (e.g. [24]). However, the relationship with DBA has not previously been quantified,

779   which has the advantage in being able to enable estimates of energy expenditure for free-

780   moving individuals [37]. As with other studies [13], our work using pygmy goats on a treadmill

781   indicated the extent to which energy expenditure was higher on a positive slope, than on level

782   substrate (Figure 2a, Table 3). On a downhill slope, the energetic cost was marginally higher,

783   in accordance with Dailey and Hobbs [23], who found energy on downhill slopes overlapped

784   with measurements on level aspects, but contradicts Lachica *et al.* [24], who found lower power

785   use for animals moving down slopes. Power use on a descending slope is more complex than

786   for that of an ascending slope: When animals move uphill they have to provide energy to

787   overcome gravity manifest in potential energy change (given by PE = mgh, where m is the

788   mass, g is the gravitational constant and h is the height increment), so the rate at which energy

789   must be provided depends directly on the speed and the extent of the incline (cf. Figure 2).

790   However, the power use for descending a slope should theoretically initially decrease at

791   shallow slopes as the cost of locomotion is initially partially subsidized by the gain in energy

792   from the realised potential energy, followed by an increase in power as the animal works to

793   brake descent at steeper slopes [25,57]. This phenomenon is clear when descent slope is varied

794   by small increments (e.g. Fancy and White [58]) but is obviously complicated when slope

795   increments are large, as in our case and other animal studies [25]. Overall, it is clear that there

796   are varying relationships between cost of transport and slope of terrain across species [25],

797   presumably as a consequence of the specifics of the animal morphologies [59].

798   Terrain type affects behaviour and energy expenditure beyond just slope because, for

799   example, whether the environment underfoot is more or less compliant changes the costs of

800   locomotion [23,60] (also demonstrated in humans cf. [61]). We were not able to correct for the

801   effects of substrate compliancy in our two enclosures, which were tarmac and grass, but we

802   note that Bidder *et al*. [60] measured an increase in $\dot{V}O_2$ of 9.1% on tarmac and 17.7% on grass,

803   compared to exercise on a treadmill in humans. This suggests estimates of energy expenditure

804   using the treadmill might underestimate the true energy expenditure although this likely also

805   depends on the precise elasticity of the treadmill substrate. For these reasons, we appreciate

806   that our own estimates of energy expenditure of the freely roaming goats are unlikely to be

807   particularly accurate but they are, we believe, a step towards a more refined approach and will

808   help as part of the overall framework attempting to determine power use by animals operating

809   in different energy landscapes [22].

810   Finally, activity levels, measured by the proportion of time predicted in active

811   behaviours compared to inactivity, were higher in the first enclosure (A). Although activity

812   levels may vary seasonally due to temperature [16,62], temperature did not explain the

813   proportion of time active observed in this study. It was accounted for by the enclosure, which

814   may be linked to a seasonal shift in environmental conditions, as enclosure A was measured in

815   summer, and enclosure B in winter. The energetic costs of thermoregulation may be moderated

816   in colder temperatures due to heat produced through activity. The individuals in this study may

817   not respond in the same way as free-living mammals, because our study animals had access to

818   shelters which they could utilise resulting in lower energetic costs to thermoregulation.

819   Ungulates living in seasonal environments may reduce their T_b to reduce the energetic costs of

820   thermoregulation [16,62], which is important to consider when estimating energetic costs of

821   free-living animals. Although temperature was measured on the animal-attached devices, other

822   environmental conditions that affect thermoregulation, such as wind speed and precipitation,

823   were not taken into consideration.

824

Conclusions

In this study, the rate of oxygen consumption was measured using indirect calorimetry and enabled the prediction of energy expenditure using biologging data that included tri-axial acceleration and temperature. Although measures of tri-axial acceleration have been shown to be a useful proxy for energy expenditure in free-ranging animals, acceleration alone cannot account for environmental variation. Furthermore, additional information required for making predictions about behaviour, energy expenditure and an animals' substrate and aspect in an animals' environment will improve what can be interpreted from biologging data. Measuring behaviour and energy expenditure can improve understanding of how species will survive in a changing environment, and provide calibration for estimates of energy expenditure. Future research should account for different elevations in terrain, including variation in substrate and as well as slope or aspect such as would be encountered in species' natural habitat (e.g. species that climb rocks such as ibex).

Methods

Study subjects

Nine female African pygmy goats (*Capra hircus aegagrus*) housed at Belfast Zoo were used in this study (Table 4). Individuals were aged between 3 and 10 years old and weighed between 13 and 32 kg (mean ± SD = 25.9 ± 6.3 kg). They were housed in their normal enclosure comprising a farm building with a level concrete yard and areas of wood mulch (area = 163 m^2, 16.6 x 7.3 m) (enclosure A). Experiments took place in an adjacent pen within the same building during the spring (April to July) and summer (March to June) of 2018 and 2019 to enable measurement of a range of temperatures (T_a ranged from 9.7 to 31.6 ˚C).

848   Daily behaviour was measured using animal-attached tags (biologgers) in both

849   enclosure A in May 2018 and a second enclosure (B) in November 2017. For logistical reasons,

850   it proved impossible to standardize protocols so that animals were studied in both enclosures

851   during one season. Enclosure B comprised a sloping grass paddock (slope = 18%, area = 2210

852   m^2, 50.1 x 35.3 m) surrounded by hedges, with a small heated building adjacent. Sampling in

853   each enclosure was opportunistic as a result of husbandry, and therefore both seasons could not

854   be measured in each enclosure. It was assumed that seasonal variation in metabolic rate was

855   accounted for in respirometry measurement because they were conducted over multiple

856   seasons.

Table 4: Body mass, age and indirect calorimetry measurements of each goat used in the study. Only four individuals could be trained to walk on the treadmill sufficiently well to take measurements of walking energy expenditure (G02, G05, G06 and G07). For more details see Additional file 2.

Goat ID	Age	Weight	Type and number of measurements
G01	6	31.4 ± 2.5	REE (n = 14)
G02	4	23.4 ± 1.6	REE (n = 20); Walking (n = 5, speed = 0.8 – 1.3 km h^{-1}, terrain slope = 0˚)
G03	6	31.5 ± 0.1	-
G04	10	28.7 ± 0.7	REE (n = 12)
G05	7	23.8 ± 1.8	REE (n = 13); Walking (n = 29, speed = 0.8 – 3.0 km h^{-1}, terrain slope = -15 ˚, 0 ˚, 15 ˚)
G06	2	15.1 ± 1.7	REE (n = 21); Walking (n = 34, speed = 0.8 – 2.6 km h^{-1}, terrain slope = -15 ˚, 0 ˚, 15 ˚)
G07	2	17.5 ± 2.3	REE (n = 18); Walking (n = 20, speed = 0.8 – 2.2 km h^{-1}, terrain slope = -15 ˚, 0 ˚, 15 ˚)
G08	7	31.2 ± 0.1	-
G09	5	30.6 ± 0.6	REE (n = 10)

Indirect calorimetry measurements were not obtained for G03 and G08 because they could not be trained to stand in the chamber.

857

Accelerometry data collection

Individuals were equipped with a collar-mounted 'Daily diary' tag [44] which incorporated a tri-axial accelerometer which recorded at a frequency of 40 Hz. Tags also measured magnetic compass heading via a tri-axial magnetometer, as well as temperature and barometric pressure. Each device was powered by a 3.6V lithium battery (LS 14250, Saft, France; 147 mm x 25 mm; 9 g) encased in a plastic housing (combined mass 15.2 g) and sealed with Tesa tape (No. 4651; Tesa AG, Hamburg, Germany). Devices were attached to the collar on the ventral side using Tesa tape and an additional weight (metal nuts; 15 g) attached to ensure the device remained in position ventrally. Collar-mounted devices weighed between 135 g and 235 g dependant on the collar size (< 1% of body mass) and were fitted to be within +3 cm of neck circumference ensuring accurate measurement of body acceleration [63].

Devices were oriented so that the y-axis corresponded to 'heave' (dorso-ventral motion), x-axis to 'sway' (lateral motion) and z-axis to 'surge' (anterio-posterior motion). Before deployment, each device was calibrated for the exact time, direction of the axis, accelerometer and magnetometer offsets.

Measuring the rate of oxygen consumption: Indirect calorimetry setup

An open-circuit indirect calorimetry system was used to measure $\dot{V}O_2$, a measure of energy expenditure with the assumption of no anaerobic respiration [37]. A treadmill intended to exercise dogs (Professional Fit Fur Life Treadmill, Surrey, UK; dimension 180x55 cm), with a respirometry chamber built on top made of polycarbonate sheet connected with aluminium panels was used (Figure 5). The chamber size was adjustable: For pygmy goat measurements, a chamber volume of 440 L was used (80 cm high by 55 cm wide by 100 cm long). Eight fans

881   were spaced across one side of the chamber to ensure complete mixing of air within the

882   chamber. The setup was tested for leaks using nitrogen leak tests [64].

883

884   **Figure 5:** Schematic diagram of the open-circuit indirect calorimetry system used to measure

885   oxygen consumption ($\dot{V}O_2$), showing the air being pumped in by the air pump (1) measured by

886   a flow meter and the air subsample being drawn out, through the drying chamber by the FoxBox

887   Respirometry System. For the highest flow rates, an additional air pump (2) was used which

888   had a built-in flow meter (not shown; Flowkit, Sable Systems, Las Vegas, USA). Temperature,

889   barometric pressure and humidity were also measured within the chamber.

890

891   Fresh air, from outside the building, was pushed through the system using an air pump

892   (Wob-L Piston Series 2660, Rietschle Thomas Sheboygan Inc., Wisconsin, USA) at a range of

893   flow rates (20-150 L/min) because the rate that oxygen declines in the chamber depends on the

894   size and activity state of the subject [65]. Flow rate was measured using a variable area

895   flowmeter (Platon NGX Glass Variable Area Flowmeter, Platon, Saint Etienne, France) before

896   entering the chamber. Inflow temperature was controlled and measured using a temperature

897   control unit (Sable Systems, Las Vegas, USA), prior to measuring the flow rate, to ensure a

898   consistent temperature within the chamber and to prevent chamber temperature increasing due

899   to heat from the air pump. A subsample of air was drawn from the chamber at a rate of 350

900   $ml.min^{-1}$, passed through a drying column (Drierite, DRIERITE, Ohio, USA) to remove water

901   vapour from the air being sampled, and analysed to determine the relative proportion of oxygen

902   and carbon dioxide (FoxBox Respirometry System, Sable Systems, Las Vegas, USA). Flow

903   into the drying column and analyser could be diverted so that it was sourced from fresh air (i.e.

904   from outside), to baseline the measured relative concentration of oxygen. For each

905   measurement, the entire system was turned on and allowed to equilibrate for one hour. Relative

906   oxygen concentration of the chamber gas was monitored until the analyser drift was deemed

907   negligible (<0.001 % O_2 per min; [66]). Data were recorded every 10 s using the FoxBox

908   internal memory and downloaded to a PC after the measurement. Temperature within the

909   chamber was dependant on T_a and was measured at three points throughout the chamber and

910   once outside the chamber (DTM-307B Dual digital thermometer, TECPEL, Taiwan and TP60S

911   Wireless Thermometer Hygrometer, ThermPro, Toronto, Canada). Humidity and pressure

912   were measured once within the chamber (Digital altimeter, Geo-master, Tianjin, China). The

913   fraction of carbon dioxide in excurrent gas was monitored to ensure the drying agent, Drierite,

914   was not exhausted. The drying agent was replaced with recharged agent prior to exhaustion.

915   Analyser drift was assumed to be linear over time and was accounted for by calculating

916   the percentage drift, which is the change in incurrent %O2 (F_iO_2) from the start to the end. The

917   percentage drift per minute was then added to %O_2 (*raw*F_eO_2). To calculate the %O_2 corrected

918   for drift (F_eO_2), the following equation was used (see 64);

919
$$F_eO_2 = rawF_eO_2 + \left| \left(\frac{\Delta F_iO_2}{\Delta\text{time}} \right) x \ time \ elapsed \right| \qquad (1)$$

920   V̇O2 was calculated by the rate of air flow (L/min) into the chamber (FR_i) by the difference in

921   the incurrent %O_2 (F_iO_2) and drift corrected excurrent %O_2 (F_eO_2) in the chamber. Flow was

922   corrected to standard temperature and pressure using;

923
$$VO_2 = FR_i \left[\frac{Fi \ O_2 - F_eO_2}{100 - F_eO_2} \right] \qquad (2)$$

924

925   *Indirect calorimetry measurements*

926   For resting measurements (n = 103, see Table 4), each individual was trained to stand within a

927   respirometry chamber for ~50 min following the provision of hay within the chamber. For

walking measurements (n = 88, see Table 4), four individuals were trained to walk on the treadmill within the chamber at different speeds (0.8 – 3.0 km/h) and angle of slope (-15˚, 0˚, +15˚) for up to 30 min (Table 4). All training was conducted using positive reinforcement training techniques [66] over a period of two months, and individuals were motivated using positive reinforcement (with a portion of their daily food) during measurements. Initially, individuals were trained to stand in the chamber. Then, they were trained to walk on the treadmill with the chamber door open, by gradually starting the treadmill for short periods. These periods were gradually increased until they walked for the desired period. The chamber door was closed when individuals were confident walking on the treadmill. Subjects were given ample time, between 5 and 30 minutes, prior to measurements to ensure they were calm. If any sign of stress was exhibited, such as restlessness, vocalisation, or shaking, before or during a measurement, the measurement was abandoned. Individuals were weighed before and after each measurement using an animal weighing scale (Adam Equipment, Milton Keynes, UK).

For each measurement, the system was turned on and allowed to equilibrate for one hour, during which oxygen concentration within the chamber was monitored until analyser drift was deemed negligible (see above). For resting measurements, subjects were placed in the chamber, and typically the system would take ~20 min to reach a steady state. A measurement was taken for 10 min after this steady state was reached and gas conditions were stable. After 10 min, the analyser reading was set to the ambient concentration of 20.95% O_2 by drawing fresh outside air into the chamber for ~5 min and then returned to sampling chamber air, to compensate for analyser drift (Lighton, 2008). The system typically took 30 min to return to steady state with the animal inside the chamber, and a second measurement was taken for 10 min. The animal was removed, and the measurement finished when a steady state was reached with an empty chamber.

952 For measurements taken whilst subjects were walking, the system would take ~12 min
953 to reach steady state chamber oxygen concentration. Animals are assumed to be in a
954 physiological steady state after 3 min of exercise [37,68]. A measurement was taken for 10 min
955 at steady state, before the animal was allowed to stop and leave the chamber. Chamber air was
956 continually measured and the system typically took 30 min to return to steady state. The animal
957 walked at speeds between 0.8 km.h^{-1} and 3.0 km.h^{-1} changing at 0.1 km.h^{-1} increments on the
958 level (level 0°) and at different slopes (positive 15° and negative -15°) dependant on their
959 ability.

960

961 *Acceleration measurement*

962 For each indirect calorimetry measurement, individuals were equipped with a collar-mounted
963 'Daily Diary' tags as described above. Static acceleration (see Additional file 1) was calculated
964 as a measure of body acceleration, using the running mean of raw acceleration over 2 seconds,
965 and subtracted from the raw acceleration to give dynamic acceleration. The vectorial sum of
966 the dynamic acceleration for the three axes was calculated giving VeDBA (hereafter referred
967 to as DBA [5,69]):

$$VeDBA = \sqrt{(A_x{}^2 + A_y{}^2 + A_z{}^2)} \quad (3)$$

969

970 Additionally, each individual was equipped with a collar-attached tag, as described above, for
971 a mean of 4.7 days (SD = ± 1.1 days) in enclosure A and 5.6 days (SD = ± 3.4 days) in enclosure
972 B. Daily acceleration data were selected from 6 hours after deployment and 2 hours before
973 removal.

974

975   *Behavioural classification*

976   To create daily behaviour profiles, each individual was video-recorded (Canon PowerShot

977   SX720 HS; Canon Inc, Japan), and the start and end time of each behaviour was time-stamped

978   using BORIS software [70]. Tri-axial accelerometry and magnetometry data were time-

979   matched with video observations at a resolution of 1 second. Five behaviours were selected

980   and individual goats recorded for a total of 10.9 hrs (mean ± SD = 38.5 ± 16.7 mins, Table 2).

981   Additional behaviours (e.g. aggression, scratching, shaking) that were observed for less than 1

982   second were grouped as 'Other'. Behaviours were classified for each enclosure; enclosure A

983   was level terrain with no slope whereas, enclosure B was sloped terrain, thus the slope of terrain

984   for locomotion behaviours was included in observations to build the model (Table 2).

985   From the raw acceleration and magnetometry axes, 14 variables that describe both the

986   posture and body movement of an individual were calculated (Additional file 1). Random forest

987   models, an extension of classification trees, were used to classify behaviour using the package

988   *randomForest* [71,72]. To build the model the data were randomly split, 60% of the observed

989   data set was used as a training set and 40% as a validation set. Random forest models use

990   classification trees (500 in this model) by building a hierarchy of decision rules to classify

991   observations [73]. Observations are subdivided at each classification node until the Gini index

992   does not decrease, and the mean Gini decrease gives the importance of each predicting variable

993   [71]. The error rate, including the Out-of-bag error estimates, were checked. Confusion

994   matrices were created using the validation dataset to validate the model and the precision and

995   recall of each behaviour were calculated (Additional file 1).

996

997   *Daily behaviour and energy expenditure*

998   Using behavioural classification template behaviours were identified for the daily acceleration

999   data to identify the behaviour of goats for each second throughout the day. The proportion of

1000   time spent conducting each behaviour per day was subsequently calculated. To measure

1001   activity levels, behaviours were classed as active (walking, running and eating) or inactive

1002   (standing and resting). The proportion of time spent in active behaviours was then calculated.

1003   Using the daily behaviour budget, energy expenditures were assigned to each

1004   behaviour, using both behaviour and DBA. Behaviours were categorised as stationary or

1005   locomotory. A relationship between DBA and $\dot{V}O_2$ was assigned to each behaviour, using

1006   resting measurements for stationary behaviours and walking measurements for locomotory

1007   behaviours. Using model predictions, energy expenditure was assigned to each behaviour using

1008   the models predicting the relationship between $\dot{V}O_2$ and DBA, including the effect of body

1009   weight, temperature and slope of terrain for locomotion behaviour. DBA was checked for each

1010   behaviour. Where the model predicted DBA outside the range observed for behavioural

1011   classification, behaviour was marked as unknown. For behaviours that were not directly

1012   measured in this study, the difference in energy expenditure compared to measured behaviour

1013   was used to estimate energy expenditure of those behaviours.

1014   To calculate DEE (kJ.day^{-1}), the time spent in each behaviour was multiplied by the

1015   energetic costs of each behaviour which was then multiplied by the calorific equivalent of 20.51

1016   kJ.L^{-1} O$_2$ consumed, assuming an RQ of 0.90 [74].

1017

1018   *Statistical analyses*

1019   *Indirect calorimetry measurements*

1020   All analyses were conducted using R version 3.6.3 [75]. General Linear models (GLM) or

1021   generalised linear mixed models (GLMM) were used to: 1) assess the effect of temperature on

1022 REE; 2) assess the relationship between $\dot{V}O_2$ and DBA and; 3) test the effect of positive and

1023 negative slopes on the relationship between $\dot{V}O_2$ and DBA. To investigate the relationship

1024 between temperature and REE, a GLMM was used with $\dot{V}O_2$ as the response variable.

1025 Temperature, humidity, body mass and DBA were included as fixed effects and the model used

1026 a quadratic term of temperature to test for a non-linear effect. Goat ID was included as a random

1027 effect. The collinearity between body mass and age was quantified using a Pearson's

1028 correlation.

1029   To model the relationship between $\dot{V}O_2$ and DBA, a GLM was used with $\dot{V}O_2$ as the

1030 response and temperature, humidity, body mass and DBA as fixed effects. To measure the

1031 effect of terrain slope, a similar model was built which included terrain slope (angle of

1032 treadmill) as a fixed effect and an interaction term between DBA and terrain slope. A random

1033 effect of individual was not included in either model due to the number of goats measured (n=4

1034 and n=3, respectively). The correlation between DBA and speed was checked for walking

1035 measurements on the level terrain and two terrain slopes. The global models were simplified

1036 using the *dredge* function in the R package "MuMin" [76] which uses AIC_c to assess the best

1037 fit model. Models within $\Delta AIC_c \leq 6$ were retained for inference and the simplest model was

1038 selected [77]. Model residuals were checked for normality.

1039

1040 *Daily behaviour and energy expenditure analysis*

1041 To explain the proportion of time spent active and daily energy expenditure, two separate

1042 GLMM's were built. In both models, individual ID and date were included as random factors

1043 and enclosure (A or B) and temperature were included as fixed effects. Model residuals were

1044 checked for normality and AICc was used to select the best model. The collinearity between

1045 enclosure and temperature was quantified using a Pearson's correlation.

1046

Supplementary information

Additional file 1: Information for each individual goat included in the study including their body weight and age, the respirometry measurements taken in the study, and the values predicted or measured for each individual in each enclosure. An ethogram of all the behaviours with additional details.

Additional file 2: Input and output of the random forest models used to classify behaviour: error rates including Out-of-bag error estimate, mean Gini decrease of each predicting variable, confusion matrix of predicted and observed behaviours, and precision and recall of each behaviour.

Additional file 3: Respirometry measurements including individual body weight, temperature, DBA, walking speed and measurement type.

Abbreviations

T_a: Ambient temperature; T_b: Body temperature; ODBA: Overall dynamic body acceleration; VeDBA: Vectorial dynamic body acceleration; DBA: Dynamic body acceleration; $\dot{V}O_2$: Rate of oxygen consumption; REE: Resting energy expenditure; GLM: General linear model; GLMM: Generalised linear mixed model

Declarations

Ethics approval and consent to participate

1067 This study was approved by the Queens University Belfast ethics committee (QUB-BS-AREC-

1068 19-004) and Belfast Zoo.

1069 *Consent for publication*

1070 Not applicable

1071 *Availability of data and materials*

1072 Respirometry measurements are available as an additional file. The daily acceleration data are

1073 available from the corresponding author on reasonable request.

1074 *Competing interests*

1075 The authors declare that they have no competing interests

1076 *Funding*

1078 *Authors' contributions*

1079 ED, NM and DMS conceived the study design. ED collected and analysed the data. ED drafted

1080 the manuscript. PS and RPW contributed to writing the manuscript. All authors provided

1081 comments and approved the final manuscript.

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